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Advances in drug-induced diseases

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Advances in drug-induced diseases

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Table of contents

05 Editorial: Advances in drug-induced diseases
Yao Liu, Jia-bo Wang, Linan Zeng, Maxine Gossell-Williams, Patricia Moriel and Miao Yan

08 Potentially Inappropriate Medication Among People With Dementia in China: A Nationwide Cross-Sectional Study
Mengnan Zhao, Zhaoyan Chen, Fangyuan Tian and Ting Xu

16 Population pharmacokinetics model for escitalopram in Chinese psychiatric patients: effect of CYP2C19 and age
Shujing Liu, Tao Xiao, Shanqing Huang, Xiaolin Li, Wan Kong, Ye Yang, Zi Zhang, Xiaojia Ni, Haoyang Lu, Ming Zhang, Dewei Shang and Yuguan Wen

30 Relationship Between Linezolid Exposure and the Typical Clinical Laboratory Safety and Bacterial Clearance in Chinese Pediatric Patients
Ben-Nian Huo, Yue-E. Wu, Ling Shu, Ruo-Qi Zhang, Jian-Wen Xiao, Qian-Bo Li, Wei Zhao, Yun-Tao Jia and Lin Song

39 Impact of pharmacist active consultation on clinical outcomes and quality of medical care in drug-induced liver injury inpatients in general hospital wards: A retrospective cohort study
Dongxuan Li, Jie Dong, Xin Xi, Guili Huang, Wenjun Li, Cheng Chen, Jun Liu, Qian Du and Songqing Liu

50 Literature review of the clinical characteristics of metformin-induced hepatotoxicity
Chunjiang Wang, Hongyi Deng, Yunfei Xu and Ying Liu

60 Analysis of clinical characteristics of mesalazine-induced cardiotoxicity
Junyu Chen, Tengfei Duan, Weijin Fang, Shikun Liu and Chunjiang Wang

69 Cardiovascular safety of febuxostat and allopurinol in patients with gout: A meta-analysis
Xudong Guan, Shengzhao Zhang, Jiayan Liu, Fengbo Wu, Lingyan Zhou, Ying Liu and Na Su

79 The pathogenesis, diagnosis, prevention, and treatment of CAR-T cell therapy-related adverse reactions
Yanping Li, Yue Ming, Ruoqiu Fu, Chen Li, Yuanlin Wu, Tingting Jiang, Ziwei Li, Rui Ni, Li Li, Hui Su and Yao Liu

105 Potential cardiotoxicity induced by Euodiae Fructus: *In vivo* and *in vitro* experiments and untargeted metabolomics research
Dan Zhang, Jintao Lü, Zhixin Ren, Xiaomeng Zhang, Huanzhang Wu, Rina Sa, Xiaofang Wang, Yu Wang, Zhijian Lin and Bing Zhang

125 Clinical features, diagnosis and management of amoxicillin-induced Kounis syndrome
Chunjiang Wang, Yulu Zhou, Weijin Fang, Zuojun Li and Shaoli Zhao

136 Do antibody–drug conjugates increase the risk of sepsis in cancer patients? A pharmacovigilance study
Shuang Xia, Yi-Chang Zhao, Lin Guo, Hui Gong, Yi-Kun Wang, Rui Ma, Bi-Kui Zhang, Yue Sheng, Mayur Sarangdhar, Yoshihiro Noguchi and Miao Yan

151 Data mining and safety analysis of BTK inhibitors: A pharmacovigilance investigation based on the FAERS database
Qing Wan, Qiang Li, Xin Lai, Tiantian Xu, Jinfang Hu and Hongwei Peng

163 Ocular adverse events associated with anti-VEGF therapy: A pharmacovigilance study of the FDA adverse event reporting system (FAERS)
Pan Ma, Xinmei Pan, Ruixiang Liu, Ya Qu, Linli Xie, Jiangchuan Xie, Liya Cao and Yongchuan Chen

176 Drug-induced liver injury in COVID-19 treatment: Incidence, mechanisms and clinical management
Xichuan Li, Wanting Wang, Suying Yan, Weipeng Zhao, Hui Xiong, Cuiping Bao, Jinqian Chen, Yuan Yue, Yanjun Su and Chunze Zhang

191 Post-marketing safety of immunomodulatory drugs in multiple myeloma: A pharmacovigilance investigation based on the FDA adverse event reporting system
Tingting Jiang, Hui Su, Yanping Li, Yuanlin Wu, Yue Ming, Chen Li, Ruoqiu Fu, Lu Feng, Ziwei Li, Li Li, Rui Ni and Yao Liu

203 Treatment for chemotherapy-induced peripheral neuropathy: A systematic review of randomized control trials
Chenkun Wang, Si Chen and Weiwei Jiang

217 Immune-mediated hepatitis induced by immune checkpoint inhibitors: Current updates and future perspectives
Zherui Liu, Yun Zhu, Huan Xie and Zhengsheng Zou

234 Efficacy and safety of concomitant use of proton pump inhibitors with aspirin-clopidogrel dual antiplatelet therapy in coronary heart disease: A systematic review and meta-analysis
Xiaofeng Luo, Min Hou, Shuangshuang He, Xue Yang, Pan Zhang, Yingxin Zhao and Haiyan Xing

245 Autoimmunity associates with severity of illness in elderly patients with drug-induced liver injury
Yu-Ting Xiong, Jian-Fei Wang, Xiao-Xia Niu, Yi-Ming Fu, Ke-Xin Wang, Chun-Yan Wang, Qian-Qian Li, Jian-Jun Wang, Jun Zhao and Dong Ji

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Editorial: Advances in drug-induced diseases

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Editorial on the Research Topic
Advances in drug-induced diseases

Introduction

Pathological damage to normal organs or tissues caused by drugs is called drug-induced disease (Wei et al., 2021). In recent years, drug-induced diseases have increased significantly and become a major global public health problem. At the second Global Ministerial Summit on Patient Safety held in Bonn, Germany, in 2017, the World Health Organization (WHO) announced its third global patient safety challenge on medication safety, which aims to reduce serious, avoidable drug-related harm by 50% globally over the next 5 years (WHO, 2017).

Strict drug safety evaluation has been carried out for new drugs before marketing, but some rare and serious adverse drug reactions (ADRs) may occur when the drug is exposed to a large number of people and used for a long time after marketing (Leslie and Schousboe, 2019). Most countries have established pharmacovigilance systems to reduce or avoid drug damage. However, due to factors such as low spontaneous reporting rate, low reporting quality and imperfect system, indeed drug safety data are available, but improvements are needed, and the incidence of drug-induced diseases is seriously underestimated (Pitts et al., 2016; Andrade et al., 2019). In addition, the different intervention and prevention efforts to reduce drug-induced diseases in various countries will lead to significant differences in the outcome of drug-induced diseases. Therefore, drug-induced diseases need more attention and research. The purpose of this Research Topic is to discuss the latest progress of drug-induced diseases and related research, with a view to further understanding and exploring new strategies for prevention and

treatment of drug-induced diseases, so as to provide reference for improving drug safety of patients.

This Research Topic contains 19 manuscripts, including six review articles and thirteen original research articles, which extensively discuss the causes of drug-induced diseases, such as drug interactions, drug metabolism, drug transport, and genetic differences between individuals. In addition, this Research Topic aims to get further insights into epidemiology, pathogenic factors, and pathogenesis of drug-induced diseases. We hope to help the authorities formulate policies for the prevention and treatment of drug-induced diseases by exploring new methods to promote the safety of drug use for patients.

Firstly, some cross-sectional studies discussed the risk factors of drug-induced diseases. Huo et al. calculated the linezolid (LZD) exposure using the population pharmacokinetics model of pediatric LZD. And they found that the hematological indexes should be carefully monitored during the treatment by LZD, especially the most common adverse reactions, including thrombocytopenia and low hemoglobin, providing a reference for the personalized drug treatment and clinical treatment risk assessment of LZD. A cross-sectional study in China (Zhao et al.) showed that potentially inappropriate drug (PIM) use in older outpatients with dementia was highly prevalent. Age>80 years, female sex, and taking multiple drugs were risk factors for an increased number of PIM. Xiong et al. found that female sex and cholestatic liver damage pattern were dominant in elderly patients with drug-induced liver injury (DILI) through a retrospective hospitalization-based cross-sectional study. Comorbidities were not the direct factors leading to the severity of DILI. On the contrary, autoimmunity can promote the disease progression of elderly patients with DILI, deserving more intensive treatment and monitoring. In addition, Li et al. conducted a retrospective cohort study. The result indicated that the pharmacist active consultation service could help patients with DILI to obtain better medical care and improve patient outcomes. Hence, they call on pharmacists to participate more in patient care.

Then, the safety of drugs after marketing was analyzed and evaluated through the FDA adverse event reporting system (FAERS). Wan et al. analyzed the safety signal of Bruton's tyrosine kinase inhibitors and found that patients treated with ibrutinib were more prone to develop adverse events than those treated with acalabrutinib. Jiang et al. summarized the different safety profiles of immunomodulators in multiple myeloma. The results provided a rationale for clinicians and pharmacists to choose suitable immunomodulators for various patients. Xia et al. conducted a pharmacovigilance study that found that antibody-drug conjugates may increase the risk of sepsis in cancer patients, resulting in high mortality. Ma et al. conducted a pharmacovigilance study showing that ocular adverse events associated with anti-VEGF drugs vary. And the results can provide a reference for clinical drug selection.

Thirdly, some literature analyses and reviews have summarized the clinical characteristics of drug-induced diseases. Wang et al. summarized the clinical characteristics of hepatotoxicity of rare ADRs caused by metformin through literature analysis, which is conducive to the diagnosis and timely treatment of hepatotoxicity caused by metformin. Chen et al. retrospectively analyzed the clinical characteristics of mesalazine induced cardiotoxicity through literature, providing basis for clinical diagnosis, treatment and prevention. Wang et al. retrospectively analyzed the clinical features of amoxicillin induced Kounis syndrome (KS), suggesting that amoxicillin induced KS should be considered when chest pain is accompanied by allergic symptoms, electrocardiogram changes and/or elevated levels of myocardial injury markers. Li et al. reviewed the common adverse reactions of CAR-T cell therapy, as well as the mechanism, risk factors, diagnostic criteria and treatment methods of these adverse reactions, providing valuable reference for the safe, effective and wide application of CAR-T therapy. Other meta-analyses evaluate the efficacy and safety of drugs. Wang et al. evaluated chemotherapy-induced peripheral neuropathy (CIPN), and discussed the differences in the efficacy of related therapeutic drugs. The results showed that duloxetine, venlafaxine, pregabalin, crocin, tetrodotoxin, and monosialotetrahexosyl ganglioside might be beneficial to the treatment of CIPN. Luo et al. through systematic review and meta-analysis, found that in patients with coronary heart disease, the co-use of proton pump inhibitors with aspirin and clopidogrel was associated with a reduced risk of gastrointestinal complications, but may increase the incidence of major adverse cardiovascular events, myocardial infarction, stroke, revascularization, and stent thrombosis. Guan et al. compared the cardiovascular safety difference between febuxostat and allopurinol in gout patients through meta-analysis. It was found that febuxostat may have similar cardiovascular characteristics to allopurinol in patients without atherosclerotic disease. However, allopurinol treatment was associated with lower cardiovascular mortality compared with febuxostat in patients with a history of cardiovascular disease.

In addition, Liu et al. successfully established a population pharmacokinetics model of escitalopram and formulated an individualization of dosing regimens based on the age of the patients, CYP2C19 genotype, and serum drug concentrations. The results emphasized that gene detection and therapeutic drug concentration monitoring during treatment were necessary to achieve dosage regimen individualization. Zhang et al. overviewed how overdosage and irrational usage of Euodiae Fructus can induce cardiac side effects at macroscopic and microscopic levels through *in vivo* and *in vitro* experiments and untargeted metabolomics research, providing evidence and reference for the safety research of herbal medicine.

Meanwhile, in our Research Topic, there are two manuscripts discussing drug-induced liver injury and immune-mediated hepatitis (IMH) respectively. Li et al. reviewed the incidence of abnormal liver function in patients with COVID-19 caused by many antiviral drugs, such as favipiravir, remdesivir, lopinavir/ritonavir, and hydroxychloroquine. At the same time, they expounded the possible basic mechanism, and finally put forward reasonable clinical treatment suggestions for such liver injury. Liu et al. introduced in detail the pathophysiology, epidemiology,

diagnosis, treatment, and prognosis of IMH caused by immune checkpoint inhibitors.

In conclusion, the Research Topic provides the theoretical basis for the current research on drug-induced diseases to improve the level of clinical medication, ensure the maximum benefit of patients while reducing drug damage, and achieve the goal of rational drug use.

Author contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Potentially Inappropriate Medication Among People With Dementia in China: A Nationwide Cross-Sectional Study

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Objectives: The purpose of this study was to explore the prevalence of potentially inappropriate medication (PIM) among older outpatients (age ≥ 65 years old) with dementia in eight cities in China using the AGS Beers criteria of 2019 and to identify the potential factor increasing the number of PIMs.

Methods: A cross-sectional study about PIM in older outpatients with dementia from January 2020 to December 2020 was carried out in eight cities in China, Chengdu, Beijing, Guangzhou, Shanghai, Shenyang, Tianjin, Zhengzhou, and Hangzhou, distributing five major geographical regions in China (east, west, north, south, central). The diagnosis of dementia was based on the International Classification of Diseases (ICD-10) to identify. Based on the 2019 AGS Beers criteria, the PIM prescriptions were evaluated. The identification of potential factors was completed using a binary logistic regression model.

Results: Of 18,624 older outpatients with dementia, 3.52% were detected with 1 PIM, and 35.91% received at least two PIMs. The antipsychotic drugs quetiapine and olanzapine were most frequently prescribed in patients with PIM, accounting for 8.01 and 7.36%, respectively. Logistic regression analyses showed that female patients with dementia aged >80 years who took more medications were exposed easily to PIM use.

Conclusion: PIM use among older outpatients with dementia in China is highly prevalent, and the associated risk factors were increasing age, female sex, and number of medications. The most frequently prescribed drugs by clinicians were anpsychotropic drugs, which were much more frequent than other drugs.

Keywords: potentially inappropriate medications, dementia, outpatient, older, psychotropic drugs

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INTRODUCTION

Dementia is a degenerative disease accompanied by impaired cognitive function. According to statistical data from the World Health Organization (WHO), approximately 50 million people are diagnosed with dementia worldwide, and 60% live in low- and middle-income countries. By 2050, the number is up to 139 million (WHO, 2020). Due to the aging global population, the growing trend will be more apparent, especially in developing countries (Prince et al., 2015; Banta et al., 2017). A study showed that the prevalence of dementia is linked with age, and females are more susceptible to

developing dementia than males (Rizzi et al., 2014). As the most populous country and the largest developing country in the world, China has a large number of dementia patients. A study performed by Jia LF showed that the prevalence of dementia in China is estimated to be 6%, and approximately 15.07 million people (age ≥60 years old) have dementia in China (Jia et al., 2020).

Potentially inappropriate medication is a global concern, especially for older people with reduced physical function. It is considered a term that those drugs should be avoided or cautioned to use when the risk of adverse events may outweigh the potential benefit (Renom-Guiteras et al., 2015). Due to higher comorbidity, number of medications and lower activities of daily living (ADL), people with dementia may have higher PIM use than people without dementia (Clague et al., 2017; Raivio et al., 2006; Kristensen et al., 2018). A recent study showed that the prevalence of PIM in older people with dementia can be as high as 60% (Renom-Guiteras et al., 2018). According to systematic analyses of global disease burden in 2017, dementia is the fifth leading cause of death in China (Zhou et al., 2019). Potentially inappropriate medication (PIM) may be one of the reasons leading to death in older people with dementia, and extensive studies have also shown that the occurrence of PIM leads to an increased risk of adverse events and hospitalizations, even death (Cross et al., 2017; Wallace et al., 2017; Murphy et al., 2020). Thus, it is necessary to determine PIM use and risk factors among older patients with dementia. To date, a growing body of studies about the prevalence of PIM and risk factors have been published, but the sample size of most studies was small (Epstein et al., 2010; Fiss et al., 2013; Hanlon et al., 2015), lacking representativeness. A large sample study by Renom-Guiteras A et al. found that PIM use in older people with dementia is highly prevalent, and patients aged >8 years with more comorbidities and functional impairment are easily exposed to PIM (Renom-Guiteras et al., 2018). However, the sample of this study is limited to European countries, and the screening tool is the European Union (7)-PIM list. Due to differences in race, prescription medication and medical policy among countries, PIM use and related factors also vary based on different screening tools. In addition, there are no large sample studies on the prevalence and risk factors for PIM use in older patients with dementia in China. Therefore, we performed a large, national study to better identify PIM use and potential factors in older patients with dementia in China.

METHODS

Setting and Sample

The cross-sectional study was carried out in eight cities of China, Chengdu, Beijing, Guangzhou, Shanghai, Shenyang, Tianjin, Zhengzhou, and Hangzhou, distributing five major geographical regions in China (east, west, north, south, central). A total of 75 hospitals were included in our study.

The participants were those diagnosed with dementia aged ≥65 years from outpatient clinics of hospitals. The diagnosis of dementia was based on the International Classification of Diseases (ICD-10) to identify Alzheimer's disease (F000-F002, F009, G300, G301, G308 and G309), vascular dementia (F010-F013, F018 and F019), dementia in Parkinson's disease (F023), dementia in Huntington's disease (F022), dementia in Pick's disease or frontotemporal dementia (F020), dementia in HIV (F024), dementia in Creutzfeldt–Jakob disease (F021) and unspecified dementias (F03X and F028). And these participants who met inclusion criteria were cluster sampled from Hospital Information System (HIS) of 75 hospitals between January 2020 and December 2020. HIS is a set of computer information management system combined with hospital management and medical activities, providing data source for numerous studies.

Data Collection

The data from electronic medical records in outpatient clinics of 75 hospitals in eight cities were collected in three parts. The first part was patients' sociodemographic information, including region, hospital, department, sex, and age. The second part was medical information, including disease diagnosis, payment form, and patient code. The third part was prescription information, including the generic name and trade name of medication, dosage, and the number of medications. Patients with incomplete or missing information were excluded from the study.

Evaluation Criteria

In our study, the 2019 AGS Beers criteria were applied to detect the prevalence of PIM in older outpatients with dementia. In addition, this data information from outpatients lacks some indicators of renal function, and the rules of PIM-based eGFR (Table six in the 2019 AGS Beers criteria) were excluded. Overall, we applied **Table 2** (PIM use in older adults), Table 3 (PIM use in older adults due to drug-disease or drug-syndrome interactions that may exacerbate the disease or syndrome), Table 4 (drugs used with caution in older adults), and Table 5 (potentially clinically important drug–drug interactions that should be avoided in older adults) of the 2019 AGS Beers criteria to evaluate PIM use in older people with dementia. In this study, two researchers independently analyzed and evaluated the prescription drugs per patient. The inconsistency between the two researchers was discussed by a third expert.

Statistical Analysis

The data were analyzed by SPSS 26, and p value <0.05 was considered statistically significant. The results of descriptive analyses are presented as the mean ± SD (standard deviation) for continuous variables, and discontinuous variables are presented as the median ± IQR (interquartile range), frequencies or percentages. Based on PIM as a dependent variable, a binary logistic regression model was applied to identify risk factors associated with the PIM use through control covariates such as age, sex, the number of drugs, and the number of diseases.

Ethics Approval

This study protocol was approved by the Sichuan University West China Hospital Research Ethics Board. All procedures performed

TABLE 1 | Basic characteristics of older outpatients with dementia in China.

Characteristic	Chengdu			Beijing			Guangzhou			Tianjin		
	Total(N = 711)	Non-PIM (N = 445)	PIM (N = 266)	Total (N = 5086)	Non-PIM (N = 3087)	PIM (N = 1999)	Total (N = 2269)	Non-PIM (N = 1410)	PIM (N = 859)	Total (N = 1092)	Non-PIM (N = 781)	PIM (N = 311)
Sex, n (%)												
Male	344(46.98)	214(48.09)	120(45.11)	2401(47.21)	1528(49.50)	873(43.67)	1060(46.72)	703(49.86)	357(41.56)	550(50.37)	382(48.91)	168(54.02)
Female	377(53.02)	231(51.91)	146(54.89)	2685(52.79)	1559(50.50)	1126(56.33)	120(53.28)	707(50.14)	502(58.44)	542(49.63)	399(51.09)	143(45.98)
Age, n (%)												
65–80	317(44.59)	220(49.44)	97(36.47)	2361(46.42)	1521(49.27)	840(42.02)	971(42.79)	633(44.89)	338(39.35)	623(57.05)	466(59.67)	157(50.48)
>80	394(55.41)	225(50.56)	169(63.53)	2725(53.58)	1566(50.73)	1159(57.98)	1298(57.21)	777(55.11)	521(60.65)	469(42.95)	315(40.33)	154(49.52)
No. of medications n (%)												
1	143(20.11)	117(26.29)	26(9.77)	1171(23.02)	1059(34.31)	112(5.60)	421(18.55)	373(26.45)	48(5.59)	463(42.40)	447(57.23)	16(5.14)
2–4	342(48.10)	227(51.01)	115(43.23)	2822(55.49)	1585(51.34)	1237(61.88)	1279(56.37)	806(57.16)	473(55.06)	486(44.51)	273(34.96)	213(68.49)
≧5	226(31.79)	101(22.70)	125(46.99)	1093(21.49)	443(14.35)	650(32.52)	569(25.08)	231(16.38)	338(39.35)	143(13.10)	61(7.81)	82(26.37)
No. of diseases n (%)												
1–4	492(69.20)	333(74.83)	159(59.77)	3239(63.68)	2101(68.06)	1138(56.93)	2024(89.20)	1271(90.14)	753(87.66)	967(88.55)	720(92.19)	247(79.42)
5–9	173(24.33)	97(21.80)	76(28.57)	1611(31.68)	880(28.51)	731(36.57)	231(10.18)	130(9.22)	101(11.76)	116(10.62)	60(7.68)	56(18.01)
≧10	46(6.47)	15(3.37)	31(11.65)	236(4.64)	106(3.43)	130(6.50)	14(0.62)	9(0.64)	5(0.58)	9(0.82)	1(0.13)	8(2.57)
Payment n (%)												
Free	177(24.89)	111(24.94)	66(24.94)	371(7.29)	222(7.19)	149(7.45)	341(15.03)	234(16.60)	7(12.46)	1(0.09)	1(0.13)	0(0.00)
Partial Fee	452(63.57)	275(61.80)	177(53.93)	4628(90.99)	2821(91.38)	1807(90.40)	1306(57.56)	786(55.74)	520(60.54)	962(88.10)	697(89.24)	265(85.21)
Full fee	82(11.53)	59(13.26)	23(21.12)	73(1.44)	37(1.20)	36(1.80)	622(27.41)	390(27.66)	232(27.01)	106(9.71)	69(8.83)	37(11.90)
Other	0(0.00)	0(0.00)	0(0.00)	14(0.28)	7(0.23)	7(0.35)	0(0.00)	0(0.00)	0(0.00)	23(2.11)	14(1.79)	9(2.89)
No. of prescription expenditure n (%)												
<500 CNY	310(43.60)	199(44.72)	111(41.73)	1674(32.91)	1188(38.48)	486(24.31)	1455(64.13)	940(66.67)	515(59.95)	520(47.62)	388(49.68)	132(42.44)
500–1000 CNY	149(20.96)	102(22.92)	47(17.67)	1548(30.44)	941(30.48)	607(30.37)	490(21.60)	301(21.35)	189(22.00)	366(33.52)	265(33.93)	101(32.48)
>1000 CNY	252(35.44)	144(32.36)	108(40.60)	1864(36.65)	958(31.03)	906(45.32)	324(14.28)	169(11.99)	155(18.04)	206(18.86)	128(16.39)	78(25.08)

Characteristic	Shanghai			Shenyang			Hangzhou			Zhengzhou		
	Total (N = 6857)	non-PIM (N = 3786)	PIM (N = 3071)	Total (N = 433)	non-PIM (N = 293)	PIM (N = 140)	Total (N = 1052)	non-PIM (N = 696)	PIM (N = 356)	Total (N = 1124)	non-PIM (N = 782)	PIM (N = 342)
Sex, n (%)												
Male	2565(37.41)	1601(42.29)	964(31.39)	255(58.89)	17(58.70)	83(59.29)	498(47.34)	329(47.27)	169(47.47)	703(62.54)	497(63.55)	206(60.23)
Female	4292(62.59)	2185(57.71)	210(68.61)	178(41.11)	12(41.30)	57(40.71)	554(52.66)	367(52.73)	187(52.53)	421(37.46)	285(36.45)	136(39.77)
Age, n (%)												
65–80	2880(42.00)	1773(46.83)	110(36.05)	135(31.18)	10(36.18)	29(20.71)	478(45.44)	333(47.84)	145(40.73)	530(47.15)	410(52.43)	120(35.09)
≧80	3977(58.00)	2013(53.17)	196(63.95)	298(68.82)	18(63.82)	111(79.29)	574(54.56)	363(52.16)	211(59.27)	594(52.85)	372(47.57)	222(64.91)
No. of medications n (%)												
1	2982(43.49)	2403(63.47)	579(18.85)	103(23.79)	92(31.40)	11(7.86)	285(27.09)	250(35.92)	35(9.83)	164(14.59)	146(18.67)	18(5.26)
2–4	3664(53.43)	1304(34.44)	236(76.85)	164(37.88)	12(41.98)	41(29.29)	572(54.37)	358(51.44)	214(60.11)	556(49.47)	412(52.69)	144(42.11)
≧5	211(3.08)	79(2.09)	132(4.30)	166(38.34)	78(26.62)	88(62.86)	195(18.54)	88(12.64)	107(30.06)	404(35.94)	180(23.02)	224(65.50)
No. of diseases n (%)												
1–4	6744(98.35)	3730(98.52)	3014(98.14)	310(71.59)	232(79.18)	78(55.71)	897(85.27)	594(85.34)	303(85.11)	940(83.63)	690(88.24)	250(73.10)
5–9	111(1.62)	55(1.45)	56(1.82)	106(24.48)	53(18.09)	53(37.86)	143(13.59)	96(13.79)	47(13.20)	162(14.41)	80(10.23)	82(23.98)
≧10	2(0.03)	1(0.03)	1(0.03)	17(3.93)	8(2.73)	9(6.43)	12(1.14)	6(0.86)	6(1.69)	22(1.96)	12(1.53)	10(2.92)
Payment n (%)												
Free	3329(48.55)	1277(33.73)	205(66.82)	296(68.36)	19(64.85)	106(75.71)	38(3.61)	21(3.02)	17(4.78)	161(14.32)	81(10.36)	80(23.39)
Partial Fee	3147(45.89)	2316(61.17)	831(27.06)	114(26.33)	85(29.01)	29(20.71)	970(92.21)	642(92.24)	328(92.13)	639(56.85)	475(60.74)	164(47.95)
Full fee	375(5.47)	191(5.04)	184(5.99)	23(5.31)	18(6.14)	5(3.57)	38(3.61)	29(4.17)	9(2.53)	324(28.83)	226(28.90)	98(28.65)

(Continued on following page)

TABLE 1 | (*Continued*) Basic characteristics of older outpatients with dementia in China.

Characteristic	Shanghai			Shenyang			Hangzhou			Zhengzhou		
	Total (N = 6857)	non-PIM (N = 3786)	PIM (N = 3071)	Total (N = 433)	non-PIM (N = 293)	PIM (N = 140)	Total (N = 1052)	non-PIM (N = 696)	PIM (N = 356)	Total (N = 1124)	non-PIM (N = 782)	PIM (N = 342)
Other	6(0.09)	2(0.05)	4(0.13)	0(0.00)	0(0.00)	0(0.00)	6(0.57)	4(0.57)	2(0.56)	0(0.00)	0(0.00)	0(0.00)
No. of prescription expenditure n (%)												
<500 CNY	5288(77.12)	2842(75.07)	244(79.65)	117(27.02)	97(33.11)	20(14.29)	483(45.91)	336(48.28)	147(41.29)	491(43.68)	352(45.01)	139(40.64)
500–1000 CNY	1182(17.24)	704(18.59)	478(15.56)	125(28.87)	97(33.11)	28(20.00)	294(27.95)	194(27.87)	100(28.09)	419(37.28)	284(36.32)	135(39.47)
>1000 CNY	387(5.64)	240(6.34)	147(4.79)	191(44.11)	99(33.79)	92(65.71)	275(26.14)	166(23.85)	109(30.62)	214(19.04)	146(18.67)	68(19.88)

in this study conformed to the standards of the 1964 Helsinki Declaration and subsequent relevant ethics.

RESULTS

Basic Characteristics of Older Outpatients With Dementia

In our study, a total of 55,904 electronic medical records was extracted, 2845 incomplete or missing medical records were excluded, including 1303 items for missing gender, 1185 items for solvents, and 385 repeated drugs in a prescription. Overall, 18,624 patients with dementia were included, distributed among 75 hospitals in eight cities in China. The mean age of the study population was 80.88 ± 7.69 years, ranging from 65 to 103, and 54.85% were female. The median number of disease diagnoses was 2 (1–3), and 16.17% (3011) were diagnosed with more than five diseases (including five). Additionally, the median number of prescribed medications was 2 (1–4), and approximately 16.15% (3007) were classified as polypharmacy (defined as five or more medications). In this study, more than half of the people spent less than CNY 500 on medical care. The basic information characteristics of the population are shown in **Table 1**.

The Prevalence of PIMs and Leading Medications

Of 18,624 older patients with dementia, 3.52% (656) were detected with 1 PIM, and 35.91% (6688) received at least two PIMs. The prevalence in the eight cities ranged from 28.48 to 44.79%. The prevalence of PIMs in eight cities is displayed in **Figure 1**.

According to the 2019 AGS Beers criteria, the antipsychotic drugs quetiapine and olanzapine were most frequently prescribed in older outpatients with PIM, accounting for 8.01% (1491) and 7.36% (1370), respectively. In addition, SSIRs (citalopram, sertraline) and sedative hypnotics (estazolam, zopiclone, alprazolam, zolpidem, lorazepam) were also observed in top drugs of PIM. **Figure 2** lists the percentages and names of the top ten drugs.

Risk Factors Associated With PIM

Table 2 displays the results of the multiple logistic regression. Considering PIM to be a dependent variable, age >80 years, the number of medications was associated with the occurrence of PIM. In addition, females with dementia were likely to receive PIM. In our study, we also found that the payment form negatively affected PIM.

DISCUSSION

In our study, a total of 18,624 participants in eight cities in China were recruited, which is larger than a previous study in China, making our results more representative. The cross-sectional study revealed that PIM in older outpatients with dementia is highly prevalent and identified three potential

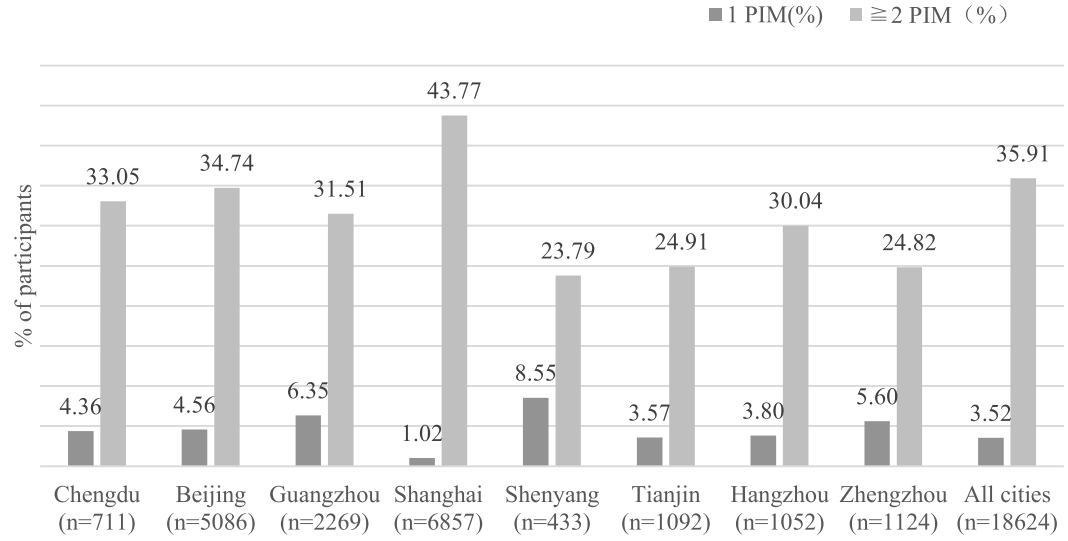

FIGURE 1 | Prescription of 1 PIM and ≥2 PIMs among older outpatients with dementia in China.

factors increasing the number of PIMs. Links exist between PIM and drug-related problems (Hagstrom et al., 2015; Muhlack et al., 2017), and the connection is more apparent in older people. Based on these results, resolving related medical problems may be useful.

The prevalence of PIM in our study was 39.43%, and 35.91% were prescribed at least two PIMs. Compared with the Ferreira et al. study using the 2019 AGS Beers criteria, our result was lower (Ferreira et al., 2021). The study population and sample size may be responsible for the difference. While the prevalence of PIM reported by Cross et al. was lower than our study results (Cross et al., 2016), it was possibly due to the difference in PIM screening tools. In our study, we applied the 2019 AGS Beers criteria to determine PIM, while Cross et al.'s study only used dementia-specific PIMs of the Beers criteria. In addition, the prevalence of PIM varied in the regions included in the study, ranging from 28.48 to 44.79%. **Figure 1** shows that Tianjin was the city with the lowest percentage of PIM. The lower number of drug medications per dementia patient in Tianjin may be the explanation of the phenomenon. In addition, the prevalence of PIM in Shanghai ranked first in eight cites, and several reasons may be responsible. First, the difference among PIM was attributed to the sample size. Of the total population included, more than one-third of participants were from Shanghai. Second, the difference in medication habits among clinicians may explain the gap in PIM use. According to the dataset comprising dementia patients from eight cities, the frequency of quetiapine use (ranking first in prescribed PIM prescriptions) in Shanghai accounted for 68.61% (1023/1491) of the total amount of quetiapine.

In our study, we found that antipsychotics were frequently prescribed by clinicians in dementia patients with PIM, similar to Renom-Guitera et al.'s study. In addition, the number of quetiapine was higher than olanzapine, ranking first in the top ten PIMs according to the 2019 AGS Beers criteria, consistent with the study by Machado-Duque et al. (Machado-Duque et al., 2021). In contrast, olanzapine was prescribed most frequently in the United States (Maust et al., 2015). It is possible that the larger number of dementia patients with Parkinson's disease in China leads to more prescriptions of quetiapine because olanzapine should be avoided in PD patients due to the adverse effects of the extrapyramidal system (Zhang et al., 2005; Li et al., 2019; Aarsland et al., 2005; American Geriatrics Society Beers Criteria® Update Expert Panel, 2019). It is worth noting that the number of antipsychotic drugs prescribed was much higher than that of other medications among the top ten PIMs. This frequent use of antipsychotic drugs may be compatible with dementia patients who always had accompanying mental symptoms such as agitation and aggressiveness. A study by Calsolaro et al. also pointed out that it is sometimes necessary to use antipsychotics to control symptoms (Calsolaro et al., 2021). Due to the side effects of antipsychotic drugs, such as the decline in cognitive function, cerebrovascular events, severe extrapyramidal effects and mortality (American Geriatrics Society Beers Criteria® Update Expert Panel, 2019; Calsolaro et al., 2021; Vinaşi et al., 2021), clinicians should be cautious when prescribing treatment for patients with dementia.

Benzodiazepines (sedative hypnotics) are frequently prescribed by clinicians to treat patients with sleep disorders. Sleep disorders can be observed in older adults, especially people with dementia (Tian et al., 2017; Gulia et al., 2018; Ward et al., 2020). In our study, estazolam, a benzodiazepine drug, was the third most commonly used PIM after quetiapine and olanzapine, accounting for 4.23% (787). The higher prescription rate could be attributed to the higher prevalence of sleep disorders in older patients with dementia. However, prolonged use of benzodiazepine may result in a series of adverse effects, such as falls, cognitive decline, and mortality (Barker et al., 2004; Pek et al., 2017). International guidelines suggest that older people with dementia should avoid the use of benzodiazepines as much

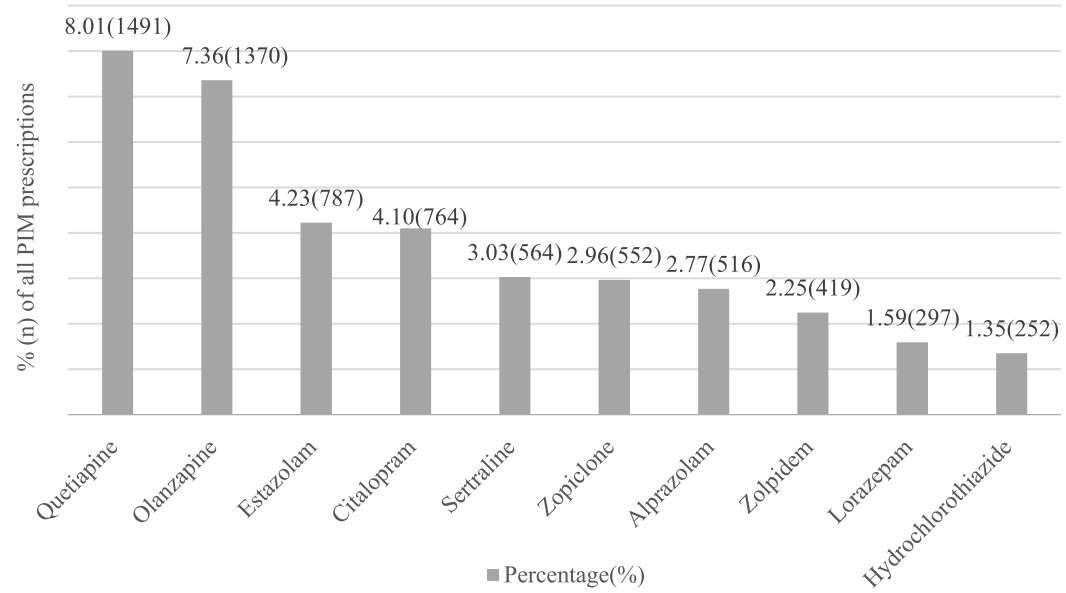

FIGURE 2 | Ten most frequently prescribed PIMs among older outpatients with dementia in China.

TABLE 2 | Multivariable analysis of risk factors associated with PIM among older outpatients with dementia in China.

Variables	Odds Ratio	CI Lower	CI Upper	*p* Value
Sex, n (%)				
Female	Reference			
Male	0.892	0.839	0.947	<0.001
Age, n (%)				
65–80	Reference			
≧80	1.074	1.011	1.142	0.021
No. of medications n (%)				
1	Reference			
2–4	1.22	1.137	1.308	<0.001
≧5	1.118	1.001	1.249	0.048
No. of diseases n (%)				
1–4	Reference			
5–9	0.951	0.866	1.045	0.296
≧10	0.73	0.577	0.923	0.009
Payment n (%)				
Full fee	Reference			
Partial Fee	0.463	0.432	0.497	<0.001
Free	0.488	0.433	0.549	<0.001
others	0.284	0.147	0.546	<0.001
No. of prescription expenditure n (%)				
<500 CNY	Reference			
500–1000 CNY	0.893	0.827	0.964	0.004
>1000 CNY	1.02	0.934	1.113	0.666

as possible. Thus, benzodiazepines are taken into consideration when clinicians comprehensively evaluate the condition of patients and there is no alternative drug treatment.

According to a binary logistic model, our study identified three risk factors associated with PIM: female sex, number of medications, and increasing age. Aging is inextricably linked to the deterioration of organ function, causing alterations in pharmacokinetics and pharmacodynamics and further causing some drug-related problems (Fried et al., 2014; Payne et al., 2016). To our knowledge, 80 years was considered a cutoff of advanced age. Compared with those aged 65–79 years, those aged >80 years easily suffered more prescription and PIM (Mo et al., 2016). In our study, age> 80 years was also considered a potential risk factor, consistent with an earlier published study (Murphy et al., 2020). Increased PIMs in females may be due to the following reasons: 1) the risk diagnosed with dementia in women was higher than men; 2) women focus more on their health issue and have more healthcare visits and complaints; 3) they are more likely to use psychotropic drugs with anticholinergic properties compared to men (Bierman et al., 2007; Johnell et al., 2009; Jia et al., 2020). Regarding the number of medications, the more drugs you take, the more likely you are to have PIM. The strong association between the number of medications and PIM has been confirmed by numerous studies, consistent with our study results (Ma et al., 2018; Tian et al., 2021). Interestingly, we found that reimbursement was negatively related to the occurrence of PIM. This may be due to reimbursement making it less expensive to have more drug options.

There are strengths and limitations in our study. First, a large samplein eight cities in China is our strengths, making the results more reliable. Second, it made up for the lack of PIM and risk factors in patients with dementia in China. However, this was a cross-sectional study, which is prone to bias the results. And some unmeasured confounding factors and lacking follow-up and other medical data might make the related risk factors not be analyzed comprehensively. In our study, we just applied **Table 2**, 3, 4,5 of 2019 AGS Beers criteria, Table 6 was excluded due to the absence of renal indicators. The prevalence of PIM in our study may be underestimated. Additionally, participants with dementia in this

study were outpatients, which was not sicker than inpatients, the finding might not apply to inpatients. Therefore, we need to further carry out a study among inpatients or nursing home patients with dementia and collected related follow-up data.

CONCLUSION

The current study shows that the prevalence of PIM among outpatients with dementia in China is high. In addition, age >80 years, female sex, and taking multiple medications are risk factors for an increasing number of PIMs. Notably, among patients with PIM, antipsychotic drugs were the most frequent and much more frequent than other drugs. This prompted us to explore the use of antipsychotics in dementia patients and the relationship between antipsychotics and adverse reactions in patients with dementia in further research.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MZ: Developing design, literature search, manuscript writing. ZC, FT, and TX: Developing design, literature search, manuscript writing, and analysis of results. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.929584/full#supplementary-material

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Population pharmacokinetics model for escitalopram in Chinese psychiatric patients: effect of CYP2C19 and age

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Objective: To establish a population pharmacokinetic model in Chinese psychiatric patients to characterize escitalopram pharmacokinetic profile to identify factors influencing drug exposure, and through simulation to compare the results with the established therapeutic reference range.

Methods: Demographic information, dosing regimen, CYP2C19 genotype, concomitant medications, and liver and kidney function indicators were retrospectively collected for inpatients taking escitalopram with therapeutic drug monitoring from 2018 to 2021. Nonlinear mixed-effects modeling was used to model the pharmacokinetic characteristics of escitalopram. Goodness-of-fit plots, bootstrapping, and normalized prediction distribution errors were used to evaluate the model. Simulation for different dosing regimens was based on the final estimations.

Results: The study comprised 106 patients and 337 measurements of serum sample. A structural model with one compartment with first-order absorption and elimination described the data adequately. The population-estimated apparent volume of distribution and apparent clearance were 815 and 16.3 L/h, respectively. Age and CYP2C19 phenotype had a significant effect on the apparent clearance (CL/F). CL/F of escitalopram decreased with increased age, and CL/F of poor metabolizer patients was significantly lower than in extensive and immediate metabolizer patients. The final model-based simulation showed that the daily dose of adolescents with poor metabolizer might be as high as 15 mg or 20 mg and referring to the therapeutic range for adults may result in overdose and a high risk of adverse effects in older patients.

Conclusion: A population pharmacokinetics model of escitalopram was successfully created for the Chinese population. Depending on the age of the patients, CYP2C19 genotype and serum drug concentrations throughout treatment are required for adequate individualization of dosing regimens. When developing a regimen for older patients, especially those who are poor metabolizers, vigilance is required.

KEYWORDS
CYP2C19 genotype, elderly, escitalopam, adolescent, population pharmacokinetics

Introduction

Depression and anxiety disorders affect a large number of people worldwide, which is placing an increasing burden on health services (Hazell, 2021). Nowadays, approaches to treatment include antidepressant and mood-stabilizing drugs, psychotherapy, and physical activity. Escitalopram is still the antidepressant of choice because of its safety, efficacy and tolerability (Cipriani et al., 2009; Sanchez et al., 2014; Cipriani et al., 2018). Escitalopram is highly selective for serotonin transporters and is active against depression (Burke et al., 2002; Wade et al., 2002; Rapaport et al., 2004) and anxiety disorders (Stahl et al., 2003; Davidson et al., 2004).

Escitalopram is an active S-enantiomer of citalopram and is one of the most commonly prescribed selective serotonin reuptake inhibitors (SSRIs). It was launched in the United States in 2002 and China in 2006. The pharmacokinetic profile of escitalopram has been studied extensively in healthy people. The maximum concentration of escitalopram is reached ~4 h after oral administration of 10–20 mg/day, with an elimination half-life ($t_{1/2}$) of ~30 h. This supports the therapeutic plan of a once-daily dose of 10–20 mg, and escitalopram is characterized by oral clearance and volume of distribution of 0.48 L/h/kg and 18.3L/kg, respectively (Søgaard et al., 2005; Rao, 2007). Escitalopram is primarily metabolized in the liver by cytochrome P (CYP)450, particularly CYP2C19, which is a highly polymorphic enzyme that causes interindividual pharmacokinetic differences (Rao, 2007; Pastoor and Gobburu, 2014), and is excreted mainly through the kidneys. The effect of age (Dolder et al., 2010; Yang and Scott, 2010), gender (Montejo et al., 2015), smoking (Oliveira et al., 2017; Scherf-Clavel et al., 2019), CYP2C19 phenotype (Huang et al., 2021), hepatic impairment (Areberg et al., 2006), and renal impairment (Dolder et al., 2010) on the pharmacokinetics of escitalopram have been investigated. The findings of these studies were instrumental in developing specific dosing recommendations for escitalopram for specific populations (Hicks et al., 2015; Brouwer et al., 2021). Escitalopram has been approved for use in China for 16 years, and it is the first-line antidepressant medication in China (Rao, 2007). As a result, it is necessary to investigate the factors that may affect the pharmacokinetics of escitalopram in the Chinese population in order to provide a basis for individualized medication in China.

Population pharmacokinetics (PopPK) modeling is a widely used tool to analyze pharmacokinetic data to individualize dosing regimens. Based on this approach, we can identify potential covariates that influence the pharmacokinetics of escitalopram and establish formulas to describe individual parameters. Compared with traditional pharmacokinetics, the advantage of PopPK is that the sparse blood drug concentrations can be used to quantify the intrinsic and extrinsic factors influencing pharmacokinetics by incorporating different covariates. There have been several studies on the PopPK of escitalopram. The PK parameters have been compared in HIV-infected and uninfected psychiatric patients (Courlet et al., 2019). A PopPK model of escitalopram in patients during the perinatal period has been established (Weisskopf et al., 2020). The effect of age, weight, gender and CYP2C19 genotype on escitalopram exposure has been studied in American and Italian patients. (Jin et al., 2010; Akil et al., 2016; Kim et al., 2021). No systematic PopPK analysis of escitalopram has been established in Chinese psychiatric patients. A PopPK/PD model has been developed in Korean healthy volunteers (Kim et al., 2021). Although the mutation frequency of CYP2C19 genotype in the Chinese population was similar to that in Korean population (Dorji et al., 2019), they did not investigate the effect of CYP2C19 genotype. Additionally, CYP2C19 *2 and *3 have much less mutation frequency in European than in East Asian population, but *17 is higher than in East Asian. Therefore, because of the difference in race and CYP2C19 variant allele frequency, investigation in the Chinese population is curial.

In the present study, we established a PopPK model of escitalopram in Chinese psychiatric patients by retrospectively collecting serum drug concentrations and related information. Compared to previous studies, in addition to the influence of age, sex, weight, height, body mass index (BMI) and CYP2C19 genotype, we included liver and kidney function-related biochemical indicators and combination therapy to complete a comprehensive pharmacokinetic evaluation of escitalopram. Simulations were also conducted to investigate whether patients needed to take different doses of escitalopram under different circumstances. The objective of the current study was to develop a PopPK model for escitalopram in Chinese psychiatric patients to explore the potential factors that contribute to variability in escitalopram pharmacokinetics. Furthermore, the model served to predict average drug exposure under various influencing factors through simulation and compared it with the established therapeutic reference range.

Methods

Subjects and data collection

The data were obtained from psychiatric inpatients in the Affiliated Brain Hospital of Guangzhou Medical University from 2018 to 2021 and monitored drug blood concentrations during this period. Patients were excluded if there was only one blood

concentration measurement, and if there was no reliable information about administration and blood sampling times. This study provided an opportunity to evaluate whether age, sex, weight, height, BMI, smoking, drinking, CYP2C19 genotype, alanine aminotransferase (ALT), mitochondrial aspartate aminotransferase (m-AST), total bilirubin (TBIL), albumin (ALB), urea, serum creatinine (Scr), and combination therapy (such as omeprazole and valproic acid) affected the pharmacokinetics of escitalopram. This study was approved by the Institutional Review Board (IRB) in the Affiliated Brain Hospital of Guangzhou Medical University (Approval number: 2021027).

Determination of escitalopram concentrations

Blood samples (three to four ml) were collected into coagulation-promoting tubes and centrifuged at 17,600 *g* for 3 min. Serum samples (100 μL) were transferred into 2-ml Eppendorf tubes and mixed with 20 μL internal standard (citalopram-d6) and 500 μL acetonitrile. After vortex-mixing for 10 s and centrifugation at 21,130 *g* for 5 min, ~100 μL supernatant was removed and transferred to autosampler vials with lining tubing. Escitalopram was measured by HPLC-tandem mass spectrometry (Shimadzu, Kyoto, Japan). Separation was performed on an Agilent Eclipse XDB-C18 column (4.6 × 50 mm, 1.8 μm) with a flow of 0.6 ml/min, and the mobile phase consisted of (A) 75% methanol with 5 mM ammonium formate and (B) methanol for 1.3 min. The injection volume was 1 μL. The linear range was 3–300 ng/ml. This analytical method has been examined by selectivity, specificity, matrix effect, stability, and intra- and inter-batch precision and accuracy.

Determination of CYP2C19 genotype

DNA was extracted utilizing DNA extraction and purification kits from Shanghai BaiO Technology Co. Ltd. The genotype of CYP2C19 was determined using a human CYP2C19 gene detection kit provided by Wuhan Youzhiyou Medical Technology Co. Ltd. DNA amplification was accomplished after extracting DNA and adding the DNA reaction solution. Following the reaction, the Ct values of various channels were calculated using the amplification curves, and the results were determined. With regard to CYP2C19 isoenzymes, patients were divided into three groups according to the predicted phenotypes: extensive metabolizer (EM) if they were homozygous for the wild-type allele *1/*1; intermediate metabolizer (IM) if they carried the *1/*2 or *1/*3 allele; and poor metabolizer (PM) if they carried the *2/*2 or *2/*3 allele.

Modeling strategy and software

The PopPK model of escitalopram was created using the nonlinear mixed-effect modeling program (NONMEM, version 7) with the first-order conditional estimation with inter- and intraindividual variability interaction (FOCE-I) method to estimate population parameters and identify candidate covariates. Pirana (version 2.9.0) was used to document and structure model development. Normalized prediction distribution errors (NPDE) test was performed using the NPDE-add on package in R (version 4.1.1). Perl-speaks-NONMEM (version 3.4.2) was used to conduct bootstrap analysis (n = 1,000). Goodness-of-fit plots were performed using GraphPad Prism (version 9.1.1). Statistical analysis was performed using SPSS (version 25.0).

PopPK model development

A basic model without any covariates was developed initially. The pharmacokinetics were described using a first-compartment model with first-order absorption and first-order elimination in terms of apparent oral clearance (CL/F), apparent volume of distribution (V_d/F), and absorption rate constant (K_a). Due to the paucity of concentration data within a few hours after oral administration, the absorption phase could not be described. We fixed K_a to 0.6 according to an established model in Chinese subjects (Chen et al., 2013). A statistical model was included to describe between-subject and residual variability. The interindividual variabilities of CL/F and V_d/F were evaluated through an exponential error model (Eq 1), and the intraindividual unexplained variability was through a mixed residual error model (Eq 2).

$$\boldsymbol{P_i} = \hat{\boldsymbol{P}} \times \boldsymbol{e}^{\boldsymbol{\eta_i}} \quad (1)$$

Where Pi represents the estimate of *i*th individual parameters (V_d/F or CL/F), $\hat{P}$ is the population value of the parameters, and η_i is a random-effects with a mean of zero and variance of ω^2 conform to normal distribution.

$$\boldsymbol{Y} = \boldsymbol{F} \times (1 + \boldsymbol{\varepsilon}_1) + \boldsymbol{\varepsilon}_2 \quad (2)$$

Where Y and F denote the model-observed and -predicted escitalopram concentrations, respectively. ε_1 and ε_2 represents proportional error and additive error, respectively, which follow a normal distribution with a mean of zero and variance of σ^2.

The selection of candidate covariates was through the method of stepwise forward selection–backward elimination resulting in the final PopPK model for escitalopram. For concomitant medication, we evaluated the effect of the CYP2C19 inhibitors taken by each patient for that several studies have demonstrated that the magnitude of drug-drug interactions with escitalopram was weak and moderate

(Gutierrez et al., 2003; Siccardi et al., 2013) with proton-pump inhibitors having a moderate effect on escitalopram pharmacokinetics (Malling et al., 2005; Gjestad et al., 2015), and we also explored the effect of CYP2C19 inducers. Missing values of weight and height were imputed to the population median value. Covariates would be incorporated into the basic model when their addition reduced the objective function value (OFV) to >6.63 ($p < 0.01$) and removed from the full model when exclusion of the covariates resulted in an increase <10.83 ($p < 0.001$). Eqs 3, 4 were applied for continuous (age, height, weight, etc.) and noncontinuous (sex, CYP2C19 genotype, and combination therapy) covariates, and Eqs 5–7 were used to investigate the influence of CYP2C19 genotypes.

The following were continuous covariates:

$$\boldsymbol{P_i} = \hat{\boldsymbol{P}} \times \boldsymbol{e}^{\eta_i} \times \left[1 + \boldsymbol{\theta}_{COV} \times \left(\boldsymbol{Cov_i} - \overline{\boldsymbol{Cov_i}}\right)\right] \quad (3)$$

The following were non-continuous covariates:

$$\boldsymbol{P_i} = \hat{\boldsymbol{P}} \times \boldsymbol{e}^{\eta_i} \times [1 + \boldsymbol{\theta}_{COV} \times \boldsymbol{COV_i}] \quad (4)$$

Where θ_{COV} represents the calibrator of parameters, COV_i and $\overline{Cov_i}$ are the ith individual value and population median value of covariates, respectively. For gender covariate, COV = 0 represents male and COV = 1 represents female. The concomitant medication covariate was 0 for patients who did not receive concomitant drugs during escitalopram sampling time, and 1 for patients who received concomitant drugs. CYP2C19*1 encodes the normal function enzyme, and *2 and *3 encode no function. Consequently, homozygous wild-type CYP2C19 *1 had the full drug-metabolizing capacity, and *1/*2 and *1/*3 had reduced metabolism compared to *1/*1. PMs possessed two null alleles, such as *2/*2 and *2/*3, in our analysis. Depending on the phenotype, the CYP2C19 genotype was grouped into three: one for *1/*1 subjects, two for *1/*2 or *1/*3 subjects, and three for *2/*2 or *2/*3 subjects.

$$\text{IF GENE} = 1\ \boldsymbol{CL} = \boldsymbol{TVCL} \times \boldsymbol{e}^{\eta_i} \times \boldsymbol{\theta}_{EM} \quad (5)$$

$$\text{IF GENE} = 2\ \boldsymbol{CL} = \boldsymbol{TVCL} \times \boldsymbol{e}^{\eta_i} \times \boldsymbol{\theta}_{IM} \quad (6)$$

$$\text{IF GENE} = 3\ \boldsymbol{CL} = \boldsymbol{TVCL} \times \boldsymbol{e}^{\eta_i} \times \boldsymbol{\theta}_{PM} \quad (7)$$

TABLE 1 Demographic data and patients characteristics.

Characteristics	Median/Number	Range/Ratio
Age (year)	45	12–83
Gender		
Male	59	55.66%
Female	47	44.34%
Weight (kg)	61	37–97
Height (cm)	165	150–180
BMI (kg/m^2)	22.4	14.87–33.91
Smoking habit		
Yes	3	2.83%
No	103	97.17%
Drinking habit		
Yes	0	0%
No	106	100%
Liver function index		
ALT (U/L)	17	5–162
m-AST (U/L)	4.31	1.51–14.71
TBIL (mg/dl)	9.4	2.6–30.1
Renal function index		
ALB	40.4	30.2–68.2
Urea	3.99	1.69–31.54
Scr	67	30–152
CYP2C19 phenotype		
EM	47	44.34%
IM	49	46.23%
PM	10	9.43%
Concomitant medication		
Omeprazole	6	5.7%
Rifampicin	2	1.9%
Buspirone	11	10.4%
Venlafaxine	1	0.9%
aripiprazole	7	6.6%
Clozapine	13	12.3%
Valproic acid	36	34.0%
Lithium Carbonate	13	12.3%
Diazepam	24	22.6%
Clonazepam	7	6.6%
Olanzapine	39	36.8%
Mirtazapine	11	10.4%
Risperidone	26	24.5%

Model evaluation

The precision of parameters and the ability of the final covariate model were assessed by goodness-of-fit plots, bootstrapping, and NPDE. At the same time, the plausibility of estimated parameters and relative standard errors, and changes in both inter- and intraindividual variability were also considered. Goodness-of-fit plots were used for the final model quality evaluation, which included: population predicted concentration versus observed concentrations (as known as dependence variables (DV)); individual predicted concentration versus observed concentrations; population predicted concentrations versus conditional weighted residuals (CWRES); and time after last dose versus CWRES. A bootstrap analysis was performed with resampling 1,000 times. The results of bootstrapping were summarized as median, and 95% confidence intervals of each parameter compared with the corresponding parameters obtained with the origin dataset. NPDE is a model evaluation approach based on the fit of

TABLE 2 Allele and Genotype frequencies of CYP2C19.

		Total (N = 106)	Frequency (%)	Phenotype
Allele	*1	143	67.5	Normal
	*2	65	30.6	None
	*3	4	1.9	None
Genotype	*1/*1	47	44.34	Extensive
	*1/*2	48	45.28	Immediate
	*1/*3	1	0.94	Immediate
	*2/*2	7	6.60	Poor
	*2/*3	3	2.84	Poor

each observation and is not easily influenced by experimental design.

Simulation

Simulation can provide escitalopram dosing guidance in Chinese psychiatric patients, and it was conducted under several regimens based on the final estimations to find optimal individualized dosing regimens. We predicted steady-state concentration profiles for the therapeutic doses of 5, 10, 15, and 20 mg qd for adolescents ≥12 and <18 years, adults ≥18 and <65 years, and elderly ≥65 years with different CYP2C19 phenotypes (EM, IM and PM). We performed the simulation to establish: 1) whether the steady-state serum levels in adult patients were in the therapeutic range after administration according to the instructions; 2) whether it was necessary to give older and PM patients half the dose of escitalopram; and 3) whether adolescent patients could be administrated the same dosing regimen as adults. Simulation was performed to ensure that >95% of the trough concentrations were within the therapeutic window during therapy.

Results

Demographic information

The final dataset for the PopPK model included 106 psychiatric patients and 337 escitalopram measurements in both steady-state and non-steady-state. And the approximate sampling times were most of around trough. All patients were given conventional tablets with 5 mg qd, 10 mg qd, 15 mg qd, 20 mg qd, 5 mg bid, or 10 mg bid. The median dose of escitalopram was 10 mg/day (range 5–30 mg/day). Details on the demographics are summarized in Table 1, and the frequency of CYP2C19 is listed in Table 2. CYP2C19*2, the main mutant and causative allele, was the most common genotype, followed by CYP2C19*3, thus making higher frequencies of *1/*2 and *2/*2 among all test samples. In accordance with the therapeutic drug monitoring guidelines in psychiatry by Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) in 2017 (Hiemke et al., 2018), we collected information on enrolled patients receiving CYP2C19 inhibitors and inducers during the sampling time, as well as drugs with potential effect. Concomitant medications are shown in Table 1. The therapeutic window and laboratory alert level of escitalopram in AGNP guideline were 15–80 ng/ml and 160 ng/ml, respectively (Hiemke et al., 2018). We summarized the blood drug concentration information in Table 3.

PopPK model for escitalopram

The pharmacokinetics of escitalopram were best described by a one-compartment model with first-order absorption and elimination (Courlet et al., 2019; Weisskopf et al., 2020). Owing to the proportional error model that could better describe the present model, we fixed the additive error to 0. The OFV of the basic model was 1923.221. The mean (relative standard error) basic model estimate parameters were 14 L/h (4%) for CL/F and 815 L (16%) for V/F. The between-subject variability was estimated to be 0.146 and 0.216 for CL/F and V/F respectively, and the intraindividual variability was 0.0289 in the proportional error model

A detailed description of the principal results of covariate analyses is presented. During the forward inclusion process, the CYP2C19 phenotype was a significant covariate for CL/F with

TABLE 3 Distribution of blood drug concentration in all patients.

Concentration	Number	Ratio (%)
<15 ng/ml	24	7.12
≥15 ng/ml, ≤80 ng/ml	274	81.31
>80 ng/ml, <160 ng/ml	37	10.98
≥160 ng/ml	2	0.59

TABLE 4 Final parameter estimates of escitalopram PopPK model.

PK parameters	Final model		Bootstrap	
	Estimate	Rse%	Median	95%CI
Fixed effect				
CL/F (L/h)	16.3	6%	16.4	14.7–18.2
V/F (L)	815	14%	803.9	581.9–1,070.8
K_a (h^{-1})	0.6, FIX	—	0.6, FIX	—
θ_{Age}	0.0077	20%	0.0077	0.0043–0.0108
θ_{IM}	0.847	7%	0.848	0.74–0.97
θ_{PM}	0.479	11%	0.478	0.38–0.59
Random effect				
CL/F	0.0877	21%	0.0809	0.0520–0.1254
V/F	0.235	29%	0.215	0.090–0.388
Residual error				
Additive error	0, FIX	—	0, FIX	—
Proportional error	0.0287	12%	0.0288	0.0226–0.0359

the model decreased by 25.58 ($p < 0.001$) to a final value of 1897.64. Age also had a significant impact on the CL/F of escitalopram with the value of OFV decreasing by 9.928 ($p < 0.01$) to 1913.293. There were no significant effects of gender, height, weight, BMI, smoking, concomitant medication, and liver or kidney function on CL/F or V/F. When we incorporated age at CL/F forward based on the CYP2C19 phenotype covariate model, the model led to a 21.10 decrease in OFV value to 1876.533, and the full model was developed. The backward elimination step each time removed a covariate from the full model. The values of OFV were increased by 36.76 ($p < 0.001$) and 21.107 ($p < 0.001$) for the CYP2C19 phenotype and age to 1913.293 and 1897.64, respectively, which meant CYP2C19 phenotype and age had significant effect on the exposure of escitalopram. And we found no correlation between age and CYP2C19 phenotype. Estimates for PK parameters of the final model are listed in Table 4.

In the final model, there was a decrease in CL/F of escitalopram with increased patient age, and it was also influenced by different CYP2C19 phenotypes. The CL/F was 20.83 L/h in adolescents aged 15 years and 15.84 L/h in adults aged 45 years. In older patients aged 75 years, CL/F decreased to 11.89 L/h. The higher CL/F in EM than in IM and PM patients resulted in the dose-related concentration of IM patients being higher than that in EM patients, while concentration in PM patients was much higher than both IM and EM patients (Figure 1). The estimated population CL/F of escitalopram was 16.73 L/h for EM, 13.96 L/h for IM, and 8.56 L/h for PM patients. CL/F in EM patients was 1.2-fold higher than in IM patients and 1.9-fold higher than in PM patients.

Model validation

Goodness-of-fit plots, NPDE, and bootstrapping illustrated the appropriateness of the covariate model. Figure 2 showed the scatter plots of the observation values versus population (Figure 2A) and individual (Figure 2B) predicted concentrations, which observed a good correlation and were distributed symmetrically around the trend line. This suggested that the final model was a good fit for the observed data. Figure 2C shows the scatter plots of the CWRES from the final PopPK model, with a range between -3.02 and 2.53, which was distributed symmetrically around 0. The plot of time after dose versus CWRES is shown in Figure 2D. The results of bootstrapping are listed in Table 3. All estimated parameters from the final model were within the 95% confidence interval calculated from the bootstrap method, indicating that the model was constructed with good robustness. The results of NPDE are shown in Figure 3.

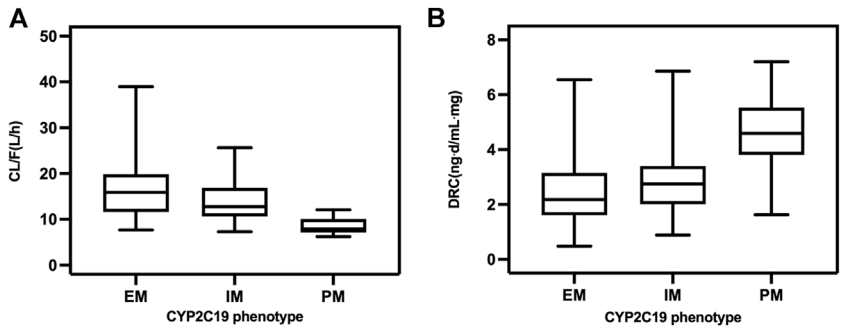

FIGURE 1
(A) CL/F and **(B)** DRC of escitalopram with different CYP2C19 phenotype.

Dosing simulation for escitalopram dose

Considering the covariates that we selected and common situations in clinical practice, the time courses of escitalopram concentrations in steady state were simulated for different ages and CYP2C19 phenotypes. The therapeutic window of escitalopram is 15–80 ng/ml and the laboratory alert level is 160 ng/ml (Hiemke et al., 2018), and applies to patients aged ≥18 and <65 years. Doses of 10, 15 and 20 mg/day were all within the range of 15–80 ng/ml for EM and IM patients (Figures 4A,B). However, the serum drug concentrations were >80 ng/ml at a daily dose of 20 mg for PM patients (Figure 4C).

The model-based simulation results in older patients showed that the drug concentration in PM patients was twice as high as that in EM patients under the same dosing regimen (Figure 5). Consistent with the above results, oral administration of 15 or 20 mg/day exceeded 80 ng/ml in PM patients. Accordingly, the recommended dose of escitalopram is no more than 10 mg/day for PM patients.

Adolescents typically have higher clearance compared to older people, which was reflected in the steady-state trough concentration being within 15–80 ng/ml when the daily dose was 15 or 20 mg in PM adolescents (Figure 6). However, caution is required for PM patients taking daily doses >10 mg.

Discussion

PopPK has been utilized extensively in clinical treatment and has become a very useful approach in optimizing individualized dosing regimens, therapeutic drug monitoring, and clinical evaluation of novel drugs. A PopPK model has been created to increase the possibility of meeting suitable pharmacokinetic/pharmacodynamic targets due to the limited therapeutic index of voriconazole and the relatively large systematic interindividual variability (Chen et al., 2019). On the other hand, due to the tendency of order patients to miss doses of medication, they developed a strategy to correct for missed doses through establishing a PopPK model and simulating (Xiao et al., 2021).

In this study, we created a PopPK model for oral administration of escitalopram in Chinese psychiatric patients, while considering demographic, genetic and physiological indicators. The model-predicted covariates of this analysis were in line with several published studies that describe the population pharmacokinetics of escitalopram (Jin et al., 2010; Akil et al., 2016; Courlet et al., 2019). We showed that CL/F of escitalopram varied nearly sevenfold, ranging from 6.26 to 38.93 L/h, which means that the pharmacokinetics of escitalopram in different populations show large interindividual variations. Some intensive sampling designs with escitalopram CL/F of 20–40 L/h, mostly in healthy

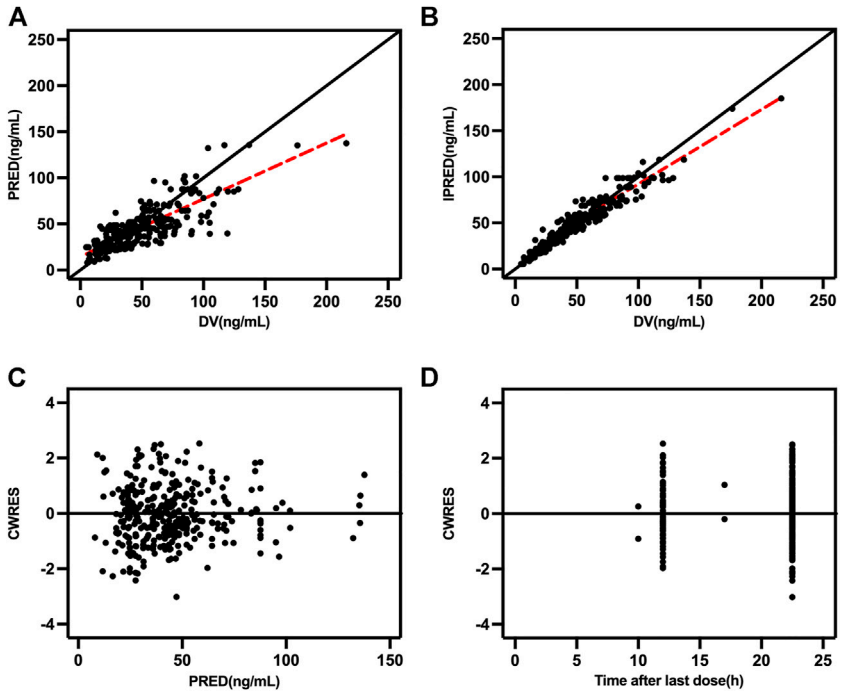

FIGURE 2
Goodness-of-fit plots **(A)** Population predicted concentration (PRED) versus observed concentrations; **(B)** individual predicted concentration (IPRED) versus observed concentrations; **(C)** population predicted concentrations versus conditional weighted residuals (CWRES); and **(D)** time-after last dose versus CWRES.

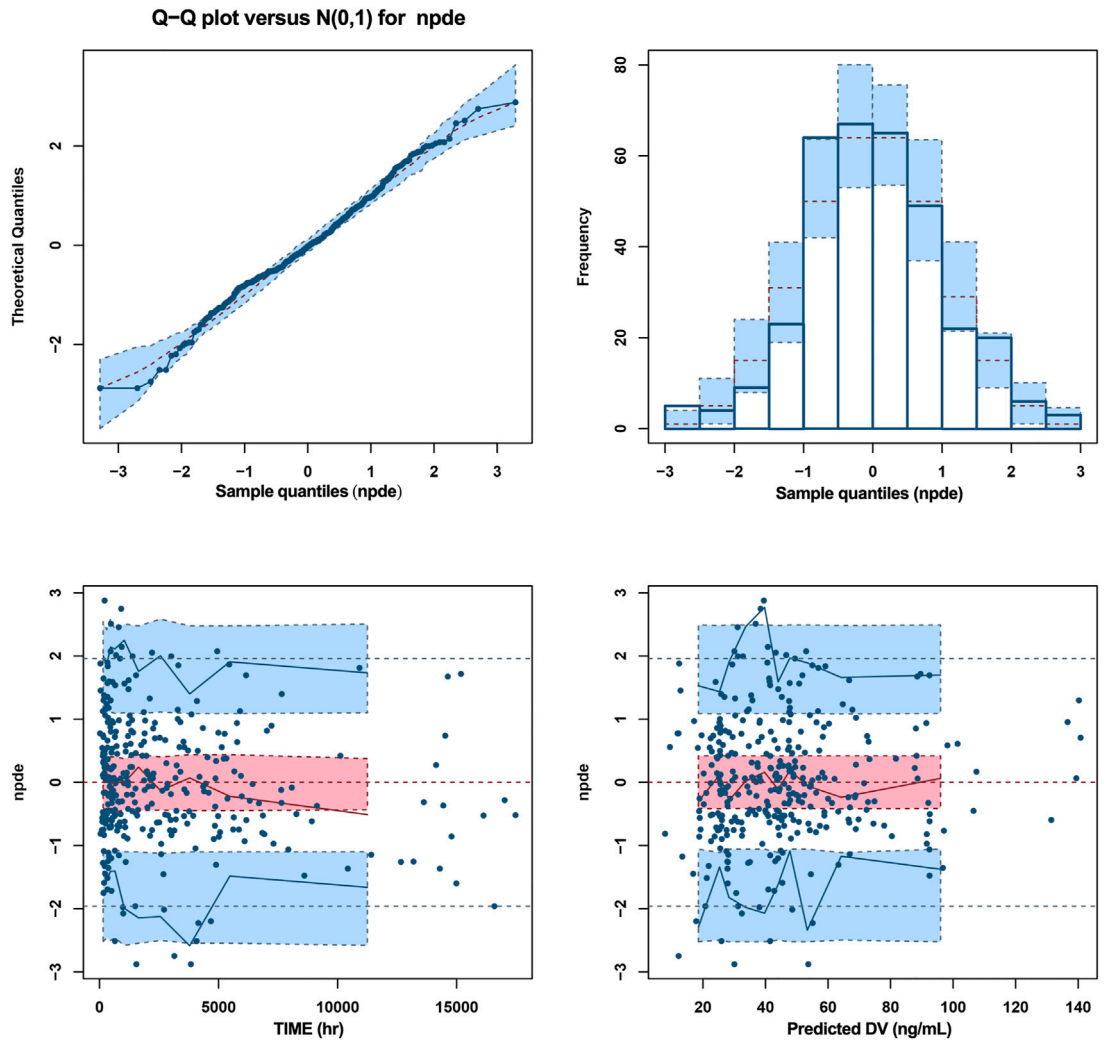

FIGURE 3
NPDE metrics for the PopPK model of escitalopram. The mean of normalized prediction distribution errors (NPDE) was 0.02359, variance was 0.9894, skewness was 0.04414, and kurtosis was 0.3027. The results of *t*-test and Fisher variance test were 0.664 and 0.911, respectively. The statistical values Shapiro-Wilk (SW) test for normality was 0.0633, and the global adjusted *p*-value was 0.19.

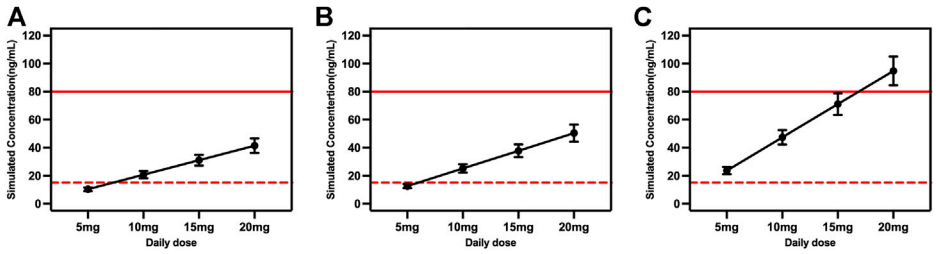

FIGURE 4
Simulated concentrations for ages ≥18 and <65 years in **(A)** extensive metabolizers; **(B)** immediate metabolizers, and **(C)** poor metabolizers at different daily doses. The red dash lines represented 15 ng/ml, and the red solid lines represented 80 ng/ml.

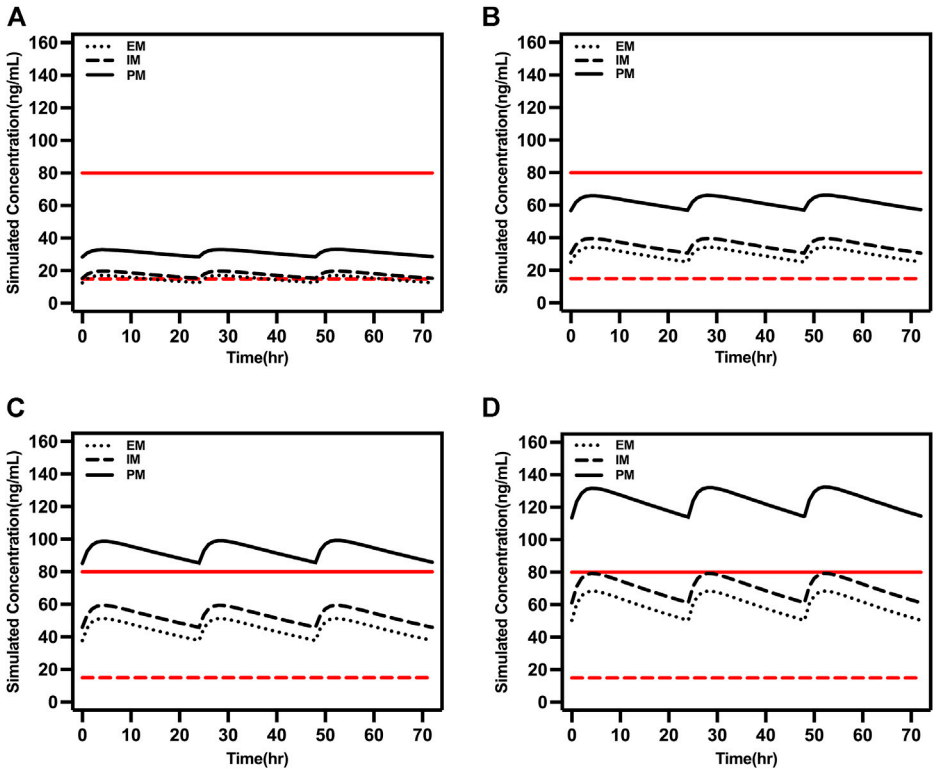

FIGURE 5
Simulated concentrations in EM, IM, and PM older patients (65 years old) at **(A)** 5 mg/day, **(B)** 10 mg/day, **(C)** 15 mg/day, and **(D)** 20 mg/day. The red dash lines represented 15 ng/ml and the red solid lines represented 80 ng/ml.

individuals (Søgaard et al., 2005; Nilausen et al., 2011; Chung et al., 2017), and published PopPK models have a CL/F > 20 or even 30 L/h (van Gorp et al., 2012; Courlet et al., 2019; Kim et al., 2021), while our study showed <20 L/h, which may have been caused by sparse data sampling.

Similar to the previous PopPK studies, our analysis revealed that age and CYP2C19 phenotype contributed differentially to the variability in the pharmacokinetics of escitalopram. Our model results showed a decrease in the CL/F of escitalopram with increasing age. This is in agreement with previously published PopPK models (Jin et al., 2010; Akil et al., 2016). Older patients ≥65 years had a significantly lower CL/F compared with younger healthy volunteers (Fredericson Overø et al., 1985; Bies et al., 2004). As reported previously, older patients had a significantly lower elimination rate than younger patients had (Dhillon et al., 2006), which was confirmed in our study. Actually, our study suggested a 10% decrease in clearance of escitalopram for every 20 years of age, which was less than the previous estimation of a decrease of 30–42% (Jin et al., 2010; Akil et al., 2016). This was consistent with the previously reported decrease in CYP2C19 activity with increasing age (Pollock et al., 1991), and was specifically quantified in our analysis of escitalopram. This might have arisen from the small number of people in each age bracket, although the age ranged from 12 to 83 years. Hence, the dose of escitalopram might need to be adjusted based on age.

In addition, the genetic polymorphism of CYP2C19 had a significant effect on the apparent clearance of escitalopram in previous studies (Areberg et al., 2006; Jin et al., 2010; Chang et al., 2014). Two single-center, randomized, open-label, two-period, two-treatment crossover bioavailability studies with 96 healthy Chinese individuals showed that the exposure of escitalopram in PM subjects and IM subjects increased by 102 and 38% respectively compared with EM, and the efficacy and toxicity of escitalopram varied among individuals with different genotypes (Chang et al., 2014; Jukić et al., 2018). In our study, EM and IM patients with CYP2C19 cleared escitalopram 48.8 and 38.7% faster than PM patients did. This means that metabolism in PMs is greatly reduced, and they experience higher systemic exposure compared with EMs and IMs that have similar clearance. Hence, genetic testing before medication and adjustment of escitalopram dose in PMs should be considered in the clinical treatment of Chinese patients. Moreover, the present findings of the CYP2C19 genotype–phenotype relationship are consistent with the previous study. When breaking the genotype into

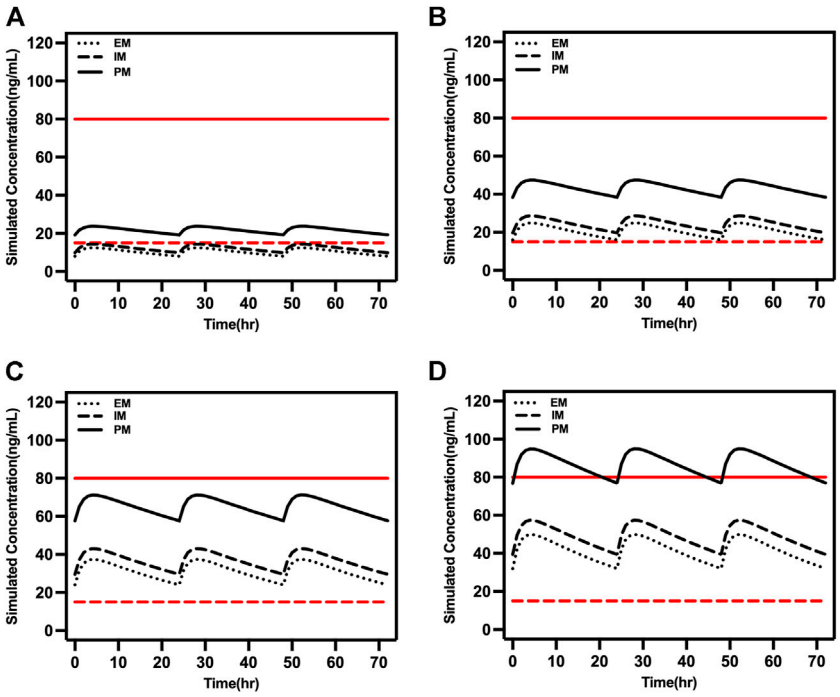

FIGURE 6
Simulated concentrations in EM, IM, and PM adolescents (16 years old) at **(A)** 5 mg/day, **(B)** 10 mg/day, **(C)** 15 mg/day, and **(D)** 20 mg/day. The red dash lines represented 15 ng/ml and the red solid lines represented 80 ng/ml.

five categories (*1/*1, *1/*2, *1/*3, *2/*2, and *2/*3), we found that estimations of CL/F for *1/*2 and *1/*3 were similar, as were those for *2/*2 and *2/*3. (Rudberg et al., 2008) showed the effect of CYP2C19*17 on the concentration of escitalopram, and patients with CYP2C19 *17/*17 alleles showed a 42% reduction in concentration. In our study, data for patients with CYP2C19*17 could not be collected because *17 was detected at a low frequency in the Chinese population, and CYP2C19 *1 is the most common allele, followed by *2 and *3 (Chen et al., 2008; Zhou et al., 2009; Tang et al., 2013). We did not consider CYP2D6 and CYP3A4 as covariates because their genetic variation has not been shown to significantly affect serum levels of escitalopram.

We found that sex was not an important factor affecting escitalopram CL/F, although a previous study with a small number of subjects suggested that CL/F of citalopram is higher in men than women (Sidhu et al., 1997). However, sex has not been found to exert a clinically significant effect on pharmacokinetics in healthy volunteers (Søgaard et al., 2005; Dhillon et al., 2006), and (Akil et al., 2016) reported that sex had no effect on escitalopram CL/F. We observed no weight-related difference in escitalopram clearance, although the influence of weight and BMI has been reported previously (Jin et al., 2010; Akil et al., 2016). This may have been caused by incomplete demographic information for some patients enrolled in our study.

Liver and kidney functions affect the metabolism and excretion of drugs. For patients receiving escitalopram with hepatic impairment, the estimated mean area under the curve (AUC) values were 51 and 69% higher for patients with mild and moderate hepatic impairment compared with healthy individuals (Areberg et al., 2006). Although all subjects in the study tolerated the treatment well and no serious adverse events were reported, careful monitoring and dose adjustment during long-term therapy are suggested. There is no conclusive evidence for the role of escitalopram in patients with depression and renal failure; however, pharmacokinetic analysis of citalopram in patients with renal insufficiency revealed that $t_{1/2}$ increased by 35% and renal clearance decreased by 40% (Joffe et al., 1998). Therefore, caution is recommended in such patients when using escitalopram. The data included a small number of patients with liver and kidney impairment; thus, evaluation of the effect of liver and kidney function in this PopPK analysis was limited.

None of the co-ingested drugs interacted pharmacokinetically with escitalopram in our study. (Malling et al., 2005) found that co-administration with cimetidine or omeprazole caused a moderate increase in exposure and $t_{1/2}$, and omeprazole and esomeprazole had a wider effect on escitalopram than sertraline and citalopram had (Gjestad et al., 2015). Proton

pump inhibitors are predominantly cleared by CYP2C19. Combination of escitalopram with drugs that are also metabolized by CYP2C19 may produce competitive inhibition between two CYP2C19 substrates. However, there were perhaps only seven of our patients treated with combined escitalopram and omeprazole, and no effect of omeprazole on escitalopram was found. Adjunctive treatment with fluvoxamine significantly increases escitalopram concentration (Yasui-Furukori et al., 2016), but there was no co-administration of fluvoxamine in our patients.

The FDA-recommended initial dose of escitalopram is 10 mg qd in adult patients, and 20 mg qd is the maximum dose. Simulation in our study reveals that the standard 5 mg/day regimen in EM and IM patients may lead to trough concentrations below the therapeutic target of 15 ng/ml, with a risk of suboptimal antidepressant efficacy. We also need to consider the effect of different CYP2C19 phenotypes. The Clinical Pharmacogenetics Implementation Consortium guidelines provide escitalopram dosing recommendations for different CYP2C19 genotypes (Hicks et al., 2015). Despite these guidelines being based on studies on the Caucasian population, they may also be suitable for the Chinese adult population. Consistent with our simulation results, EM or IM patients should initiate therapy with the recommended starting dose and maintenance dose up to 20 mg/day. Although IM patients may have elevated serum concentrations of escitalopram, there is little difference compared to EM patients. For PM patients with lower clearance and higher drug serum levels, the starting dose should be reduced by 50% (5 mg/day) and the maximum maintenance dose is 10 mg/day, or selecting drugs not predominantly metabolized by CYP2C19. Simulation results showed that the steady-state trough concentration was within the therapeutic window at a daily dose of 15 mg, but there is a risk of exceeding 80 ng/ml; thus, the maximum dose of 10 mg is recommended for PM patients, which is consistent with the guidelines. When escitalopram does not reach the target clinical efficacy, an increase in dose to 15 mg can be considered, but blood concentrations and adverse effects should be closely monitored.

Older patients are a special population. Although a single-dose clinical study confirmed that the pharmacokinetics of escitalopram were similar between young and older patients, $t_{1/2}$ and AUC were ~50% higher than in patients aged 18–35 years. Our study suggests that a daily dose of 10 mg escitalopram gives approximately the same steady-state serum levels in older individuals as a dose of 15 mg in adolescents, and that this is due to the reduced rates of metabolism in the former. Long-term excessive exposure in older people can lead to an increased rate of bradycardia (Barak et al., 2003), falls and fragility fractures (Gorgas et al., 2021); therefore, the starting and maintenance doses need to be fully considered and adjusted by genetic testing and therapeutic drug monitoring. Especially for older PM individuals, trough concentrations are higher than the minimum toxic concentration (80 ng/ml) with 15 or 20 mg/day. The FDA recommends 10 mg as the maximum daily dose for older patients, which implies that referring to the therapeutic range for adults may result in overdose and lead to a high risk of adverse effects. According to the results of the simulation, the therapeutic window on the AGNP guidelines does not extrapolate to people aged ≥65 years and needs to be reformulated. However, it requires to be validated in a large number of clinical trials. The results of the current study can provide a reference for future research.

The FDA approved escitalopram in 2009 for the acute and maintenance treatment of adolescents with major depressive disorder aged 12–17 years. The maximum recommended daily dose for adolescents was 20 mg, which is the same as for adults. Escitalopram was found to be efficacious and well-tolerated in the adolescent population with major depressive disorder when given at a daily dose of 10–20 mg in two clinical trials (Emslie et al., 2009; Findling et al., 2013). The pharmacokinetic differences showed no clinical significance in adolescents compared with adult healthy individuals (Rao, 2007). Furthermore, although the mean $t_{1/2}$ of escitalopram is shorter in adolescents, there are no differences in maximum concentration and AUC (Bareggi et al., 2007), hence the dose regimen was not affected. Our simulation results in adolescents were mostly consistent with those in adults and not significantly influenced by CYP2C19 genotype which was evidenced by serum blood concentrations within the therapeutic window at 15 mg/day and 20 mg/day for PM subjects. However, the efficacy and tolerance needed further investigation. Nevertheless, the risk of manic conversion during antidepressant treatment is highest in patients aged 10–14 years (Martin et al., 2004). Monitoring for suicidality during pharmacotherapy is necessary, and the frequency of monitoring based on each patient's particular risk.

There were several limitations that need to be considered. First, the sample size was small and most of the samples were at trough concentrations, which did not sufficiently reflect the absorption and distribution characteristics of escitalopram. Second, the small number of PM patients may have been related to the low frequency of mutations, which needs to be confirmed in further studies. Third, there were few cases of combined medication in our analysis, so it will be necessary to explore other drugs that might affect the pharmacokinetics of escitalopram. Notwithstanding, we obtained systematic data to develop a PopPK model in Chinese psychiatric patients for the first time and performed a simulation. These results provide guidance for making a better therapeutic decision on escitalopram dosing regimen to minimize excessively high exposure to this selective serotonin reuptake inhibitor through incorporating age and CYP2C19 genotype into this assessment.

Conclusion

Our PopPK model demonstrated the influence of age and CYP2C19 phenotype on escitalopram pharmacokinetics in Chinese psychiatric patients. Using a one-compartment model with first-order absorption and elimination achieved good predictive power. According to the simulation results, in contrast to patients ≥18 years, the daily dose for adolescents with PM might be as high as 15 mg or 20 mg and the current therapeutic window of escitalopram might not be suitable for older patients, both of which required further study. Our results emphasized the necessity for genetic testing and therapeutic drug monitoring during treatment for optimal dosage regimen individualization.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by The Affiliated Brain Hospital of Guangzhou Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

All authors contributed to the article. XN, HL, and MZ designed the study. WK, YY, and ZZ collected the data. SL, TX, SS, and XL analyzed the data and established the population pharmacokinetics model. SL wrote the manuscript and DS and YW revised it.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Relationship Between Linezolid Exposure and the Typical Clinical Laboratory Safety and Bacterial Clearance in Chinese Pediatric Patients

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Objectives: There have been limited studies concerning the safety and efficacy of linezolid (LZD) in children. This study aimed to evaluate the association between LZD exposure and clinical safety and efficacy in Chinese pediatric patients.

Methods: This retrospective cross-sectional study included patients ≤18 years of age who received ≥3 days of LZD treatment between 31 January 2015, and 31 December 2020. Demographic characteristics, medication information, laboratory test information, and bacterial culture results were collected from the Hospital Information System (HIS). Exposure was defined as AUC_{24} and calculated by the non-linear mixed-effects modeling program (NONMEM), version 7.2, based on two validated population pharmacokinetic models. Binary logistic regression analyses were performed to analyze the associations between AUC_{24} and laboratory adverse events, and receiver operating characteristic curves were used to calculate the cut-off values. Efficacy was evaluated by bacterial clearance.

Results: A total of 413 paediatric patients were included, with an LZD median (interquartile range) dose, duration, clearance and AUC_{24} of 30.0 (28.1-31.6) mg/kg/day, 8 (4–15) days,1.31 (1.29-1.32) L/h and 81.1 (60.6-108.7) mg/L·h, respectively. Adverse events associated with TBil, AST, ALT, PLT, hemoglobin, WBC, and neutrophil count increased during and after LZD treatment when compared with before medication ($p < 0.05$), and the

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC24, 24 h area under the concentration-time curve; BUN, blood urea nitrogen; CIs, confidence intervals; HIS, hospital information system; IQR, interquartile range; LZD, linezolid; MIC, minimal inhibition concentration; NONMEM, nonlinear mixed-effects modeling program; ORs, odds ratios; PLT, platelet; ROC, receiver operating characteristic; TBil, total bilirubin; WBC, white blood cell count.

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most common adverse events were thrombocytopaenia (71/399, 17.8%) and low hemoglobin (61/401, 15.2%) during the LZD treatment. Patients with AUC_{24} higher than 120.69 mg/L h might be associated with low hemoglobin 1–7 days after the end of the LZD treatment, and those with an AUC_{24} higher than 92.88 mg/L·h might be associated with thrombocytopaenia 8–15 days after the end of the LZD treatment. A total of 136 patients underwent bacterial culture both before and after LZD treatment, and the infection was cleared in 92.6% (126/136) of the patients, of whom 69.8% (88/126) had AUC_{24}/MIC values greater than 80.

Conclusion: Hematological indicators should be carefully monitored during LZD treatment, especially thrombocytopaenia and low hemoglobin, and a continuous period of monitoring after LZD withdrawal is also necessary. Since the AUC_{24} cut-off values for laboratory adverse events were relatively low, a trade-off is necessary between the level of drug exposure required for treatment and safety, and the exposure target (AUC_{24}/MIC) in pediatric patients should be further studied, especially for patients with complications and concomitant medications.

Keywords: linezolid, exposure, safety, efficacy, paediatric

INTRODUCTION

LZD is an oxazolidinone antibiotic that inhibits bacterial protein synthesis and prevents bacterial reproduction by binding to the bacterial 23S ribosomal RNA of the 50S subunit blocking the formation of a functional 70S initiation complex (Daniel and Ronald, 2001). The absolute oral bioavailability of LZD is approximately 100%, with good tissue penetration and non-susceptibility to drug resistance (Roger et al., 2018). It is commonly used to treat severe Gram-positive bacterial infections. It is considered clinically effective but is usually difficult to manage because of its large individual pharmacokinetic differences and related adverse events, especially in pediatric patients, and it is an antibiotic with a narrow therapeutic window and dose-dependent toxicity (Sotgiu et al., 2012; Peyrani et al., 2014).

A meta-analysis showed that approximately one out of every two patients experienced adverse events due to LZD (4), but the incidence of LZD-related adverse reactions in Chinese children has rarely been reported. Hematological toxicity, hyperlacticaemia, and optic neuropathy are the main adverse reactions to LZD (3, 4, 5), and thrombocytopaenia is a significant adverse drug reaction with the highest risk in the clinic (Han et al., 2021). The incidence of LZD-induced thrombocytopaenia varies from 3.8% to 15.7% in children worldwide (Meissner et al., 2003; Garazzino et al., 2011; Garazzino and Tovo, 2011), which is lower than that in adults (range 16.7–60.5%) but higher than the drug label reported (2.4% in children) (Natsumoto et al., 2014; Hirano et al., 2014; U.S. Department of Health and Human Services, 2021). In addition, the risk of adverse reactions increased with exposure and duration of LZD treatment (Matsumoto et al., 2014; Rao et al., 2020), the incidence of thrombocytopaenia in adult patients was significantly higher when the trough concentration was greater than 7.5 mg/L (Nukui et al., 2013), and children with thrombocytopaenia had a significantly higher average trough concentration than those without thrombocytopaenia (19.8 vs. 6.8 mg/L) (Ogami et al., 2019), but the relationship between LZD exposure and adverse reactions in Chinese children has not been studied.

Population pharmacokinetic models of LZD in children have been widely established and have been used to calculate drug exposure, and their extrapolated predictive performance has been confirmed (Vinks, 2002; Jungbluth et al., 2003; Rao et al., 2020; Ogami et al., 2021). Therefore, in this study, we aimed to calculate LZD exposure using population pharmacokinetic models of LZD in children and then evaluated the relationship between drug exposure and adverse events in Chinese pediatric patients. The efficacy of LZD was also evaluated for personalized drug therapy using LZD and risk assessment in clinical therapy.

PATIENTS AND METHODS

Study Design

We conducted a retrospective cross-sectional study of hospitalized children who received LZD treatment in the Children's Hospital of Chongqing Medical University (Chongqing, China) from 31 January 2015, to 31 December 2020. This study was approved by the Ethics Committee of the Children's Hospital of Chongqing Medical University with an informed consent exemption considering the observational and retrospective nature of the study, and the data were collected without identifiers (Approval No. 2020–282). We used the STROBE checklist as the main reference in reports of this cross-sectional study.

Study Subjects

Patients younger than 18 years of age that were intravenously or orally administered LZD for at least three consecutive days were included. The criteria for patient exclusion were a lack of

TABLE 1 | The definition of laboratory adverse events[a].

	Normal Baseline Values[b]	Abnormal Baseline Values
Liver dysfunction		
High TBil	>2 times of ULN	>1.5 times of the baseline value
High AST	>2 times of ULN	>2 times of the baseline value
High ALT	>2 times of ULN	>2 times of the baseline value
Renal dysfunction		
High Scr	>2 times of ULN	>2 times of the baseline value
High BUN	>2 times of ULN	>2 times of the baseline value
Hematology properties		
Thrombocytopenia	<75% of LLN	<75% of LLN
Low hemoglobin	<75% of LLN	<75% of LLN and <90% of baseline value
Low WBC	<75% of LLN	<75% of LLN
Low neutrophils	<50% of LLN	<50% of LLN

[a]*Laboratory adverse events were defined based on the Food and Drug Administration label of linezolid, and the corresponding reference is 13.*
[b]*LLN, and ULN, values of each parameter were considered based on the normal baseline value ranges defined by the department of clinical laboratory in our hospital.*
Abbreviations: ALT, alanine amimotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; LLN, lower limit of the normal; Scr, serum creatinine concentration; TBil, total bilirubin; ULN, upper limit of normal; WBC, white blood cell count.

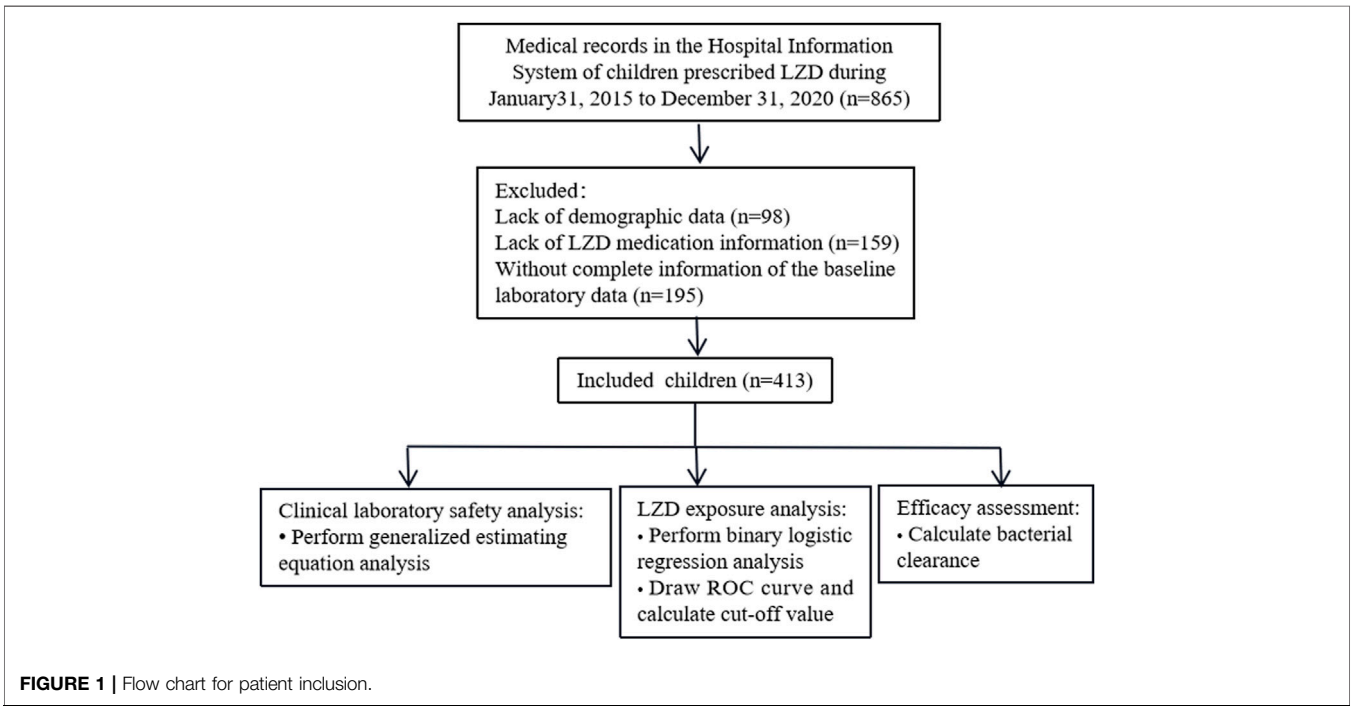

FIGURE 1 | Flow chart for patient inclusion.

demographic data, LZD medication information, or baseline laboratory data for safety assessment.

Data Collection

Medical records in the hospital information system (HIS) database of the patients who matched the inclusion criteria were extracted by an information centre engineer, and then, two of the authors manually screened the information for inclusion and identified and recorded reasons for exclusion. Any disagreements were resolved through discussion or by consulting a third author. The HIS database is a comprehensive, integrated information system includes detailed clinical and demographic information about all pediatric patients, and the information from the HIS database are derived from daily notes recorded by clinicians of all the patients, which helps to improve patient care by assessing data and making recommendations for care. The following information of the included patients was extracted and recorded.

1) Demographic parameters, medication information, and serum creatinine concentration (Scr): sex, age, body weight, height, clinical diagnosis, LZD medication route, dosage, administration time and duration of LZD treatment, and serum creatinine concentrations measured during LZD medication. These data were used for the LZD exposure calculations.

TABLE 2 | Demographic characteristics, medication information, and exposure of linezolid according to the AUC_{24} quartile (n = 413).

	Total	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Number of participants	413	106	102	102	103
Characteristic					
Age, year, median (IQR)	1.2 (0.4–3.6)	0.2 (0.1–0.4)	0.9 (0.5–1.2)	2.0 (1.1–2.9)	6.0 (4.0–9.7)
Sex, n (%)					
Men	252 (61.0)	53 (50.0)	64 (62.7)	67 (65.7)	68 (66.7)
Women	161 (39.0)	53 (50.0)	38 (37.3)	35 (34.3)	35 (34.3)
Weight, kg, median (IQR)	9.5 (6.5–14.0)	5.2 (4.3–6.8)	8.3 (7.0–9.9)	11.5 (9.0–13.0)	20.0 (15.0–25.5)
Height, cm, median (IQR)	73.0 (61.0–96.0)	57.0 (52.0–63.4)	71.0 (63.0–75.0)	85.0 (73.0–91.5)	109.0 (99.3–123.5)
Medication information					
Linezolid dose, mg/kg/day, median (IQR)	30.0 (28.1–31.6)	30.0 (27.9–30.3)	30.0 (26.7–31.0)	30.0 (27.3–31.2)	30.0 (29.1–32.6)
Duration of treatment, day, median (IQR)	8 (4–15)	8 (4–14)	9 (4–15)	6 (4–15)	11 (5–17.5)
Clearance, L/h, median (IQR)	1.31 (1.29–1.32)	1.31 (1.30–1.32)	1.30 (1.07–1.31)	1.30 (1.04–1.32)	1.31 (1.30–1.32)
Exposure of linezolid					
AUC_{24}, mg/L.h, median (IQR)	81.1 (60.6–108.7)	43.5 (34.1–53.3)	72.6 (64.9–76.9)	92.2 (85.5–100.0)	149.3 (122.1–189.4)

Abbreviations: AUC_{24}, 24-h area under the concentration-time curve; IQR, interquartile range.

2) Data for safety assessment: total bilirubin (TBil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Scr, blood urea nitrogen (BUN), platelet (PLT), hemoglobin, white blood cell count (WBC), and neutrophil count measured before LZD medication, during treatment, and 1–7 days and 8–15 days after the last dose of LZD administration.
3) Data for efficacy assessment: bacterial culture results and the measured LZD minimal inhibitory concentration (MIC) values.

Exposure Analysis

Two population pharmacokinetic models established by Garcia-Prats AJ et al. (Garcia-Prats et al., 2019) and Si-Chan Li et al. (Li et al., 2019) were chosen to calculate the exposure of LZD in this study, and the predictive performance of the two models has been validated and used in our hospitals. The NONMEM, version 7.2 (Icon Development Solutions, Columbia, MD, United States), was used to perform the simulations and calculate the LZD exposure. The related formulas of the two models are shown in **Supplementary Table S1**. Exposure to LZD was defined as a 24-h area under the concentration-time curve (AUC_{24}) in the steady state, and AUC_{24} = daily dose/clearance.

Safety and Efficacy of LZD

In this study, the safety of LZD was evaluated by laboratory adverse events, which were defined based on the Food and Drug Administration label for LZD (13), see **Table 1**. Efficacy was evaluated by comparing the bacterial culture results before and after LZD treatment, and the proportion of AUC_{24}/MIC values greater than 80 was also calculated, as previous studies have shown that higher success rates for LZD might occur at AUC_{24}/MIC values greater than 80 (22,23,24,25).

Statistical Analysis

The Shapiro–Wilk test was performed to assess whether the data were normally distributed. Continuous outcomes with abnormal distributions are expressed as medians and interquartile ranges. Categorical outcomes are reported as counts and percentages. AUC_{24} was calculated to indicate the *in vivo* exposure of LZD, and patients were divided into four groups according to the interquartile range (IQR) of AUC_{24}: quartile 1, quartile 2, quartile 3, and quartile 4. A generalized estimating equation (GEE) was used to analyze the incidence of changes in the laboratory adverse events over time before and after LZD treatment. Binary logistic regression analyses were performed to analyze the associations between AUC_{24} and safety, and age, sex, and laboratory parameters measured before the LZD medication were considered potential confounding factors based on the preliminary analysis and a literature review (Chang et al., 2013; Mullins et al., 2013; Chuang et al., 2014) and were included for adjustment. The covariates were evaluated continuously, and by the quartile of exposure, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Receiver operating characteristic (ROC) curves were used to estimate the exposure cut-off values for the laboratory adverse events. The sensitivity and specificity and the maximum Youden's index of the ROC curve were calculated, and the maximum Youden's index was selected as the optimal exposure cut-off value. Youden's index equals the result of subtracting one from the sum of sensitivity and specificity (Rui et al., 2016). p values less than 0.05 were considered to be statistically significant. Data were gathered using the Microsoft Excel software (Redmond, WA, United States), and all analyses were performed using the IBM SPSS statistical software package, version 22.0 (SPSS, Chicago, IL, United States).

RESULTS

Patient Characteristics

Medical records of 865 patients who received LZD therapy were extracted and screened, and 413 patients who met our inclusion criteria were included in this study (**Figure 1**). Demographic characteristics, medication information, and exposure to LZD after drug administration are shown in **Table 2**. Counts and percentages of the laboratory adverse events are shown in **Table 3**. The most common adverse events were thrombocytopaenia (71/399, 17.8%) and low hemoglobin (61/401, 15.2%) during LZD treatment.

TABLE 3 | Counts and percentages of the laboratory adverse events and the change over time before and after linezolid treatment according to the AUC_{24} quartile.

	Total	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Laboratory adverse event, n (%)					
High TBil, umol/L					
Before medication	13/413 (3.1)	8/106 (7.5)	2/102 (2.0)	1/102 (1.0)	2/103 (1.9)
During treatment	25/374 (6.7)[a]	9/94 (9.6)	5/95 (5.3)	6/91 (6.6)	5/94 (5.3)
1–7 days after end of treatment	19/341 (5.6)[a]	10/86 (11.6)	2/86 (2.3)	3/80 (3.8)	4/89 (4.5)
8–15 days after end of treatment	11/292 (3.8)	7/67 (10.4)	1/74 (1.4)	1/72 (1.4)	2/79 (2.5)
High AST, U/L					
Before medication	26/413 (6.3)	10/106 (9.4)	6/102 (5.9)	8/102 (7.8)	2/103 (1.9)
During treatment	39/383 (10.2)[a]	14/97 (14.4)	6/95 (6.3)	11/96 (11.5)	8/95 (8.4)
1–7 days after end of treatment	26/352 (7.4)	9/86 (10.5)	3/90 (3.3)	9/85 (10.6)	5/91 (5.5)
8–15 days after end of treatment	23/314 (7.3)	5/73 (6.8)	5/78 (6.4)	8/81 (9.9)	5/82 (6.1)
High ALT, U/L					
Before medication	23/413 (5.6)	8/106 (7.5)	3/102 (2.9)	7/102 (6.9)	5/103 (4.9)
During treatment	48/380 (12.6)[a]	12/94 (12.8)	6/95 (6.3)	17/93 (18.3)	13/98 (13.3)
1–7 days after end of treatment	26/353 (7.4)	9/86 (10.5)	2/90 (2.2)	8/84 (9.5)	7/93 (7.5)
8–15 days after end of treatment	26/316 (8.2)	8/72 (11.1)	4/82 (4.9)	7/78 (9.0)	7/84 (8.3)
High Scr, mg/ml					
Before medication	11/413 (2.7)	6/106 (5.7)	1/102 (1.0)	3/102 (2.9)	1/103 (1.0)
During treatment	11/378 (2.9)	6/95 (6.3)	0/93 (0)	1/91 (1.1)	4/99 (4.0)
1–7 days after end of treatment	7/340 (2.1)	3/83 (3.6)	1/85 (1.2)	0/81 (0)	3/91 (3.3)
8–15 days after end of treatment	6/292 (2.1)	2/69 (2.9)	1/73 (1.4)	0/70 (0)	3/80 (3.8)
High BUN, mmol/L					
Before medication	4/413 (1.0)	2/106 (1.9)	0/102 (0)	1/102 (1.0)	1/103 (1.0)
During treatment	4/326 (1.2)	1/81 (1.2)	0/74 (0)	0/79 (0)	3/92 (3.3)
1–7 days after end of treatment	6/236 (2.5)	1/50 (2.0)	0/58 (0)	0/53 (0)	5/75 (6.7)
8–15 days after end of treatment	7/174 (4.0)	2/34 (5.9)	1/48 (2.1)	1/41 (2.4)	3/51 (5.9)
Thrombocytopenia, $\times10^9$/L					
Before medication	23/413 (5.6)	3/106 (2.8)	4/102 (3.9)	7/102 (6.9)	9/103 (8.7)
During treatment	71/399 (17.8)[a]	17/104 (16.3)	15/98 (15.3)	18/97 (18.6)	21/100 (21.0)
1–7 days after end of treatment	102/399 (25.6)[a]	30/104 (28.8)	24/98 (24.5)	27/97 (27.8)	21/100 (21.0)
8–15 days after end of treatment	70/361 (19.4)[a]	5/86 (5.8)	10/94 (10.6)	21/90 (23.3)	34/91 (37.4)
Low hemoglobin, g/L					
Before medication	18/413 (4.4)	9/106 (8.5)	5/102 (4.9)	2/102 (2.0)	2/103 (1.9)
During treatment	61/401 (15.2)[a]	12/104 (11.5)	12/101 (11.9)	20/97 (20.6)	17/99 (17.2)
1–7 days after end of treatment	57/387 (14.7)[a]	12/99 (12.1)	7/96 (7.3)	14/95 (14.7)	24/97 (24.7)
8–15 days after end of treatment	54/350 (15.4)[a]	16/85 (18.8)	10/92 (10.9)	8/87 (9.2)	20/86 (23.3)
Low WBC, $\times10^9$/L					
Before medication	24/413 (5.8)	2/106 (1.9)	4/102 (3.9)	7/102 (6.9)	11/103 (10.7)
During treatment	43/389 (11.1)[a]	7/101 (6.9)	9/98 (9.2)	13/91 (14.3)	14/99 (14.1)
1–7 days after end of treatment	49/383 (12.8)[a]	9/96 (9.4)	9/96 (9.4)	16/94 (17.0)	15/97 (15.5)
8–15 days after end of treatment	50/346 (14.5)[a]	6/84 (7.1)	11/90 (12.2)	13/85 (15.3)	20/87 (23.0)
Low neutrophil count, $\times10^9$/L					
Before medication	8/413 (1.9)	0/106 (0)	2/102 (2.0)	2/102 (2.0)	4/103 (3.9)
During treatment	30/403 (7.4)[a]	2/103 (1.9)	5/99 (5.1)	9/98 (9.2)	14/103 (13.6)
1–7 days after end of treatment	22/385 (5.7)[a]	3/99 (3.0)	4/95 (4.2)	7/93 (7.5)	8/98 (8.2)
8–15 days after end of treatment	16/361 (4.4)[a]	2/84 (2.4)	2/91 (2.2)	5/92 (5.4)	7/94 (7.4)

Abbreviations: ALT, alanine amimotransferase; AST, aspartate aminotransferase; AUC_{24}, 24 h area under the concentration-time curve; BUN, blood urea nitrogen; Scr, serum creatinine concentration; TBil, total bilirubin; WBC, white blood cell count.

[a]*Significantly different from the before medication group by generalized estimation equation analysis (p < 0.05).*

Association Between LZD Exposure and Safety

The incidence of changes in the laboratory adverse events over time, before, and after medication is shown in **Table 3**. Adverse events associated with TBil, AST, ALT, PLT, hemoglobin, WBC, and neutrophil count increased during and after LZD treatment when compared with previous medication ($p < 0.05$). The association between LZD exposure and laboratory adverse events is shown in **Table 4**. The AUC_{24} quartile four group was associated with increased odds of low hemoglobin 1–7 days after LZD treatment compared with the quartile one group (adjusted OR: 4.768, 95% CI: 1.323-17.184, $p = 0.017$), and the AUC_{24} quartile three and quartile four groups were associated with increased odds of thrombocytopaenia 8–15 days after LZD treatment compared with the quartile one group (adjusted OR: 3.306, 95% CI: 1.126-9.709, $p = 0.030$ and adjusted OR: 3.770, 95% CI: 1.079-13.171, $p = 0.038$, respectively).

TABLE 4 | Association between AUC_{24} and the laboratory adverse events during treatment, and 1–7 days, 8–15 days after the end of linezolid administration[a].

Laboratory Outcomes	Continuous	Quartile 1	Quartile 2	Quartile 3	Quartile 4
During treatment					
High TBil, umol/L	1.012 (0.995–1.030) $p = 0.163$	Ref[b]	0.729 (0.205–2.588) $p = 0.625$	1.329 (0.369–4.795) $p = 0.664$	2.346 (0.327–16.836) $p = 0.396$
High AST, U/L	0.998 (0.987–1.009) $p = 0.694$	Ref	0.401 (0.134–1.202) $p = 0.103$	0.553 (0.196–1.554) $p = 0.261$	0.221 (0.045–1.086) $p = 0.063$
High ALT, U/L	1.000 (0.990–1.010) $p = 0.823$	Ref	0.577 (0.176–1.892) $p = 0.364$	1.635 (0.583–4.583) $p = 0.350$	0.839 (0.194–3.630) $p = 0.814$
Thrombocytopenia, $\times10^9$/L	0.995 (0.987–1.003) $p = 0.201$	Ref	0.727 (0.321–1.647) $p = 0.445$	0.679 (0.291–1.587) $p = 0.372$	0.429 (0.133–1.377) $p = 0.155$
Low hemoglobin, g/L	1.001 (0.993–1.009) $p = 0.889$	Ref	1.378 (0.551–3.446) $p = 0.492$	2.227 (0.880–5.635) $p = 0.091$	1.249 (0.356–4.381) $p = 0.728$
Low WBC, $\times10^9$/L	0.996 (0.986–1.006) $p = 0.388$	Ref	1.354 (0.457–4.012) $p = 0.584$	2.114 (0.721–6.199) $p = 0.173$	2.324 (0.566–9.532) $p = 0.242$
Low neutrophil count, $\times10^9$/L	1.000 (0.992–1.009) $p = 0.923$	Ref	2.352 (0.439–12.604) $p = 0.318$	4.284 (0.864–21.241) $p = 0.075$	5.414 (0.881–33.277) $p = 0.068$
1–7 days after end of treatment					
High TBil, umol/L	0.997 (0.975–1.019) $p = 0.777$	Ref	0.242 (0.040–1.482) $p = 0.125$	0.748 (0.152–3.693) $p = 0.722$	1.917 (0.191–19.212) $p = 0.580$
Thrombocytopenia, $\times10^9$/L	0.994 (0.986–1.001) $p = 0.104$	Ref	1.053 (0.535–2.071) $p = 0.882$	0.878 (0.425–1.815) $p = 0.726$	0.439 (0.154–1.251) $p = 0.123$
Low hemoglobin, g/L	1.005 (0.997–1.014) $p = 0.182$	Ref	0.675 (0.201–2.261) $p = 0.524$	2.484 (0.863–7.152) $p = 0.092$	4.768 (1.323–17.184) $p = 0.017$
Low WBC, $\times10^9$/L	0.997 (0.988–1.006) $p = 0.490$	Ref	0.890 (0.319–2.486) $p = 0.824$	1.467 (0.547–3.933) $p = 0.446$	0.734 (0.193–2.793) $p = 0.650$
Low neutrophil count, $\times10^9$/L	1.001 (0.990–1.012) $p = 0.870$	Ref	1.294 (0.277–6.033) $p = 0.743$	2.398 (0.567–10.135) $p = 0.234$	2.664 (0.431–16.477) $p = 0.292$
8–15 days after end of treatment					
Thrombocytopenia, $\times10^9$/L	1.008 (1.001–1.016) $p = 0.033$	Ref	1.631 (0.522–5.095) $p = 0.400$	3.306 (1.126–9.709) $p = 0.030$	3.770 (1.079–13.171) $p = 0.038$
Low hemoglobin, g/L	1.001 (0.993–1.009) $p = 0.889$	Ref	0.643 (0.244–1.694) $p = 0.371$	0.580 (0.204–1.649) $p = 0.307$	1.601 (0.457–5.608) $p = 0.462$
Low WBC, $\times10^9$/L	1.009 (1.001–1.017) $p = 0.037$	Ref	1.820 (0.630–5.255) $p = 0.269$	2.073 (0.705–6.093) $p = 0.185$	2.415 (0.638–9.146) $p = 0.194$
Low neutrophil count, $\times10^9$/L	1.003 (0.995–1.010) $p = 0.509$	Ref	0.813 (0.108–6.125) $p = 0.841$	2.373 (0.414–13.601) $p = 0.322$	4.970 (0.604–40.909) $p = 0.136$

Values given are Odds ratios and 95% confidence intervals estimates.

[a]*The binary logistic regression model was adjusted for variables including age (continuous, years), sex (male/female) and whether adverse events occurred before medication (yes/no).*

[b]*The Ref means taking quartile 1 as the reference category and comparing the data of quartile 2, quartile three and quartile four to those of quartile 1.*

Abbreviations: ALT, alanine amimotransferase; AST, aspartate aminotransferase; AUC_{24}, 24 h area under the concentration-time curve; TBil, total bilirubin; WBC, white blood cell count.

Exposure Cut-Off Values for Laboratory Adverse Events

An ROC analysis was subsequently performed to calculate the cut-off points of AUC_{24} for low hemoglobin and thrombocytopenia. The ROC curves and the associated results are shown in **Figure 2**. The cut-off with the largest Youden index of low hemoglobin 1–7 days after the end of LZD treatment was 120.69 mg/L h with a sensitivity of 83.8% and a specificity of 67.3%, and the cut-off with the largest Youden index of thrombocytopenia 8–15 days after the end of LZD treatment was 92.88 mg/L h with a sensitivity of 75.7% and a specificity of 72.5%.

Efficacy Assessment

The most common site of infection was pulmonary [254 (61.5%)], followed by skin [99 (24.0%)], blood [90 (21.8%)], and endocarditis [83 (20.1%)](**Supplementary Table S2**). A total of 86.4% (357/413) of the included patients underwent bacterial culture before LZD treatment, and bacteria were found in 56.0% (200/357) of the patients. The species and MIC distributions of the bacterial strains isolated from patients before LZD treatment are presented in **Table 5**. Bacterial culture was performed in 68% (136/200) of the aforementioned patients by the end of the LZD therapy, and infections of 92.6% (126/136) of the patients were cleared, of whom 69.8% (88/126) of the patients had AUC_{24}/MIC values greater than 80. Bacterial infections in 7.4% (10/136) of the patients were not cleared, of whom 90.0% (9/10) of the patients had AUC_{24}/MIC values greater than 80.

DISCUSSION

In this study, the patients who were intravenously or orally administered with LZD were all included, and the pharmacokinetic models of the corresponding route of administration were used to calculate the drug exposures. Studies have shown that there were no significant racial differences in the pharmacokinetic process of LZD in

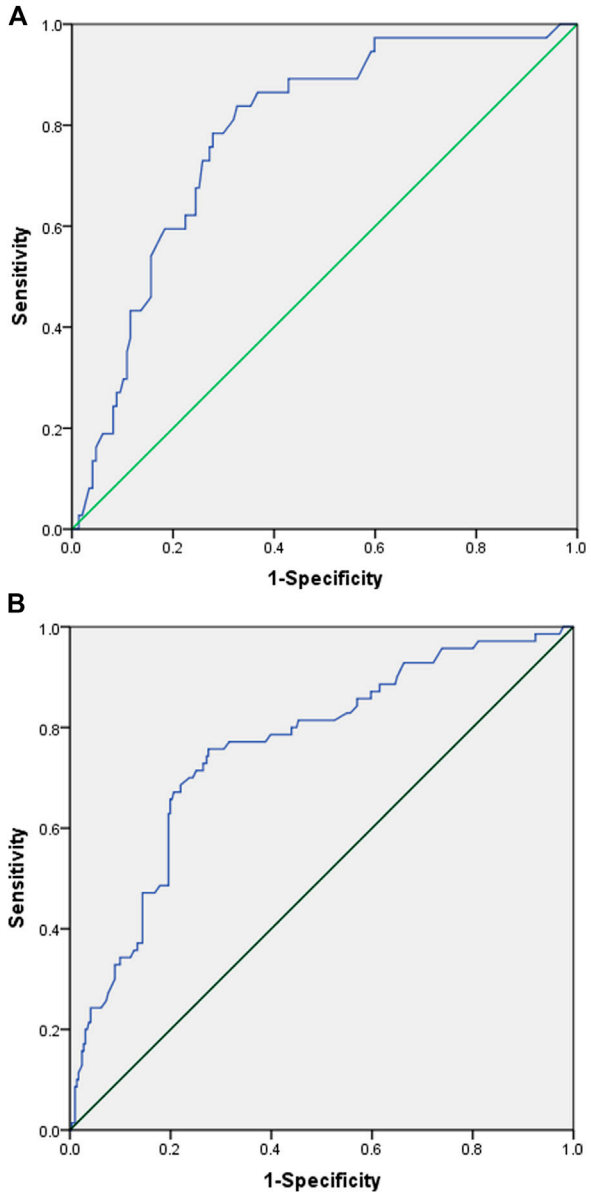

FIGURE 2 | Receiver operating characteristic (ROC) curve of linezolid-induced adverse effect. **(A)** ROC curve of low hemoglobin 1–7 days after the end of linezolid administration. (Youden index = 0.511, cut-off values = 120.69, area under the ROC curve = 0.783, 95% confidence interval=(0.706-0.860), $p < 0.001$, sensitivity = 0.838, specificity = 0.673). **(B)** ROC curve of thrombocytopenia 8–15 days after the end of linezolid administration. (Youden index = 0.482, cut-off values = 92.88, area under the ROC curve = 0.756, 95% confidence interval=(0.693-0.820), $p < 0.001$, sensitivity = 0.757, specificity = 0.725).

pediatrics (Jungbluth et al., 2003), so, we chose the pharmacokinetic model of oral administration, which was established based on a multiracial population, with race not considered a significant covariant (Garcia-Prats et al., 2019). Moreover, the pharmacokinetic model of intravenous administration of LZD was established based on Chinese pediatrics (Li et al., 2019).

TABLE 5 | The species and MIC distribution of bacterial strains isolated from patients before linezolid treatment (n = 200).

Isolates	N (%)	MIC (Ug/mL)				
		≤0.5	≤0.064	≤1	≤2	4
Staphylococcus aureus	78 (39.0)			12	65	1
Staphylococcus epidermidis	39 (19.5)			13	26	
Streptococcus pneumoniae	38 (19.0)	2		9	27	
Human staphylococcus	14 (7.0)			1	10	3
Enterococcus faecium	9 (4.5)				9	
Streptococcus pallidus	8 (4.0)	1	1		6	
Enterococcus faecalis	7 (3.5)	1		3	3	
Staphylococcus haemolyticus	3 (1.5)				3	
Staphylococcus Coriolis	3 (1.5)			1	2	
Streptococcus pyogenes	1 (0.5)			1		

MIC, minimal inhibitory concentration.

We included patients with no "normal baseline values" in our study, as no "normal baseline values" does not mean it has reached the level of adverse events as defined in the study, and by comparing the incidence of associated adverse events before and after medication, we could see if there was a statistically significant increase in the incidence of related adverse events after medication. In fact, we did not find that patients with no "normal baseline values" were more prone to develop adverse events from our data. When analyzing the association between AUC_{24} and safety, laboratory parameters measured before LZD were considered as a potential confounding factor and were included as covariates in the binary logistic regression analyses, to avoid the influence of parameter differences between individuals before LZD medication on the statistical analysis, and to keep the validity of the results.

The hematological toxicity of LZD is widely known (Sasaki et al., 2011; Bayram et al., 2017), and in this study, thrombocytopenia and low hemoglobin were particularly significant. Pediatric patients with the treatment duration more than 28 days were more likely to have laboratory adverse events of low hematological indicators after using LZD. Therefore, hematological indicators should be carefully monitored during LZD treatment, especially for patients with long-term treatment (Dong et al., 2016). A previous study reported that one patient developed grade 4 neutropoenia 7 days after the end of LZD administration (Yasu et al., 2021), but the other influencing factors were unclear. In our study, low hemoglobin, thrombocytopenia, low WBC, and low neutrophil count occurred after the end of the LZD treatment in a significant proportion of the patients. The related mechanisms and other influencing factors deserve further study, but it seems a continuous period of monitoring after LZD withdrawal is also necessary.

Studies have reported that an adequate exposure to LZD was an AUC_{24} ranging between 160 and 300 mg/L h in adults (Pea et al., 2012; Cojutti et al., 2019), but the AUC_{24} cut-off value of LZD-associated thrombocytopenia was 280.7 mg/L h in adult patients (Pea et al., 2012) and 93.4 mg/L h for mitochondrial toxicity in infants and toddlers (Srivastava et al., 2016). In our study, we calculated the AUC_{24} cut-off values of 120.69 and 92.88 mg/L h for low hemoglobin and thrombocytopenia,

respectively. It is suggested that for patients, especially pediatric patients, a trade-off is necessary between the level of drug exposure required for treatment and safety since an AUC_{24}/MIC value greater than 80 is commonly recommended in clinics (Andes et al., 2002; Rayner et al., 2003; Pea et al., 2010; Li et al., 2019).

In accordance with the drug labels, the dosage of LZD was approximately 30.0 mg/kg/day for both intravenous and oral administrations in this study (U.S. Department of Health and Human Services, 2021), and we found that, although the infections in 92.6% (126/136) of the patients were cleared, 30.2% (38/126) of the patients had an AUC_{24}/MIC value lower than 80. Since patient characteristics, peculiar pathophysiological conditions (e.g., cystic fibrosis, burn injuries, and sepsis), and combination medications could all affect the drug pharmacokinetic process and the apparent pharmacokinetic parameters (Di Paolo et al., 2010), and clearance of LZD in children younger than 12 years of age was greater than adults, with a correspondingly lower AUC_{24} (Jungbluth et al., 2003; Principi and Esposito, 2019), a previous study suggested that the LZD exposure target was an AUC_{24}/MIC ratio of 62 with combination therapy (faropenem, LZD, and moxifloxacin) for disseminated and intrathoracic *tuberculosis* in infants and toddlers (Srivastava et al., 2016). Although concomitant medication was not a significant covariant in either of the two population pharmacokinetic models, when considering the efficacy, the exposure target of LZD in pediatrics might require further study, especially for pediatric patients with complications and concomitant medications.

This study had several limitations. First, the results were potentially only biased by the LZD that we analyzed being used at a limited centre. Second, our study only included patients younger than 13 years of age, limiting our ability to comprehensively assess LZD's safety. Additionally, this study had a short follow-up period; therefore, large-scale, randomized clinical trials with longer follow-ups are still needed to further verify the safety and clinical efficacy of LZD.

CONCLUSION

Hematological indicators should be carefully monitored during LZD treatment, especially thrombocytopenia and low hemoglobin, and a continuous period of monitoring after LZD withdrawal is also necessary. Since the AUC_{24} cut-off values for laboratory adverse events were relatively low, a trade-off is necessary between the level of drug exposure required for treatment and safety, and the exposure target (AUC_{24}/MIC) in pediatrics should be further studied, especially for patients with complications and concomitant medications.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

B-NH and Y-EW contributed equally to the manuscript. LS and Y-TJ drafted the study protocol. B-NH, LS, R-QZ, and Q-BL performed the study and obtained the data. Y-EW performed the pharmacometric analysis. B-NH and LS made a statistical analysis of the data. All authors contributed to the data, revised the manuscript critically for content, and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.926711/full#supplementary-material

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Impact of pharmacist active consultation on clinical outcomes and quality of medical care in drug-induced liver injury inpatients in general hospital wards: A retrospective cohort study

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The utility of pharmacist consultation for drug-induced liver injury (DILI) management has not been explored. This retrospective cohort study evaluated the impact of a pharmacist active consultation (PAC) service on the management and outcome in patients with DILI. Consecutive patients meeting clinical biochemical criteria for DILI were enrolled at a tertiary teaching hospital between 1 January 2020 and 30 April 2022. The Roussel Uclaf Causality Assessment Method was used to assess causality between drug use and liver injury for each suspected DILI patient. Included patients were grouped according to whether they received PAC, and a proportional hazard model with multivariate risk adjustment, inverse probability of treatment weighting (IPTW), and propensity score matching (PSM) was used to assess DILI recovery. In the PSM cohort, the quality of medical care was compared between PAC and no PAC groups. A total of 224 patients with DILI (108 who received PAC and 116 who did not) were included in the analysis. Of these patients, 11 (10%) were classified as highly probable, 58 (54%) as probable, and 39 (36%) as possible DILI in the PAC group, while six patients (5%) were classified as highly probable, 53 (46%) as probable, and 57 (49%) as possible DILI in the no PAC group (p = 0.089). During patient recovery, PAC was associated with a ~10% increase in the cumulative 180-day recovery rate. The PAC group had a crude hazard ratio (HR) of 1.73 [95% confidence interval (CI): 1.23–2.43, p = 0.001] for DILI 180-day recovery, which remained stable after multivariate risk adjustment (HR = 1.74, 95% CI: 1.21–2.49, p = 0.003), IPTW (HR = 1.72, 95% CI: 1.19–2.47, p = 0.003), and PSM (HR = 1.49, 95% CI: 1.01–2.23, p = 0.046). In the PSM cohort, PAC was more likely to identify suspect drugs (90% vs. 60%, p < 0.001) and lead to timely withdrawal of the medication (89% vs. 57%, p < 0.001). Thus, PAC is associated with a better quality of medical care for patients with DILI and can improve patient outcomes.

KEYWORDS

drug-induced liver injury, clinical pharmacist, pharmacist active consultation, Roussel Uclaf Causality Assessment Method (RUCAM), patient recovery, medical care quality

Introduction

Drug-induced liver injury (DILI) is an adverse reaction induced by small-molecule drugs, biological agents, traditional Chinese medicines, and herbal and dietary supplements (Yu et al., 2017). The incidence of DILI ranges from 12.0/1,00,000 to 19.1/1,00,000 in the general population and varies according to region, study design, and patient inclusion and exclusion criteria (Sgro et al., 2002; Suk et al., 2012; Bjornsson et al., 2013). DILI is an increasingly important clinical problem for which diagnosis and treatment guidelines have been developed in recent years (Yu et al., 2017; European Association for the Study of the Liver. Electronic address et al., 2019; Chalasani et al., 2021; Devarbhavi et al., 2021). However, there are more than 1,000 drugs and dozens of diseases that can cause liver damage (Giannini et al., 2005; Malakouti et al., 2017; Thakkar et al., 2020), and the diagnosis of DILI mainly relies on the exclusion of other etiologies of liver disease and identification of suspect drugs, which requires clinical and pharmaceutical expertise. As such, the management of DILI patients remains challenging, especially for inexperienced medical personnel.

Clinical pharmacists are an important part of the patient-centered diagnosis and treatment team with professional pharmacy knowledge and the ability to provide comprehensive medication management (Saseen et al., 2017). Pharmacists have played a positive role in the prevention of cardiovascular events; anticoagulant treatment; preconception care; and management of infection, pain, cancer treatment adverse reactions, and type 2 diabetes (Saokaew et al., 2010; Dunn et al., 2015; DiPietro Mager, 2016; Sakeena et al., 2018; Durrer et al., 2021; Homan et al., 2021; Thapa et al., 2021). However, there have been no studies to date evaluating the impact of pharmacist involvement in the management of patients with DILI.

In order to explore and optimize the model of DILI management, we established a pharmacist active consultation (PAC) service at our hospital that consists of spontaneous active consultation conducted by clinical pharmacists for suspected DILI patients, with the intent of providing optimal and timely treatment recommendations. Herein, we describe the impact of PAC on DILI patient outcomes.

Methods

This study was conducted at the Third Affiliated Hospital of Chongqing Medical University, a 1350-bed tertiary teaching hospital in Chongqing, China, with approximately 40,000 annual patient admissions.

PAC service for DILI patients

On 1 March 2021, clinical pharmacists at our center began implementing the PAC service for hospitalized patients with suspected DILI. Clinical pharmacists identified patients with DILI according to the following clinical biochemistry criteria: 1) alanine aminotransferase (ALT) ≥5× the upper limit of the normal range (ULN), 2) ALT ≥3× ULN and total bilirubin >2 × ULN, or 3) alkaline phosphatase (ALP) ≥2 × ULN and gamma-glutamyl transferase >1 × ULN (Aithal et al., 2011).

Every working day, a clinical pharmacist reviewed each case that met the abovementioned criteria along with medical history, medication history, and LiverTox (https://www.ncbi.nlm.nih.gov/books/NBK547852) and immediately initiated PAC for patients with suspected DILI. This included the following steps: 1) explaining the possible reason for liver injury to patients and doctors; 2) identifying the possible causative drugs; 3) discontinuing, adjusting the dose of, or continuing treatment with the drug depending on the patient's condition; 4) selecting appropriate drugs for liver injury treatment; 5) conducting a 10-min education session for the patient; and 6) monitoring changes in liver function parameters and proposing interventions when necessary. Clinical pharmacists participated in routine ward rounds.

Study design and patient population

Using a retrospective cohort study design, consecutive patients were enrolled from 1 January 2020 to 30 April 2022 if they had at least one liver function test meeting one of the aforementioned clinical biochemistry criteria for DILI.

Patients with unambiguous alternative etiologies for liver injury were excluded; these included liver injury in infants, viral liver disease, alcoholic liver disease, autoimmune liver disease, cholestatic liver diseases, infection (e.g., liver abscess, sepsis), hemodynamic abnormality, hepatobiliary pancreatic tumor, pancreatitis, direct liver injury, osteopathy, liver cirrhosis, intestinal disease, and other nondrug or unknown causes of liver injury (Giannini et al., 2005; Malakouti et al., 2017; European Association for the Study of the Liver. Electronic address et al., 2019; Chalasani et al., 2021; Devarbhavi et al., 2021). Patients admitted to the hospital's Hepatology Department were excluded as they were treated by a specialist experienced in DILI management, and, therefore, PAC was not performed by the clinical pharmacist. Additionally, as the Chinese Society of Hepatology strongly recommends the use of the Roussel Uclaf Causality Assessment Method (RUCAM) to establish causality in the clinical diagnosis of DILI, this was

applied to each suspected case (Danan and Teschke, 2016; Yu et al., 2017). Patients were classified as highly probable (RUCAM score ≥ 9), probable (6–8), possible (3–5), unlikely (1 or 2), or excluded (≤0). Patients who were categorized as "unlikely" and "excluded" (<3) and those without follow-up liver function test data were excluded from the analysis. The remaining patients were divided into no PAC and PAC groups based on whether they received the PAC intervention.

Ethics

This study was approved by the Third Affiliated Hospital of Chongqing Medical University Review Board with a waiver for informed consent (No. 2021-37).

Definitions

The R-value [(ALT/ALT ULN)/(ALP/ALP ULN)] was used to categorize the injury pattern of DILI as hepatocellular (R ≥ 5), cholestatic (R ≤ 2), or mixed (2 < R < 5) (Danan and Benichou, 1993; Aithal et al., 2011; Danan and Teschke, 2016). The severity of DILI was categorized into four grades, namely, mild, moderate, severe, and fatal/transplantation, according to the DILI severity grading scale developed by the International DILI Expert Working Group (Supplementary Table S1) (Aithal et al., 2011).

Patient recovery and follow-up

Patient recovery was defined as a return to normal of the patient's serum biochemical parameters (1 × ULN) (Ashby et al., 2021). As patients with DILI whose liver function did not return to normal for >6 months were considered to have a chronic liver injury (Yu et al., 2017), we set 180 days as the cutoff point for follow-up. Time to recovery or follow-up time was calculated in days from the day the patient met the clinical biochemical criteria for DILI to the date of normalization of liver serum biochemical parameters (1 × ULN) or the last day of follow-up. Patients with serum ALT, aspartate aminotransferase, ALP, or bilirubin that did not return to 1 × ULN were censored at the date of their last recorded follow-up.

Inpatient DILI management quality

Seeking expert consultation is helpful to ascertain the diagnosis of DILI and attribute causality to a suspect drug (Chalasani et al., 2021). In this study, the expert was a pharmacologist or hepatologist. The appearance of the term "drug-induced liver injury" in medical records indicated that the physician was aware of the possibility of DILI, and the appearance of a specific drug name indicated that the causative drug had been identified.

Timely discontinuation was defined as discontinuation of the suspect drug within 24 h of the patient meeting the clinical biochemical criteria for DILI. Drugs, treatment measures, and liver function monitoring intervals were recorded for each group to assess differences in patient management. The time interval from meeting the clinical biochemical criteria for DILI to receiving expert consultation was calculated in hours for each patient to evaluate the efficiency of PAC service delivery.

Outcome assessment

The primary outcome of this study was a 180-day patient recovery rate and hazard ratio (HR). The secondary outcome was the quality of inpatient DILI management.

Data collection

Data were obtained from patients' electronic and paper medical records and entered into a standardized case report form, which included demographics, comorbidities, suspect drug, DILI clinical characteristics, treatment and management measures, and clinical outcomes.

Statistical analysis

Continuous variables were compared with the Student's *t*-test when normally distributed or with the Mann–Whitney U test. The chi-squared test or Fisher's exact test was used for categorical variables where appropriate. Kaplan–Meier survival analysis with the log-rank test was performed and cumulative events in the 180-day follow-up period were compared between the groups.

In the Cox proportional hazards model, potential predictors of 180-day recovery from liver injury were first assessed in a univariate analysis. Covariates were included in the final model if the *p*-value was ≤0.2 or if they were clinically important. Cox regression analysis was performed to assess the impact of PAC on the rate of 180-day recovery from liver injury, with results presented as HR with a 95% confidence interval (CI).

In a second analysis, using the variables from the univariate analysis, the inverse probability of treatment weighting (IPTW) and propensity score matching (PSM) were performed to control for selection bias and potential confounding factors between groups. A propensity score (PS) was calculated for each patient as the predicted probability of PAC from multivariate logistic regression. Based on

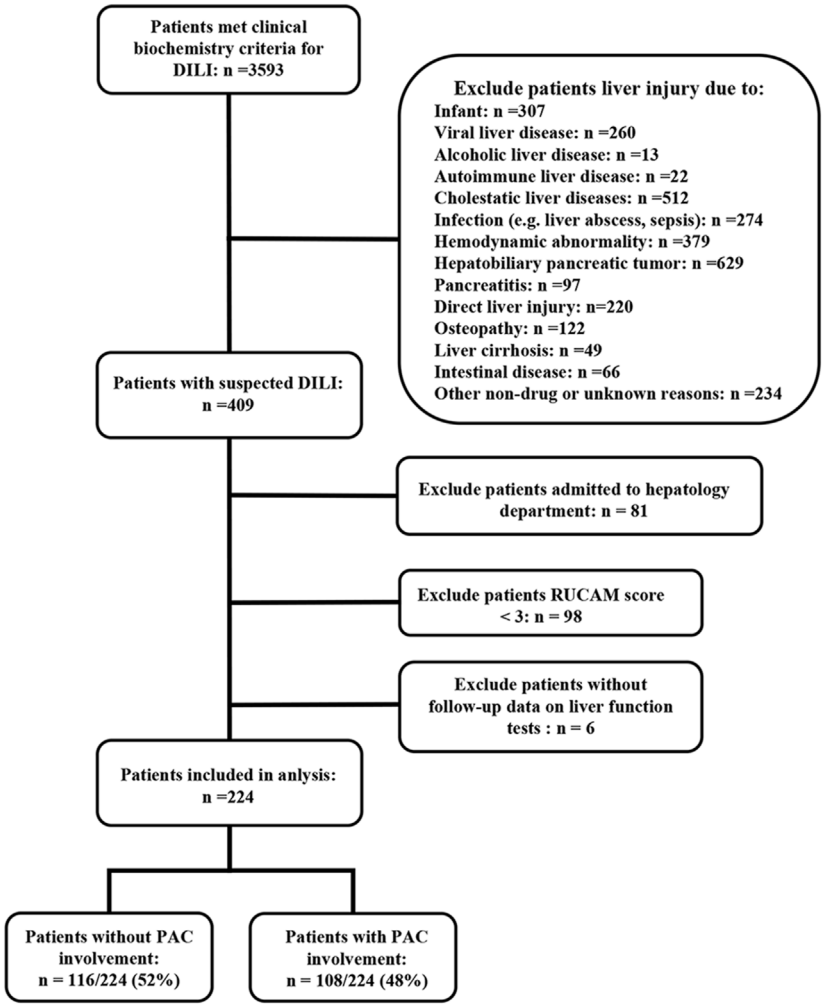

FIGURE 1
Flow diagram of patients included in the study. Abbreviations: DILI, drug-induced liver injury and PAC, pharmacist active consultation.

individual PSs, a Cox regression model was generated using the IPTW approach with PAC as the only covariate. In addition, based on individual PSs, we performed a 1: 1 nearest neighbor matching without replacement with a caliper width of 0.2, yielding a PSM cohort. A Cox regression model was generated for the matched cohort with PAC as the only covariate. Standardized mean differences were used to assess the performance of the IPTW and PSM, with a value <0.10 considered as evidence of balance (Austin and Stuart, 2015). Finally, using PSM cohorts, differences in management quality for patients with DILI were assessed to determine the utility of PAC services.

All statistical tests were two-sided, and $p < 0.05$ was set as the level of statistical significance. Data were analyzed using R v4.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population and clinical characteristics

Of the 3,593 patients meeting the clinical biochemical criteria for DILI, 3,184 were excluded. Of the remaining 224 eligible patients, 116 (52%) were assigned to the no PAC group and 108 (48%) to the PAC group (Figure 1). Using the updated RUCAM causality assessment method, 11 patients (10%) were classified as highly probable, 58 (54%) as probable, and 39 (36%) as possible DILI in the PAC group and six patients (5%) were classified as highly probable, 53 (46%) as probable, and 57 (49%) as possible DILI in the no PAC group ($p = 0.089$). Among patients with possible alternative causes of liver injury, the diagnosis was mostly viral hepatitis

TABLE 1 Patient baseline and drug-induced liver injury clinical characteristics.

Characteristic	All patients (N = 224)	No PAC service (N = 116)	PAC service (N = 108)	p-value
Age, median, years [IQR]	55.0 [46.0, 65.0]	55.5 [45.0, 65.0]	55.0 [49.8, 64.0]	0.921
Age ≥ 60 years	84 (37.5)	50 (43.1)	34 (31.5)	0.075
Male	130 (58.0)	68 (58.6)	62 (57.4)	0.893
DILI onset site				0.023**
Community-acquired[a]	101 (45.1)	61 (52.6)	40 (37.0)	
Hospital-acquired	123 (54.9)	55 (47.4)	68 (63.0)	
Drinking history	25 (11.2)	17 (14.7)	8 (7.4)	0.094
Comorbidity				
Cardiovascular diseases	63 (28.1)	35 (30.2)	28 (25.9)	0.552
Nervous system disease	34 (15.2)	18 (15.5)	16 (14.8)	>0.999
Chronic lung disease	18 (8.0)	8 (6.9)	10 (9.3)	0.625
Chronic kidney disease	3 (1.3)	3 (2.6)	0 (0.0)	0.248
Liver underlying disease[b]	28 (12.5)	16 (13.8)	12 (11.1)	0.687
Gastrointestinal diseases	29 (12.9)	16 (13.8)	13 (12.0)	0.843
Autoimmune disease	14 (6.2)	7 (6.0)	7 (6.5)	>0.999
Diabetes	13 (5.8)	7 (6.0)	6 (5.6)	>0.999
Hyperlipidemia	12 (5.4)	6 (5.2)	6 (5.6)	>0.999
Traumatic diseases	17 (7.6)	10 (8.6)	7 (6.5)	0.619
Malignant tumor	80 (35.7)	40 (34.5)	40 (37.0)	0.780
Biochemical patterns of DILI				0.352
Hepatocellular type	130 (58.0)	64 (55.2)	66 (61.1)	
Mixed type	43 (19.2)	21 (18.1)	22 (20.4)	
Cholestatic type	51 (22.8)	31 (26.7)	20 (18.5)	
Accompanying symptoms				
Jaundice	30 (13.4)	24 (20.7)	6 (5.6)	0.001**
Anorexia	23 (10.3)	18 (15.5)	5 (4.6)	0.008**
Nausea	17 (7.6)	14 (12.1)	3 (2.8)	0.010**
Vomiting	9 (4.0)	8 (6.9)	1 (0.9)	0.036*
Abdominal discomfort	16 (7.1)	11 (9.5)	5 (4.6)	0.198
Rash	8 (3.6)	2 (1.7)	6 (5.6)	0.159
Severity grading				<0.001***
Mild	160 (71.4)	71 (61.2)	89 (82.4)	
Moderate	57 (25.4)	38 (32.8)	19 (17.6)	
Severe	7 (3.1)	7 (6.0)	0 (0.0)	

Data are presented as no. of patients (%) unless otherwise specified.
[a]Community-acquired DILI was defined as a liver injury occurring in a community setting with the patient admitted to the hospital on the first liver biochemical test above the threshold.
[b]Liver malignancies were not included in underlying liver disease but were classified as malignant tumors.
Abbreviations: DILI, drug-induced liver injury; IQR, interquartile range; and PAC, pharmacist active consultation.

and recent hemodynamic abnormality (Supplementary Table S2).

Patient baseline demographic and clinical characteristics are shown in Table 1. There were significant differences between groups in the DILI onset site (p = 0.023), jaundice (p = 0.001), anorexia (p = 0.008), nausea (p = 0.010), vomiting (p = 0.036), and severity grade (p < 0.001). Among the 224 patients included in the analysis, because of the use of multidrug combinations, 260 drugs were considered causative drugs for DILI; the most common drug classes were "antineoplastic and immunomodulating agents" (Supplementary Table S3).

Pharmacist interventions in the PAC group

For the 108 patients in the PAC group, a clinical pharmacist made treatment recommendations based on the patients' condition (Table 2). Clinical pharmacists conducted 10-min

TABLE 2 Recommendations of clinical pharmacists on the management of drug-induced liver injury.

Recommendation	No. (%) of 108 PAC cases
Without intervention—patient education only[a]	11 (10.2)
Discontinue suspect drug	56 (51.9)
Adjust drug dose	2 (1.9)
Switch to alternative medicines	6 (5.6)
Cautious drug rechallenge	10 (9.3)
Add hepatoprotective drugs	68 (63.0)
Treatment with glucocorticoids[b]	7 (6.5)
Screening for viral hepatitis	15 (13.9)
Screening for autoimmune liver disease	10 (9.3)
Abdominal imaging	9 (8.3)
Repeat liver biochemistry in 2–4 days	97 (89.8)

[a]The reason for no intervention was that the clinical pharmacist believed that the management of drug-induced liver injury was appropriate and no further intervention was required.
[b]Glucocorticoids were used to treat immune checkpoint inhibitor-related hepatotoxicity.
Abbreviation: PAC, pharmacist active consultation.

patient education sessions for 89 patients (82%); the remaining 19 (18%) were unable to communicate because of the loss of consciousness. Clinical pharmacists and physicians discussed the management of DILI for all patients in the PAC group in order to collaboratively develop an optimal regimen.

IPTW weighting and PSM cohort

After IPTW, covariates were well-balanced between the PAC and no PAC groups (Figure 2). A total of 164 patients were matched by PSM (82 per group), which improved the balance of covariates between groups (Table 3).

Outcomes

The cumulative recovery rate over the 180 days follow-up period was 96.3% in the PAC group and 86.2% in the no PAC group (Figure 3A). That is, PAC increased the recovery rate by approximately 10%; this increase persisted after controlling for confounding factors (Figure 3B). We also examined 19 patients in the original cohort whose liver function did not return to normal within 180 days; information on these patients is shown in Supplementary Table S4.

Ten covariates were included in the multivariate analysis with a Cox proportional hazards model that included PAC, age ≥ 60 years, nervous system disease, chronic kidney disease, underlying liver disease, autoimmune disease, biochemical patterns of DILI, nausea, abdominal discomfort, and

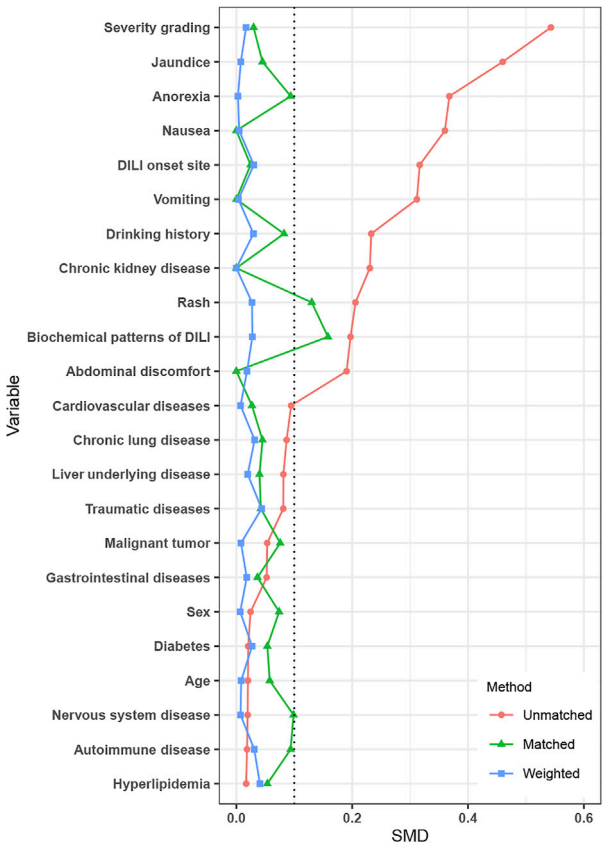

FIGURE 2
Standardized mean difference between the no PAC and PAC groups in unmatched, PSM, and IPTW cohorts. Abbreviations: PAC, pharmacist active consultation; PSM, propensity score matching; and IPTW, inverse probability of treatment weighting.

severity grade. PAC was associated with a higher crude HR (1.73, 95% CI: 1.23–2.43, $p = 0.001$) and adjusted HR (1.74, 95% CI: 1.21–2.49, $p = 0.003$) for DILI recovery (Table 4), whereas no statistically significant differences between the PAC and no PAC groups were observed for the other nine covariates. The higher HRs for the PAC group persisted with IPTW (1.72, 95% CI: 1.19–2.47, $p = 0.003$) and PSM (1.49, 95% CI: 1.01–2.23, $p = 0.046$).

We compared the quality of DILI management between the PAC and no PAC patients in the PSM cohort (Table 5). All of the patients in the PAC group (reference) were considered to have received professional advice and be aware of the possibility of DILI. In contrast, not all patients in the no PAC group were aware of the possibility of DILI (100% vs. 70.7%, $p < 0.001$), and these patients did not benefit from expert consultation (100% vs. 36.7%, $p < 0.001$). PAC was associated with a higher rate of identification of suspect drugs (90.2% vs. 59.8%, $p < 0.001$) and timely withdrawal of medication (89% vs. 57.3%, $p < 0.001$). However, there were no significant differences between the two

TABLE 3 Patient baseline and drug-induced liver injury characteristics in the propensity score-matched cohort.

Characteristic	All patients (*N* = 164)	No PAC service (*N* = 82)	PAC service (*N* = 82)	*p*-value
Age, median, years [IQR]	55.0 [45.8, 64.0]	55.0 [43.5, 65.0]	55.0 [49.2, 63.8]	0.760
Age ≥ 60 years	58 (35.4)	34 (41.5)	24 (29.3)	0.141
Male	92 (56.1)	47 (57.3)	45 (54.9)	0.875
DILI onset site				>0.999
Community-acquired[a]	71 (43.3)	35 (42.7)	36 (43.9)	
Hospital-acquired	93 (56.7)	47 (57.3)	46 (56.1)	
Drinking history	16 (9.8)	9 (11.0)	7 (8.5)	0.793
Comorbidity				
Cardiovascular diseases	47 (28.7)	23 (28.0)	24 (29.3)	>0.999
Nervous system disease	27 (16.5)	12 (14.6)	15 (18.3)	0.674
Chronic lung disease	13 (7.9)	7 (8.5)	6 (7.3)	>0.999
Liver underlying disease[b]	17 (10.4)	9 (11.0)	8 (9.8)	>0.999
Gastrointestinal diseases	21 (12.8)	10 (12.2)	11 (13.4)	>0.999
Autoimmune disease	12 (7.3)	7 (8.5)	5 (6.1)	0.766
Diabetes	9 (5.5)	4 (4.9)	5 (6.1)	>0.999
Hyperlipidemia	9 (5.5)	4 (4.9)	5 (6.1)	>0.999
Traumatic diseases	15 (9.1)	8 (9.8)	7 (8.5)	>0.999
Malignant tumor	61 (37.2)	29 (35.4)	32 (39.0)	0.747
Biochemical patterns of DILI				0.543
Hepatocellular type	43 (26.2)	23 (28.0)	20 (24.4)	
Mixed type	91 (55.5)	42 (51.2)	49 (59.8)	
Cholestatic type	30 (18.3)	17 (20.7)	13 (15.9)	
Accompanying symptoms				
Jaundice	12 (7.3)	6 (7.3)	6 (7.3)	>0.999
Anorexia	12 (7.3)	7 (8.5)	5 (6.1)	0.766
Nausea	6 (3.7)	3 (3.7)	3 (3.7)	>0.999
Vomiting	2 (1.2)	1 (1.2)	1 (1.2)	>0.999
Abdominal discomfort	11 (6.7)	6 (7.3)	5 (6.1)	>0.999
Rash	6 (3.7)	2 (2.4)	4 (4.9)	0.682
Severity grading				>0.999
Mild	160 (71.4)	71 (61.2)	89 (82.4)	
Moderate	35 (21.3)	18 (22.0)	17 (20.7)	

Data are presented as no. of patients (%) unless otherwise specified.
[a]Community-acquired DILI was defined as a liver injury occurring in a community setting with the patient admitted to the hospital on the first liver biochemical test above the threshold.
[b]Liver malignancies were not included in underlying liver disease but were classified as malignant tumors.
Abbreviations: DILI, drug-induced liver injury; IQR, interquartile range; and PAC, pharmacist active consultation.

groups in consultation interval, liver function monitoring interval, number of hepatoprotective drugs used, and glucocorticoid use.

Discussion

We examined the utility of a PAC service provided by clinical pharmacists for the identification and management of patients with DILI. We found that proactive pharmacy consultation improved patients' 180-day cumulative recovery rate. Adjusted Cox multivariate analysis, IPTW weighting, and PS matching further supported these results.

The cumulative rate of recovery over 180 days of follow-up was 86% in the no PAC group and 96% in the PAC group. Patients without the PAC service followed the natural course of recovery from DILI. The estimated probability of recovery by six months was previously reported as ranging from 0.46 to 0.93 in DILI patients with different clinical characteristics (Ashby et al., 2021), which is similar to the recovery rate observed in the no PAC group. PAC was associated with an approximately 10% increase in recovery

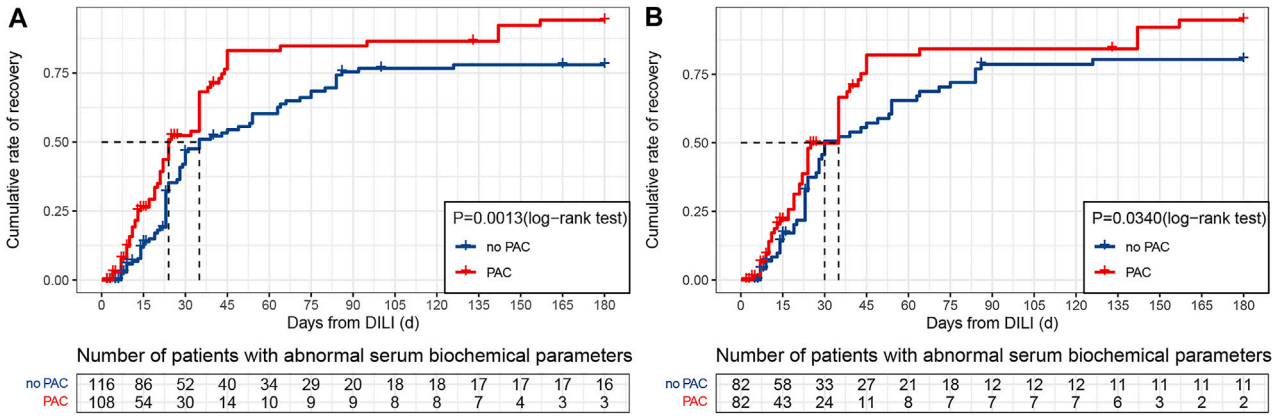

FIGURE 3
Kaplan–Meier cumulative event rates for time to recovery. **(A)** Original cohort. **(B)** PSM cohort. Abbreviations: DILI, drug-induced liver injury and PAC, pharmacist active consultation.

rate. There are two possible explanations for this result. First, PAC improved the quality of medical care for DILI patients, which accelerated their recovery. Second, as this was a real-world study with possible confounders and selection bias, there may have been an imbalance in patient baseline and clinical characteristics between the two groups. In fact, some characteristics were imbalanced between the two original cohorts (Table 1). To minimize the impact of confounding factors on outcomes, we performed an adjusted Cox multivariate analysis and used IPTW and PS matching. The higher recovery rate of the PAC group remained robust after controlling for confounders, suggesting that it was mainly due to improved management of DILI.

In the PSM cohort, the coverage of specialist consultation in the no PAC group was just 30%, implying that most patients did not experience the benefit of evidence-based treatment guidelines. This could in theory be resolved if all patients with suspected DILI sought expert consultation with a hepatologist. However, as skilled doctors at tertiary hospitals in China are greatly overworked (Hu and Zhang, 2015), only a limited number of patients can receive such consultation. The clinical pharmacist, who has medication management skills, is uniquely trained to assist individual patients through effective dispensing of medications, which can prevent adverse drug-related outcomes (Mansur, 2016). Thus, clinical pharmacists can share the workload of skilled doctors by assuming the responsibility of expert consultation.

Based on the PSM cohort, we found that the PAC was associated with higher rates of DILI diagnosis and identification of suspect drugs, as well as timely drug discontinuation, which is the preferred management strategy for suspected DILI although it is predicated on correct identification of the causative drug (Yu et al., 2017; European Association for the Study of the Liver. Electronic address et al., 2019; Chalasani et al., 2021; Devarbhavi et al., 2021). To this end, and in order to provide appropriate recommendations, clinical pharmacists referred to the available evidence-based resources for the diagnosis and management of DILI (Isaacson and Babich, 2020). In some cases, an appropriate recommendation is not limited to accurate identification of the causative drug; a more challenging decision is that of drug continuation or rechallenge under the precondition of DILI. Drug rechallenge may be appropriate under the following circumstances: 1) when no safer alternatives are available, 2) the objective benefits exceed the risks, and 3) patients are fully informed and provide consent, adhere to their treatment for the duration of follow-up, and alert healthcare providers to symptoms of hepatitis (Hunt et al., 2017). Of the 108 patients in the PAC group, cautious drug rechallenge was recommended by the clinical pharmacist in 10 cases. These decisions were evidence-based and in accordance with guideline recommendations for specific drugs (e.g., hepatotoxicity related to immune checkpoint inhibitors or antituberculosis drugs) (Senousy et al., 2010; Remash et al., 2021).

In this study, clinical pharmacists were involved in the treatment of all patients in the PAC group, including the screening of alternative etiologies for DILI, attributing causality to a specific agent, deciding to continue or discontinue the drug, and administering appropriate drugs for DILI therapy. This is in line with the pharmacist's responsibility to engage in comprehensive drug management and share the workload of clinicians. However, these are secondary to providing high-quality medical care to patients through PAC services. The present study also summarized DILI prevention and treatment strategies used at our institution that allows clinical pharmacists to correctly identify patients requiring

TABLE 4 Univariate and multivariate analyses to predict recovery in drug-induced liver injury patients.

Variable	Univariate analysis		Multivariate analysis	
	Crude HR (95% CI)	*p*-value	aHR (95% CI)	*p*-value
PAC	1.73 (1.23–2.43)	0.001	1.74 (1.21–2.49)	0.003**
Age ≥ 60 years	0.78 (0.55–1.11)	0.166	0.90 (0.62–1.31)	0.595
Female	0.86 (0.61–1.20)	0.360	—	—
DILI onset site[a]				
Hospital-acquired	0.90 (0.65–1.26)	0.552	—	—
Community-acquired	Reference			
Drinking history	0.80 (0.47–1.36)	0.409	—	—
Comorbidity				
Cardiovascular diseases	0.99 (0.68–1.42)	0.939	—	—
Nervous system disease	0.67 (0.42–1.08)	0.097	0.71 (0.43–1.16)	0.172
Chronic lung disease	0.84 (0.41–1.73)	0.642	—	—
Chronic kidney disease	0.23 (0.03–1.67)	0.147	0.30 (0.04–2.31)	0.249
Liver underlying disease[b]	1.42 (0.89–2.27)	0.140	1.45 (0.88–2.38)	0.146
Gastrointestinal diseases	1.07 (0.67–1.72)	0.778	—	—
Autoimmune disease	1.63 (0.88–3.04)	0.123	1.52 (0.80–2.86)	0.200
Diabetes	1.46 (0.76–2.78)	0.253	—	—
Hyperlipidemia	0.69 (0.32–1.47)	0.336	—	—
Traumatic diseases	0.79 (0.37–1.69)	0.546	—	—
Malignant tumor	0.93 (0.66–1.32)	0.688	—	—
Biochemical patterns of DILI				
Hepatocellular type	0.98 (0.65–1.49)	0.940	0.94 (0.61–1.45)	0.772
Mixed type	1.11 (0.67–1.84)	0.689	1.11 (0.64–1.95)	0.708
Cholestatic type	Reference			
Accompanying symptoms				
Jaundice	0.80 (0.49–1.29)	0.360	—	—
Anorexia	1.23 (0.74–2.05)	0.425	—	—
Nausea	1.62 (0.95–2.79)	0.079	1.93 (0.98–3.81)	0.057
Vomiting	1.51 (0.70–3.23)	0.292	—	—
Abdominal discomfort	2.40 (1.32–4.37)	0.004	1.72 (0.84–3.51)	0.140
Rash	0.85 (0.37–1.93)	0.703	—	—
Severity grading				
Mild	Reference			
Moderate	1.19 (0.83–1.72)	0.347	0.86 (0.53–1.40)	0.534
Severe	0.50 (0.16–1.58)	0.237	0.64 (0.19–2.15)	0.467

[a]Community-acquired DILI was defined as a liver injury occurring in a community setting with the patient admitted to the hospital on the first liver biochemical test above the threshold.
[b]Liver malignancies were not included in underlying liver disease but were classified as malignant tumors.
Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval; HR, hazard ratio; DILI, drug-induced liver injury; and PAC, pharmacist active consultation.

attention and appropriate drugs in order to provide optimal pharmaceutical care.

To our knowledge, this is the first study to assess the impact of PAC on DILI patient recovery, and it had several advantages. Confounding factors were well controlled and the impact of PAC on 180-day recovery was demonstrated. Our results also showed a new way to manage DILI through clinical pharmacist involvement and can serve as a reference to medical institutions for improving the quality of medical care. However, this study also had several limitations. First, it was based on data from a retrospective review of medical records, and causality between the intervention (PAC) and the outcomes of DILI patients was assessed using the updated RUCAM, which is best applied to a prospective study design; thus, we could not ensure data completeness and high RUCAM scores. Second, because of the retrospective nature of the study, there may have been unrecognized confounding variables linking PAC and patient recovery. Third, this was a single-center study and the findings may not apply to other centers. Fourth, because our

TABLE 5 Quality of medical care in the propensity score-matched cohort of drug-induced liver injury patients.

Management quality	No PAC service (N = 82)	PAC service (N = 82)	p-value
Expert consultation[a]	26 (31.7)	82 (100.0)[b]	<0.001***
Recognition of the possibility of DILI	58 (70.7)	82 (100.0)[b]	<0.001***
Identification of suspect drug	49 (59.8)	74 (90.2)	<0.001***
Timely discontinuation of the suspect drug	47 (57.3)	73 (89.0)	<0.001***
Expert consultation interval, h [IQR][c]	27.4 [5.3, 78.6]	13.5 [6.4, 30.5]	0.294
Liver function monitoring interval, h [IQR]	72.9 [48.2, 116.1]	90.5 [69.2, 119.8]	0.239
Number of hepatoprotective drug use			0.337
0	10 (12.2)	7 (8.5)	
1	17 (20.7)	27 (32.9)	
2	31 (37.8)	32 (39.0)	
3	20 (24.4)	14 (17.1)	
4	4 (4.9)	2 (2.4)	
Glucocorticoid use	16 (19.5)	25 (30.5)	0.149

Data are presented as no. of patients (%) unless otherwise specified.
[a]Expert was defined as a pharmacologist or hepatologist.
[b]With the PAC group as a reference, all patients in the PAC group were considered as having received professional consultation advice and being aware of the possibility of DILI.
[c]In the no PAC group, only the matched 26 patients who received hepatologist consultation were assessed.
Abbreviations: PAC, pharmacist active consultation; IQR, interquartile range; and DILI, drug-induced liver injury.

cohorts included many patients with other alternative causes of DILI categorized as "possible" in the RUCAM causality assessment, the identification of DILI patients is still ambiguous and the results remain controversial. Finally, the PAC service mainly targeted patients with mild to moderate DILI, while those with serious DILI were treated at the Hepatology Department by hepatologists and were not included in the analysis. Therefore, the effect of PAC on the recovery of patients with severe DILI is unclear and requires further validation in a well-designed study.

Conclusion

Our study provides evidence that DILI patients can benefit from PAC services. Clinical pharmacists can share the responsibility of drug management for DILI with doctors by providing evidence-based treatment recommendations. Our findings can encourage greater pharmacist involvement in patient care and collaboration with other healthcare providers to improve the outcome for patients with DILI. We also recommend the use of the updated RUCAM in future DILI cases and similar studies to assist DILI patient identification and enrollment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

Ethics statement

This study was approved by the Third Affiliated Hospital of Chongqing Medical University Review Board with a waiver of informed consent.

Author contributions

SL and QD planned the project. SL, QD, and DL designed the details of the study. DL, JD, XX, GH, WL, CC, and JL contributed to data collection and analysis. SL, QD, and DL contributed to the writing and editing of the manuscript. DL generated the figures of the manuscript. All authors corrected and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.972800/full#supplementary-material

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Literature review of the clinical characteristics of metformin-induced hepatotoxicity

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Background: Knowledge of metformin-induced hepatotoxicity is based on case reports. The aim of this study was to investigate the clinical features of metformin-induced hepatotoxicity.

Methods: We collected relevant literature on metformin-induced hepatotoxicity published from January 1994 to February 2022 by searching Chinese and English databases.

Results: Thirty patients (19 males and 11 females) from 29 articles were included, with a median age of 61 years (range 29–83). The median time to onset of liver injury was 4 weeks (range 0.3–648) after metformin administration. Clinical symptoms occurred in 28 patients, including gastrointestinal reactions (56.7%), jaundice (50.0%), fatigue (36.7%), anorexia (23.3%), pruritus (13.3%), dark urine (13.3%), and clay-colored stools (10.0%). Serum alanine transaminase, aspartate transaminase, γ-glutamyl transferase, total bilirubin and alkaline phosphatase were elevated to varying degrees. Liver imaging in 26 patients showed hepatic steatosis (6 cases, 23.1%) and gallbladder wall thickening (11.5%). Liver biopsies from 13 patients showed portal phlebitis (61.5%), cholestatic hepatitis (38.5%), and parenchymal inflammation (38.5%). After metformin discontinuation, liver function returned to normal levels at a median of 6 weeks (range 2–16).

Conclusions: Metformin-induced hepatotoxicity is a rare adverse reaction. Physicians and patients should be alert to metformin-induced hepatotoxicity.

KEYWORDS

metformin, cholestasis, hepatotoxicity, hepatocellular, liver injury

Introduction

Metformin, a biguanide drug, is an insulin-sensitizing agent with potent antihyperglycemic properties. Metformin is currently the initial drug of choice for the treatment of type 2 diabetes mellitus (T2DM) because it reduces the risk of cardiovascular events and death in people who are overweight or obese (Holman et al., 2008).

Metformin has good safety and tolerability, and it does not increase the risk of hypoglycemia when used alone. The most common side effects of metformin are gastrointestinal reactions such as diarrhea, nausea, and/or abdominal discomfort. They are usually mild, transient, and dose-related but may occur in up to 50% of patients taking the drug (Bouchoucha et al., 2011). Lactic acidosis and vitamin B12 deficiency are additional potential side effects of metformin (Misbin et al., 1998; Out et al., 2018). Hepatotoxicity secondary to metformin is rare. Knowledge of metformin-induced hepatotoxicity is based on case reports. The clinical features and prognosis of metformin-induced hepatotoxicity are unknown. Furthermore, The diagnosis of metformininduced liver injury is difficult because of the heterogeneity of clinical presentations and the absence of established criteria. The purpose of this study was to explore the characteristics of metformin-induced hepatotoxicity and to provide reference for the diagnosis, treatment and prognosis of metformin-induced hepatotoxicity.

Materials and methods

Search strategy

We searched original studies, clinical reports, case reports, and reviews published in Wanfang, CNKI, VIP,PubMed, EMBASE, Web of Science, the Cochrane Library and Medline before February 2022. No language restrictions were applied. The MeSH terms and keywords were the following: metformin, hypoglycemic agents, hepatitis, hepatotoxicity, jaundice, liver injury, pruritus, cholangitis, and cholestasis. We conducted an initial assessment of the titles and abstracts of the papers and then read the full text of all potentially eligible papers. Only papers that met the following inclusion criteria were included: 1) the research subjects were humans; 2) the papers were published online; and 3) the case report included a detailed medical history, laboratory tests, treatment and prognosis.

Data extraction

According to a self-designed table, we extracted country, age, sex, disease history, concomitant medications, indication, dose, onset time, clinical manifestations, laboratory tests, imaging studies, biopsy, treatment, and prognosis. Laboratory tests included alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transferase (GGT), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP), international normalized ratio (INR) and albumin (ALB).

Relevance evaluation

The CIOMS/RUCAM (Council of International Organizations of Medical Sciences/Roussel Uclaf Method for Assessment of Causality) is used to assess drug-induced hepatotoxicity causality and states the following ratios: excluded (≤0), unlikely (1–2), possible (3–5), probable (6–8), highly probable (>8) (Danan and Benichou, 1993).

Drug-induced liver injury severity classifications

The DILI severity classification is based on the International DILI Expert Working Group's Severity Index (Aithal et al., 2011): 1) mild, ALT ≥5 or ALP ≥2 and TBIL<2 times the upper limit of normal (ULN); 2) moderate, ALT ≥5 or ALP ≥2 and TBIL≥2 ULN, or symptomatic hepatitis; 3) severe, ALT ≥5 or ALP ≥2 and TBIL ≥2 ULN, or symptomatic hepatitis and one of the following criteria: INR ≥1.5 or ascites and/or encephalopathy, disease duration <26 weeks, absence of underlying cirrhosis, or other organ failure due to DILI; and 4) fatal/transplantation, death or liver transplantation due to DILI.

Pattern of liver injury

Three types of liver damage were defined: 1) hepatocellular (isolated ALT >5×ULN, or R > 5); 2) cholestatic (isolated ALP≥2×ULN, or R ≤ 2); and 3) mixed hepatocellular and cholestatic patterns (2 < R < 5). The R was defined as (measured ALT/ALT ULN)/(measured ALP/ALP ULN).[6]

Statistical analysis

Data were analyzed descriptively. Count data are expressed as numbers and percentages, and measurement data are expressed as the median (minimum, maximum).

Results

Basic information

A total of 1876 relevant studies were initially identified. After removing duplicate documents and screening the titles and abstracts, 29 studies were identified for a full-text assessment

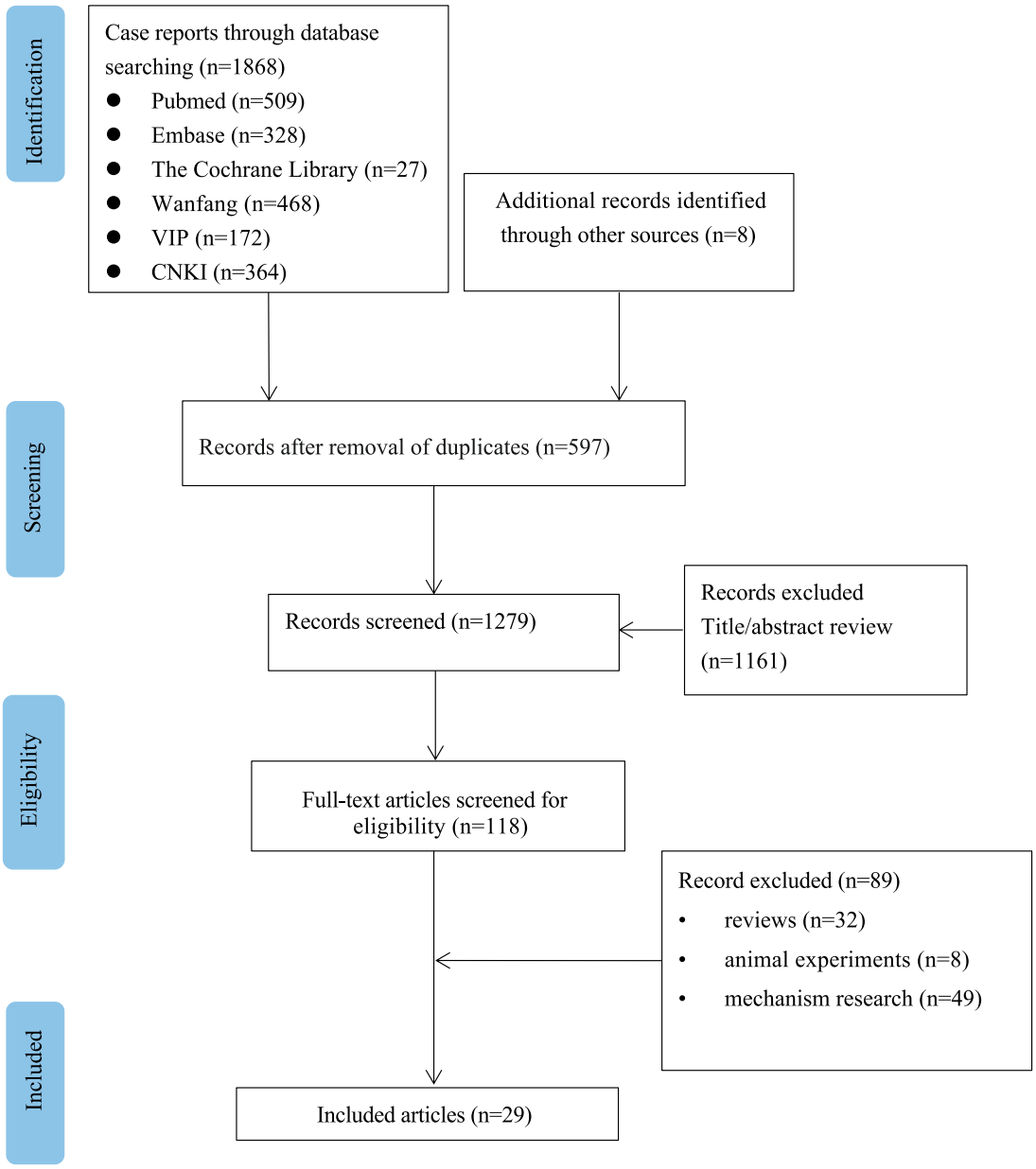

FIGURE 1
Flow chart of the study selection process for reported cases of metformin-induced hepatotoxicity.

(Figure 1). The clinical characteristics of the 29 studies are summarized in Table 1. (Babich et al., 1998; Swislocki and Noth, 1998; Parikh et al., 2000; Desilets et al., 2001; Mcnear et al., 2002; Nammour et al., 2003; Deutsch et al., 2004; Barquero Romero and Pérez Miranda, 2005; Kutoh, 2005; Aksay et al., 2007; Battula et al., 2007; de la Poza Gómez et al., 2008; Biyyani et al., 2009; Cone et al., 2010; Olivera-González et al., 2010; Zhu and Xu, 2010; Hashmi, 2011; Lee et al., 2011; Mallari et al., 2011; Alston et al., 2012; Miralles-Linares et al., 2012; Ren et al., 2012; Saadi et al., 2013; Mancano, 2014; Dayanand et al., 2015; Pinto et al., 2016; Zheng, 2016; Benito et al., 2019; Chen, 2020) The median age of the 30 patients (19 males and 11 females) was 61 years (range 29–83). In addition to being used to treat type 2 diabetes, metformin was also used for weight loss in one patient (3.3%). The median time to onset of liver injury was 4 weeks (range 0.3–648). The median daily dose of metformin at the onset of liver injury was 1 g (range 0.5–2.25). Medical history information was available for 20 patients (66.7%), four of whom had a history of liver disease. Twenty-two patients (73.3%) received an average of 3.5 drugs in addition to metformin, and one patient (3.3%) had a history of drinking.

TABLE 1 Clinical data of 30 patients with metformin-induced hepatotoxicity.

Reference	Sex/age	Daily dose (g)	Duration	Clinical presentation	ALT	AST	TBIL	DBIL	GGT	ALP	RUCAM score	Type of injury	Resolution
Zhu and Xu, (2010)	m/48	1.5	2d	fatigue	240	104	na	na	na	na	6	H	1 m
Chen, (2020)	m/82	0.5	2 m	fatigue, bloating, loss of appetite, AD, N	158	102	1.4	0.6	68	94	9	H	2 w
Ren et al. (2012)	f/29	1.5	1y	fever, yellow urine, clay-colored stool, AD, V	782	346	1.8	na	na	na	7	H	15 d
Battula et al. (2007)	m/63	1	4 w	fever, AP, N, V	169	38	4.2	na	na	437	7	C	na
Deutsch et al. (2004)	f/67	1	6 w	fatigue, bloating, J, W	905	1152	4.8	3.5	248	121	12	C	3 m
Swislocki and Noth, (1998)	m/75	1	8 w	felt well	413	322	normal	normal	na	684	7	H	4 w
Babich et al. (1998)	f/53	2	4 w	lower extremity edema, lethargy, fatigue, diarrhea, J	651	583	14.4	na	na	500	8	M	1 m
Desilets et al. (2001)	m/64	1	2 w	fatigue, anorexia, weight loss, J	289	214	21.3	na	na	994	10	C	3 m
Nammour et al. (2003)	m/68	1.7	4 w	weight loss, pruritus, J	109	36	15.7	10	809	383	9	C	8 w
Kutoh, (2005)	f/73	0.5	3 w	fatigue, anorexia, AP, N, V, J	772	689	6.5	na	na	635	10	M	7 w
Cone et al. (2010)	m/61	1	2 w	fatigue, weight loss, N	571	623	1.8	na	325	143	9	H	2 m
de la Poza Gómez et al. (2008)	m/83	1.7	11 w	hypoxia, weight loss, J, W	47	36	2.3	na	740	586	9	C	4 m
Olivera-González et al. (2010)	f/73	2.55	2 w	anorexia, dyspnea, polyuria, polydipsia, V, W	4506	8091	1.4	na	29	95	9	H	1 m
Barquero Romero and Pérez Miranda, (2005)	f/80	1.7	8 w	loss of appetite, yellow urine, pruritus, AP, J, W	596	1198	15	12.3	442	164	8	H	3 m
Biyyani et al. (2009)	m/61	1	4 w	AP, N, V, J	169	38	4.2	1.2	na	437	9	M	4 w
Miralles-Linares et al. (2012)	m/61	1.7	6 w	J	861	290	2.9	2.4	861	622	9	M	30 d
Hashmi, (2011)	f/44	1	1 m	na	738	na	na	na	42	normal	8	H	1 m
Saadi et al. (2013)	m/78	0.85	2 w	fatigue, diarrhea, anorexia, pruritus, AP, N, V, J	1050	496	22.2	15.2	1264	1001	9	M	2 m
Zheng, (2016)	f/70	1	4 w	AD, N	1093	1152	2.2	na	na	176	8	H	10 d
Mancano, (2014)	m/41	1.5	4 w	fatigue, dark urine, clay-colored stool, J	863	419	18.1	na	2181	479	8	H	2 m
Aksay et al. (2007)	m/52	0.85	2 w	fever, N, V, W	1469	1843	2.76	1.43	na	na	9	H	10 d
Alston et al. (2012)	f/59	NA	1 m	J, N, V	85	130	5.5	na	na	293	8	C	na
Pinto et al. (2016)	f/46	2	10 y	na	92	89	0.5	0.1	na	483	8	C	na
Dayanand et al. (2015)	m/56	NA	1 m	AD, J	4701	4422	20.7	17	na	192	7	H	na
Dayanand et al. (2015)	m/61	NA	2 m	pruritus, J	1269	916	25.6	20	na	916	5	M	na
Lee et al. (2011)	m/35	NA	3 w	malaise, desquamation, AP, N, V	NA	NA	NA	na	na	NA	6	M	na
Mallari et al. (2011)	m/48	1	2 w	fatigue, malaise, AP	3165	1833	3.3	na	na	208	8	H	3 w
Parikh et al. (2000)	m/54	2	2w	fatigue, anorexia, clay colored stool, N	4ULN	4ULN	4 ULN	na	na	5 ULN	8	C	6 w
Mcnear et al. (2002)	m/55	1	few weeks	J	NA	NA	15.8	7.7	na	na	7	C	4 m
Benito et al. (2019)	f/65	NA	6 w	dark urine, AP	1414	NA	NA	na	na	na	9	H	na

Abbreviations: F, female; M, male; AD, abdominal discomfort; AP, abdominal pain; N, nausea; V, vomiting; J, jaundice; W, weakness; ALT, alanine transaminase; AST, aspartate transaminase; GGT, γ-glutamyl transferase; TBIL, total bilirubin; DBIL, direct bilirubin; ALP, alkaline phosphatase; ULN, upper limit of normal; H, Hepatocellular; C, Cholestatic; M, mixed hepatocellular and cholestatic pattern; RUCAM, roussel Uclaf Causality Assessment Method; na, not available.

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TABLE 2 Basic data of 30 patients with metformin-induced hepatotoxicity.

Parameters		Value
Sex	Male	19 (63.3%)
	Female	11 (36.7%)
Age	Year	61 (29, 83)
Country	United States	17 (56.7%)
	Spain	5 (16.7%)
	China	3 (10.0%)
	Turkey, Greece, Japanese, Israel, Saudi Arabia	1 (3.3%)
Daily dose (25)[a]	g	1 (0.5,2.25)[b]
Onset time	week	4 (0.3, 648)[b]
Indication	T2DM	29 (96.7%)
	weight loss	1 (3.3%)
HbA 1c (7)[a]	%	7.6 (7.3,11.8%)[b]
Duration	Year	0.14 (0.04, 30)[b]
Medical history (20)[a]	liver disease	4 (13.3%)
	hypertension	13 (43.3%)
	coronary heart disease	5 (16.7%)
	hyperlipidemia	5 (16.7%)
	osteoarthropathy	4 (13.3%)
	obesity	4 (13.3%)
	atrial fibrillation	2 (6.7%)
	depression	2 (6.7%)
	hypothyroidism, COPD, Crohn's disease	1 (3.3%)
Combination therapy (22)[a]	average number of drugs	3.5
	aspirin	9 (30.0%)
	CCB	8 (26.7%)
	sulfonylureas	8 (26.7%)
	ACEI/ARB	6 (20.0%)
	statins	5 (16.7%)
	β receptor blocker	5 (16.7%)
	diuretics	5 (16.7%)
	pioglitazone	2 (6.7%)
	nateglinide, trazodone, tramadol, clomezepam hydroxychloroquine, gemfibrozil, risperidone, escitalopram, lithium carbonate, omeprazole	1 (3.3%)

Abbreviations: CCB, calcium channel blocker; ACEI/ARB, angiotensin-converting enzyme inhibitor and angiotensin receptor blocker; COPD, chronic obstructive pulmonary disease; T2DM, type 2 diabetes mellitus.
[a]Represents the number of patients out of 30 for whom information regarding this particular parameter was provided.
[b]Median (minimum, maximum).

Clinical manifestations

The clinical presentation of the patients is summarized in Table 2. Twenty-eight patients (93.3%) developed symptoms at the onset of liver injury. The most common symptoms were jaundice (15 cases, 50.0%), fatigue (11 cases, 36.7%), nausea (11 cases, 36.7%), vomiting (9 cases, 30.0%), abdominal pain (8 cases, 26.7%), and anorexia (7 cases, 23.3%). Other symptoms included pruritus (4 cases, 13.3%), dark urine (4 cases, 13.3%), clay-colored stools (3 cases, 10.0%), abdominal discomfort (3 cases, 10.0%) and fever (3 cases, 10.0%). Lactic acidosis occurred in two patients (6.7%).

Laboratory tests

Laboratory test results are summarized in Table 2. The median serum ALT level was 694.5 U/L (range 47–4701), and the median serum AST level was 382.5 U/L (range 36–8091). The

TABLE 3 Clinical manifestations and laboratory tests of 30 patients with metformin-induced hepatotoxicity.

Parameters		Value
clinical manifestations (28)[a]	jaundice	15 (50.0%)
	fatigue	11 (36.7%)
	nausea	11 (36.7%)
	vomiting	9 (30.0%)
	abdominal pain	8 (26.7%)
	anorexia	7 (23.3%)
	weakness	5 (16.7%)
	weight loss	4 (13.3%)
	pruritus	4 (13.3%)
	dark urine	4 (13.3%)
	clay colored stool	3 (10.0%)
	abdominal discomfort	3 (10.0%)
	fever	3 (10.0%)
	bloating	2 (6.7%)
	malaise	2 (6.7%)
	diarrhea	2 (6.7%)
	lactic acidosis	2 (6.7%)
	desquamation, dyspnea, polyuria, polydipsia, lower extremity edema, lethargy, hypoxia	1 (3.3%)
ALT (28)[a]	U/L	694.5 (47, 4701)[b]
AST (26)[a]	U/L	382.5 (36, 8091)[b]
TBIL (27)[a]	mg/dl	4.5 (0.5, 25.6)[b]
DBIL (14)[a]	mg/dl	3.5 (0.1, 20)[b]
ALP (24)[a]	U/L	437 (94, 1001)[b]
GGT (11)[a]	U/L	442 (29, 1264)[b]
ALB (7)[a]	g/L	39 (30, 44)[b]
Imaging examination (26)[a]	ultrasound	
	hepatosteatosis	6 (23.1%)
	thickening of the gallbladder wall	3 (11.5%)
	gallstones	1 (3.8%)
	CT	
	pancreatitis	1 (3.8%)
	hepatomegaly	1 (3.8%)
	normal	15 (57.7%)
Liver biopsy (13)[a]	portal inflammation	8 (61.5%)
	cholestatic hepatitis	5 (38.5%)
	parenchymal inflammation	5 (38.5%)
	bile duct inflammation with epithelial destruction and compensatory bile duct proliferation	3 (23.1%)
	fibrosis	2 (15.4%)
	chronic hepatitis, lymphocytic vasculitis, steatosis, severe hepatitis, pericentral necrosis	1 (7.7%)

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; GGT, γ-glutamyl transferase; ALB, albumin; CT, computed tomography; NA, not available.

[a]Represents the number of patients out of 30 for whom information regarding this particular parameter was provided.

[b]Median (minimum, maximum).

median TBIL level in 27 patients was 4.5 mg/dl (range 0.5–25.6), and the median DBIL level in 14 patients was 3.5 mg/dl (range 0.1–20). The median GGT level in 11 patients was 442 U/L (29–1264), and the median ALP level in 24 patients was 437 U/L (range 94–1001).

Imaging examination

Liver imaging examination results are summarized in Table 3. Abdominal imaging was performed in 26 patients. Imaging findings were normal in 15 patients. Ultrasound in

TABLE 4 Treatment and prognosis of 30 cases of metformin-induced hepatotoxicity.

Parameters		Value
Treatment	Discounted	30 (100.0%)
	Rechanllenge	4 (13.3%)
	liver protection treatment	1 (3.3%)
	Cholecystectomy	2 (6.7%)
	Hemodialysis	1 (3.3%)
Prognosis	recover	30 (100.0%)
Resolution (24)[a]	week	6 (2, 16)[b]
RUCAM score	probable	16 (53.3%)
	highly probable	13 (43.3%)
	possible	1 (3.3%)
Pattern of liver injury	hepatocellular	14 (46.7%)
	cholestatic	9 (30.0%)
	mixed hepatocellular and cholestatic pattern	7 (23.3%)
Severity classifications	mild	5 (16.7%)
	moderate	18 (60.0%)
	severe	3 (10.0%)
	na	4 (13.3%)

Abbreviations: RUCAM, roussel Uclaf Causality Assessment Method; na, not available.
[a]Represents the number of patients out of 30 for whom information regarding this particular parameter was provided.
[b]Median (minimum, maximum).

10 patients showed hepatosteatosis (6 cases, 23.1%), gallbladder wall thickening (3 cases, 11.5%), and gallstones (1 case, 3.8%). Computed tomography (CT) in two patients showed pancreatitis (1 case, 3.8%) and hepatomegaly (1 case, 3.8%).

Liver biopsy

Liver biopsy results are summarized in Table 3. Liver biopsies were performed in 13 patients, mainly showing portal inflammation (8 cases, 61.5%), cholestatic hepatitis (5 cases, 38.5%), parenchymal inflammation (5 cases, 38.5%) and bile duct inflammation with epithelial destruction and compensatory bile duct proliferation (3 cases, 23.1%).

Treatments and outcomes

Metformin was discontinued immediately in all 30 patients (100%), one patient (3.3%) received the compound glycyrrhizin and a traditional Chinese medicine injection, two patients (6.7%) underwent cholecystectomy, and one patient (3.3%) underwent hemodialysis (Table 4). Liver function returned to normal levels in 30 patients (100%) at a median time of 6 weeks (range 2–16). Two patients (6.7%) had persistently high levels of ALP. Four patients (13.3%) were rechallenged with metformin, two of whom had relapsed hepatotoxicity.

Causality assessment and pattern of liver injury

According to the CIOMS/RUCAM score, 16 patients (53.3%) had probable hepatotoxicity related to metformin, 13 (43.3%) had highly probable hepatotoxicity, and one patient (3.3%) had possible hepatotoxicity. Of the 30 patients, 14 (46.7%) presented with hepatocellular, 9 (30.0%) presented with cholestatic, and 7 (23.3%) presented with mixed hepatocellular and cholestatic patterns.

Discussion

The hypoglycemic effect of metformin is mainly mediated by reducing hepatic glucose production by inhibiting gluconeogenesis and increasing glucose uptake in skeletal muscle and adipocytes. The maximum approved total daily dose of metformin for the treatment of diabetes is 2.5 g (Natali and Ferrannini, 2006). The absolute oral bioavailability of metformin is 40%–60%, and it is rapidly distributed after absorption in the small intestine and is not bound to plasma proteins. (Scheen, 1996) Hepatic uptake of metformin is mainly mediated by OCT1 (SLC22A1), and OCT3 (SLC22A3) on the hepatocyte membrane (Shu et al., 2008; Chen et al., 2015). Metformin is not metabolized by the liver and has a half-life of approximately 5 h, and 80% of the dose is excreted in the urine via the kidneys (Shu et al., 2008; Chen et al., 2015). This

elimination is prolonged in patients with renal insufficiency. Epidemiological studies have shown ethnic and geographical differences in the metformin response. (Williams et al., 2014). For example, African Americans seem to have a better glycemic response to metformin than European Americans (Williams et al., 2014). Genetic and environmental factors influence individual differences in metformin adverse effects and treatment responses (Florez, 2017). Metformin-induced hepatotoxicity was seen in 20% of Asians in our analysis, with the remainder being more common in North America. More prospective studies are needed to confirm whether there are ethnic differences in metformin-induced hepatotoxicity.

The exact incidence of metformin-induced hepatotoxicity is not known, but the medication label states that liver injury is very rare (<0.01%). The type of metformin-induced hepatotoxicity is not specific and can result in hepatocellular, cholestasis, or mixed hepatocellular liver injury. The latency of metformin-induced hepatotoxicity varied widely, from 10 days to 10 years after administration. There may be differences in the susceptibility of patients of different ethnic groups to drug-induced liver injury (DILI), while metformin-induced hepatotoxicity has no obvious regionality. Advanced age may be an important susceptibility factor for DIL1 (Shu et al., 2008; Chen et al., 2015). Metformin-induced hepatotoxicity occurs mainly in diabetic patients over 50 years of age. Therefore, it is important for these patients to undergo frequent monitoring of changes in liver function during metformin use. Although sex does not appear to be a risk factor for DILI in general, it has been noted that women may show a higher susceptibility to certain drugs, such as minocycline and methyldopa (deLemos et al., 2014). In contrast, metformin-induced hepatotoxicity seems to be more common among male patients. Diabetes mellitus does not seem to increase the risk for DILI in general. It is unclear whether nonalcoholic fatty liver disease (NAFLD) and obesity increase the risk of DILI. In an alcoholic patient, liver function returned to normal after discontinuation of metformin, which ruled out an effect of alcohol on the liver (Shu et al., 2008; Chen et al., 2015).

Patients with T2DM, especially older adults, often use multiple medications due to comorbidities and complications (Zaman Huri and Fun Wee, 2013). It was brought to our attention that patients received an average of 3.5 medications other than metformin. Accompanying treatment drugs such as sulfonylureas, gemfibrozil, and statins have been reported to cause hepatotoxicity (May et al., 2002; Domínguez Tordera et al., 2011). These concomitant drugs were used before the addition of metformin, which could rule out the possibility of hepatotoxicity based on time correlation and recovery of liver function after metformin discontinuation. Nevertheless, it cannot be excluded that multidrug combination therapy contributes to metformin-induced hepatotoxicity. Data from the Swedish Prescription Drug Registry including more than 600,000 elderly (≥75 years old) patients revealed that the number of drugs was significantly associated with the occurrence of drug–drug interactions (DDIs) (Johnell and Klarin, 2007). Metformin often interacts with a variety of drugs that may affect plasma concentrations of metformin. However, the effect of elevated plasma concentrations of metformin on liver injury is unclear.

Most of the patients with metformin-induced hepatotoxicity appeared acutely, and only serum ALT, AST, ALP, GGT, and other liver biochemical indices increased to varying degrees. Some patients may experience jaundice, fatigue, and gastrointestinal symptoms such as abdominal pain, nausea, vomiting, loss of appetite, and epigastric discomfort. Those with obvious jaundice may have yellow skin and sclera, dark urine, pale stool and pruritus. Liver biopsy demonstrated a mixed inflammatory infiltrate of the portal vein, characterized by lymphocytes, neutrophils, and numerous eosinophils. In contrast, acute inflammatory cells infiltrate the bile ducts with epithelial destruction and compensatory bile duct proliferation.

The pathophysiological mechanism of metformin-induced hepatotoxicity remains unclear. Metformin is not hepatically metabolized and is generally not considered to be toxic to the liver. Possible mechanisms of injury are direct, idiosyncratic, or a drug–drug interaction leading to acute liver injury in this susceptible individual. Some patients with fever and liver biopsy showed eosinophilic infiltration, supporting this point of view. Due to the direct blood supply from the portal vein, the concentrations of metformin in the liver may be much higher than those in the systemic circulation and other organs. Although metformin is concentrated in the liver, there is no evidence of dose-dependent hepatotoxicity (Wilcock and Bailey, 1994). However, the effect of elevated plasma concentrations of metformin on liver injury is unclear. The relationship between metformin-induced hepatotoxicity and gene polymorphisms still needs further research.

Timely discontinuation of suspected liver injury drugs is the most important treatment measure for DILI, and rechallenging suspected or similar drugs should be avoided as much as possible. Appropriate drug therapy is selected according to the clinical type of DILI (May et al., 2002; Domínguez Tordera et al., 2011). However, most patients with DILI will spontaneously recover without any treatment or specific measures after discontinuation of the suspected drug. A small number of patients develop chronic liver disease, and very few develop acute liver failure or even die (May et al., 2002; Domínguez Tordera et al., 2011). In our study, all patients had normal liver function within 4 months after discontinuation of metformin without any intervention. Persistently high levels of ALP in two patients were thought to be associated with long-term cholestatic effects (Nammour et al., 2003; Biyyani et al., 2009). Cholecystectomy may be required for metformin-induced cholangiohepatitis (Battula et al., 2007; Biyyani et al., 2009). One patient with acute kidney injury and lactic acidosis secondary to acute liver failure underwent hemodialysis (Battula et al., 2007; Biyyani et al., 2009). The effects of readministration of metformin remains uncertain, as some patients do not experience recurrent hepatotoxicity after

metformin rechallenge (Swislocki and Noth, 1998; Zhu and Xu, 2010).

Conclusion

Metformin-induced liver injury is rare and easily overlooked due to its insidious onset. Given the increasing prevalence of T2DM and the widespread use of metformin, clinicians should be alert to metformin-induced hepatotoxicity, a rare but potentially serious adverse effect. It should be reminded that when the patients have symptoms such as jaundice, fatigue, anorexia, pruritus, and dark urine during the medication, they should seek medical attention in time for necessary examinations, especially about 1 month after starting the medication.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author

Ethics statement

This study did not require an ethical board approval because the study was a retrospective study and did not involve sensitive personal information.

Author contributions

CW and YL conceived of the presented idea. CW, HD, YX, and YL wrote the manuscript. All authors discussed the results and contributed to the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Analysis of clinical characteristics of mesalazine-induced cardiotoxicity

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Background: Mesalazine is the first-line inflammatory bowel disease (IBD) treatment. However, it can cause fatal cardiotoxicity. We aimed to analyze the clinical characteristics of mesalazine-induced cardiotoxicity and provide evidence for clinical diagnosis, treatment, and prevention.

Methods: We collected Chinese and English literature on mesalazine-induced cardiotoxicity from 1970 to 2021 for retrospective analysis.

Results: A total of 52 patients (40 males and 12 females) were included, with a median age of 24.5 years (range 9–62) and a median onset time of 14 days (range 2–2880). Cardiotoxicity manifested as myocarditis, pericarditis, and cardiac pericarditis. The main clinical manifestations are chest pain (82.7%), fever (46.2%), and respiratory symptoms such as dyspnea and cough (40.4%). The levels of troponin T, creatine kinase, C-reactive protein, leukocyte count, erythrocyte sedimentation rate, and other biochemical markers were significantly increased. Cardiac imaging often suggests myocardial infarction, pericardial effusion, myocardial necrosis, and other symptoms of cardiac injury. It is essential to discontinue mesalamine immediately in patients with cardiotoxicity. Although corticosteroids are a standard treatment option, the benefits remain to be determined. Re-challenge of mesalamine should be carefully considered as cardiotoxic symptoms may reoccur.

Conclusion: Mesalazine may cause cardiotoxicity in patients with inflammatory bowel disease, which should be comprehensively diagnosed based on clinical manifestations, biochemical indicators, and cardiac function imaging examinations. Mesalazine should be immediately discontinued, and corticosteroids may be an effective treatment for cardiotoxicity.

KEYWORDS

mesalazine, cardiotoxicity, inflammatory bowel disease, myocarditis, pericarditis

1 Introduction

Inflammatory bowel disease (IBD) is characterized by chronic recurrent gastrointestinal inflammation, including ulcerative colitis and Crohn's disease. Ulcerative colitis symptoms include diarrhea, proctorrhagia, tenesmus, urgency, and fecal incontinence, depending on the extent and severity of the disease (Magro et al., 2017). The symptoms of Crohn's disease vary but typically include abdominalgia, weight loss, and chronic diarrhea (Gomollon et al., 2017). 5-aminosalicylate (5-ASA) is the first-line recommended drug for IBD treatment. Other therapeutic drugs include corticosteroids, immunosuppressants, and tumor necrosis factor (TNF) therapies (Bressler et al., 2015).

5-ASA, also termed mesalazine, is often associated with fever, diarrhea, abdominalgia, and hematochezia (Matsumoto and Mashima, 2020). However, cardiotoxicity has been reported as a rare but potentially fatal adverse reaction (Kristensen et al., 1990). At present, mesalazine-related cardiotoxicity is reported primarily as case reports. Its incidence, clinical features, treatment, and prognosis are still unclear. This study aimed to summarize and analyze the clinical characteristics of mesalazine-associated cardiotoxicity. Data were synthesized based on published studies to provide a reference for the rational use of mesalazine in practice.

2 Methods

2.1 Search strategy

The following databases were searched: China National Knowledge Infrastructure (CNKI), Wanfang Data, Chinese VIP databases, Web of Knowledge, PubMed, Elsevier, and Embase. The search keywords were "salazosulfapyridine" OR "mesalazine" OR "mesalamine" OR "balsalazide" OR "olsalazine" AND "myocarditis" OR "pericarditis" OR "carditis." The publication languages were restricted to Chinese and English, and the publication period was from 1 January 1970 to 31 December 2021.

2.2 Inclusion and exclusion criteria

Inclusion criteria were case reports and analyses published as full text in peer-reviewed journals. Exclusion criteria were reviews, animal studies, mechanism studies, preclinical studies, duplicate reports, and articles with insufficient data.

2.3 Data extraction

Two investigators independently selected the articles based on inclusion and exclusion criteria, followed by a panel discussion. The following data were extracted using a self-designed data extraction table: country, sex, age, primary disease, concomitant medication, mesalazine use and dosage, administration route, onset time, clinical manifestations, laboratory examination, imaging examination, treatment, and prognosis.

2.4 Literature quality evaluation

The quality of the 51 studies included was evaluated using the case series evaluation scale recommended by the National Institute for Clinical Excellence (NICE). The assessment consists of whether: 1) the cases originated from multiple treatment centers; 2) the research objectives were clearly described; 3) the inclusion and exclusion criteria were clear; 4) the definitions of the reported outcomes were clear; 5) prospective studies were performed; 6) patients were recruited continuously; 7) the main findings were clearly described; 8) the results were stratified analyses. A "yes" or "no" decision was assigned to each item, with 1 or 0 points. The scores were then aggregated.

3 Results

3.1 Basic information

After retrieval and screening, 51 studies involving 52 patients (40 men and 12 women) were included, all published in English. The specific methodology for article selection is illustrated in Figure 1. The quality of the 51 articles was evaluated, 50 were rated 3 points, and one rated 2 points. The median age of these patients was 24.5 years (range 9–62), and the median onset time of cardiotoxicity was 14 days (range 2–2880) (Table 1). Thirty-six patients reported the use and dosage of mesalamine in the literature, out of which 6 cases (16.7%) received <2 g/d, 3 (8.3%) received >4 g/d, and the remaining 27 patients (75.0%) received 2–4 g/d. All 36 patients (100.0%) received oral administration, and 5 (13.9%) received rectal administration. Thirty-five cases (67.3%) were indicated for ulcerative colitis (UC), 15 (28.8%) for Crohn's disease (CD), and 2 (3.9%) for IBD. The underlying diseases of 5 cases (31.2%) were infectious diseases, 5 (31.2%) had blood system disease, 3 (18.8%) had cardiovascular disease, and 3 (18.8%) had skin disease. Twenty-eight cases (53.8%) received concomitant corticosteroids, 6 (11.5%) received antibacterials, and 9 (17.3%) received other drugs for UC, such as azathioprine, sulfasalazine, and others. The other combined medications are shown in Table 1.

3.2 Clinical manifestations

The clinical manifestations of the 52 patients are shown in Table 2. Forty-three (82.7%) patients had (82.7%) chest pain, 24 (46.2%) had fever, and 21 (40.4%) had respiratory symptoms.

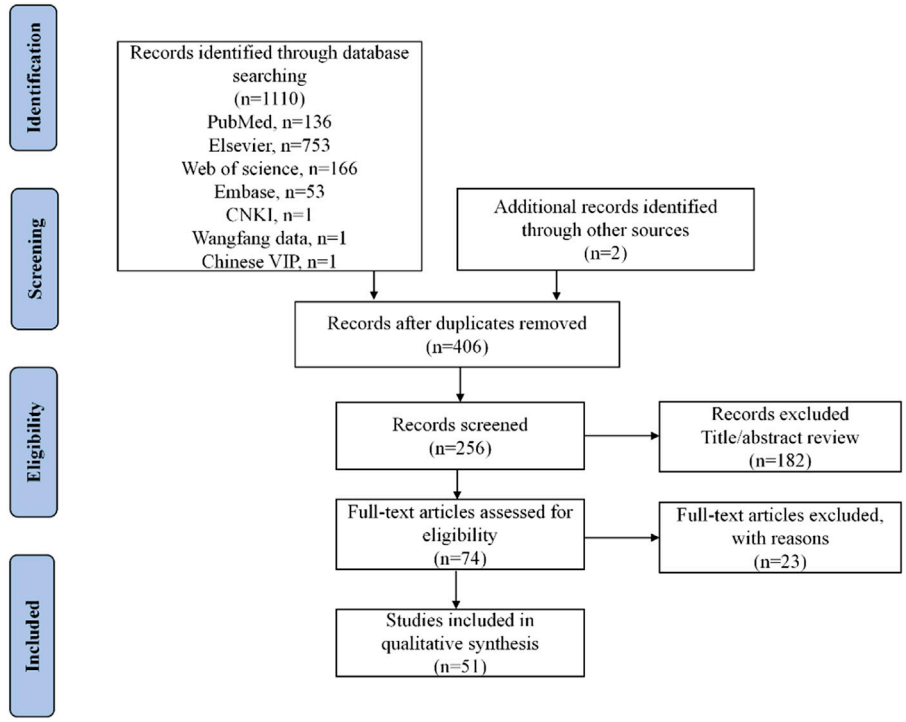

FIGURE 1
Flow diagram of the selection of studies for inclusion.

Fourteen (26.9%) patients had autonomic symptoms such as tachycardia and weakness. Thirteen (25.0%) patients had digestive symptoms such as hemafecia and belly ache. There were 21 cases (40.4%) of myocarditis, 17 cases (32.7%) of pericarditis, and 14 cases (26.9%) of myopericarditis. There were 9 cases (17.3%) with neurological symptoms, such as headache, lethargy, and syncope. Cardiovascular symptoms occurred in 6 (11.5%) patients. Eight cases (15.4%) had other clinical manifestations such as myalgia, arthralgia, and weight loss. Pericardial effusion was detected in 19 cases (36.5%).

3.3 Laboratory examination

Cardiac Troponin T (cTnT) was detected in 37 patients, including elevated levels in 33 cases (89.2%) and normal levels in 4 cases (10.8%). The median cTnT value was 1.8 μg/L (range 0.1–165.0). The serum creatine kinase (CK) assay was performed on 24 patients. Eight (33.3%) patients had normal values, and 16 (66.7%) had elevated CK levels. The median CK level was 441 U/L (range 3–16000). The C-reactive protein (CRP) test was normal in 2 cases (6.9%), and elevated in 27 cases (93.1%), totaling 29 cases. The median CRP was 97.1 mg/L (range 12.0–2580.0). Of 27 patients with leukocyte counts, 22 (81.5%) were elevated, and 5 (18.5%) were normal. The median leukocyte count was 15000/μL (range 7820–26200). The erythrocyte sedimentation rate (ESR) was determined in 20 patients. Of these, 19 cases (85.0%) were elevated, and 1 (5.0%) was normal. The median ESR was 66.5 mm/h (16.0–121.0).

3.4 Image examination

The summary of the imaging examination results is shown in Table 3. Twenty-three (44.2%) cases had ST-elevation on electrocardiogram (ECG), 9 (17.3%) had T wave inversion, 7 (13.5%) had sinus tachycardia, 5 (9.6%) had nonspecific ST-T wave change, 3 (5.8%) had PR interval decrease, 3 (5.8%) had biphasic T wave, 3 (5.8%) had normal ECG, 2 (3.8%) had T-wave flatness, 1 (1.9%) had an atrioventricular block, 1 (1.9%) had an increase in the Q wave, and 1 (1.9%) had trifascicular block. Eleven (91.7%) patients showed normal coronary angiography (CA), and 1 (8.3%) had atherosclerosis. Cardiac magnetic resonance imaging (CMRI) was performed in 28 cases: 12 (42.8%) had myocardial necrosis, 7 (25.0%) had myocardial edema, 2 (7.1%) had minimal pericardial effusion, 2 (7.1%) had myocardial fibrosis, 1 (3.6%) had interatrial septal hypertrophy, 1 (3.6%) had a benign pericardial cyst, 1 (3.6%) had diffuse hyperkinesia, and 1 (3.6%) had anterior septal hypertrophy. One (3.6%) patient had normal CMRI. Fifty

TABLE 1 General data of 52 patients reported in case series/reports.

Parameter		Value
Age (52)[a]	Years	24.5 (9,62)[b]
Sex (52)[a]	Male	40 (76.9%)
	Female	12 (23.1%)
Region (52)[a]	Europe (United Kingdom, Germany, Portugal, Denmark, Spain, Italy, France)	26 (50.0%)
	Americas (America, Canada)	19 (36.5%)
	Asia (Japan, China, Turkey, Israel)	7 (13.5%)
Onset time (52)[a]	days	14 (22,880)[b]
Use and dosage (36)[a]	Daily dose	
	<2 g	6 (16.7%)
	2g~4 g	27 (75.0%)
	>4 g	3 (8.3%)
	Usage	
	Oral	36 (100.0%)
	Rectal	5 (13.9%)
Indication (52)[a]	UC	35 (67.3%)
	CD	15 (28.8%)
	IBD	2 (3.9%)
Diseases (16)[a]	Infectious diseases: pancreatitis, arthritis, otitis media	5 (31.2%)
	Blood system diseases: anemia, idiopathic thrombocytopenic purpura, thrombophlebitis	5 (31.2%)
	Cardiovascular diseases: hypertension, non-ischemic stress cardiomyopathy	3 (18.8%)
	Skin: psoriasis, chickenpox	3 (18.8%)
Concomitant medications (52)[a]	Steroids: prednisone, budesonide, prednisolone, methylprednisolone, beclomethasone, hydrocortisone	28 (53.8%)
	Antibacterials: amoxicillin, ceftriaxone, clavulanate potassium, cefazolin, levofloxacin, ciprofloxacin, metronidazole, fluconazole	6 (11.5%)
	ACEIs: captopril, benazepril	2 (3.8%)
	NSAIDs: indomethacin, aspirin	2 (3.8%)
	CCB: nifedipine	1 (1.9%)
	Thyroid hormones: levothyroxine	1 (1.9%)
	Hypoglycemic agent: metformin	1 (1.9%)
	Antidepressant: escitalopram	1 (1.9%)
	Antiepileptic drug: clonazepam	1 (1.9%)
	Anticoagulant: low-molecular-weight heparin	1 (1.9%)
	Other drugs for UC: azathioprine, sulfasalazine, infliximab, immunoglobulin, balsalazide	9 (17.3%)

[a]Represents the number of patients out of 52 in whom information regarding this particular parameter was provided.
[b]Median (minimum-maximum).
UC, ulcerative colitis; CD, Crohn's disease; IBD, inflammatory bowel disease; ACEIs, angiotensin-converting enzyme inhibitors; CCB, calcium channel blockers; 5-ASA, 5-aminosalicylic acid; NSAIDs, Non-steroidal anti-inflammatory drugs.

patients had ultrasound cardiogram (UCG) performed: 15 (30.0%) had pericardial effusion, 12 (24.0%) had ventricular dysfunction, 7 (14.0%) had decreased ejection fraction, 7 (14.0%) had normal UCG, 6 (12.0%) had abnormal wall motion, and 3 (6.0%) had thickening of the ventricular wall.

3.5 Treatment and prognosis

The treatment and prognosis of the 52 patients are shown in Table 4. A total of 48 patients (92.3%) eventually discontinued mesalazine, and 4 (7.7%) continued to receive mesalazine. After discontinuation of mesalazine, 25 (48.1%) patients received corticosteroids, 5 (9.6%) received azathioprine (AZA), 1 (1.9%) received balsalazide, 1 (1.9%) received infliximab (IFX), and 1 (1.9%) received cyclosporine (CsA) to treat IBD. Cardiotoxicity treatment included the use of non-steroidal anti-inflammatory drugs (NSAIDs) in 18 cases (34.6%), antibiotics in 12 cases (23.1%), surgery in 6 cases (11.5%), hypotensives in 6 cases (11.5%), analgesics in 4 cases (7.7%), vasoactive drugs in 1 case (1.9%), cardiac stimulants in 1 case (1.9%), and antianginal agents in 1 case (1.9%). Fourteen cases (33.3%) had clinical symptoms that disappeared immediately after treatment, 22 cases (52.4%) had symptoms that disappeared

TABLE 2 Clinical information of 52 included patients.

Parameter		Value
Disease type (52)[a]	Myocarditis	21 (40.4%)
	Pericarditis	17 (32.7%)
	Myopericarditis	14 (26.9%)
Clinical manifestations (52)[a]	Chest pain	43 (82.7%)
	Fever	24 (46.2%)
	Respiratory system: dyspnea, cough, flu-like symptoms, throat ache	21 (40.4%)
	Autonomic system: tachycardia, weakness, paleness, chills, sweating, fatigue	14 (26.9%)
	Digestive system: hemafecia, belly ache, nausea, vomiting, dysphagia	13 (25.0%)
	Neurological system: headache, lethargy, syncope, facial numbness	9 (17.3%)
	Cardiovascular system: hypotension, angina pectoris, heart failure, elevated blood pressure, palpitations	6 (11.5%)
	Skin: diffuse maculopapular rash, skin rash	2 (3.8%)
	Other: myalgia, arthralgia, weight loss	8 (15.4%)
Pericardial effusion (52)[a]	YES	19 (36.5%)
	NO	33 (63.5%)
Laboratory examination		
Troponin T (37)[a]	μ g/L	1.8 (0.1,165.0)[b]
	Elevated	33 (89.2%)
	Normal	4 (10.8%)
CK (24)[a]	U/L	441 (3,16000)[b]
	Elevated	16 (66.7%)
	Normal	8 (33.3%)
CRP (29)[a]	mg/L	97.1 (12.0,2580.0)[b]
	Elevated	27 (93.1%)
	Normal	2 (6.9%)
Leukocyte count (27)[a]	μ L	15,000 (7820,26,200)[b]
	Elevated	22 (81.5%)
	Normal	5 (18.5%)
ESR (20)[a]	mm/h	66.5 (16.0, 121.0)[b]
	Elevated	19 (85.0%)
	Normal	1 (5.0%)

[a]Represents the number of patients out of 52 in whom information regarding this particular parameter was provided.
[b]Median (minimum-maximum).
CK, creatine kinase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

within a week, and 6 (14.3%) had symptoms that disappeared after more than a week. Except for one case (1.9%) whose outcome was death, all 51 cases (98.1%) recovered after treatment. Cardiotoxicity symptoms occurred in 11 cases after rechallenging with mesalazine.

4 Discussion

The FDA first approved mesalazine in 1992 to treat IBD (Lahiff et al., 2011). Its dosage forms include tablets, suppositories, capsules, and granules. The recommended dose for oral administration of mesalazine is 2 g/day, and the recommended dose for rectal administration is 3 g/week in divided doses (Harbord et al., 2017). Mesalazine inhibits prostaglandin formation by inhibiting cyclooxygenase (COX). It reduces signaling through the peroxisome proliferator-activated receptor gamma (PPAR-γ) pathway, decreasing nuclear factor κB activity and colon inflammation (Kim et al., 2006). Most of the cases in this study took the recommended dose, and only three patients received a higher amount. Therefore, cardiotoxicity due to mesalazine may not be correlated with the dose.

The onset time of cardiotoxicity for most patients was 2–4 weeks after taking mesalazine, suggesting that we need to pay attention to patients who develop fever, chest pain, and breathing difficulties, especially in the early stages (Radhakrishnan et al., 2018; Shergill, 2021). However, in some patients, the onset of cardiotoxicity was delayed for several months to several years (Okoro et al., 2018; Caio et al., 2021). It is important to note that the onset time does not shorten after increasing the dose of mesalazine, which may indicate

TABLE 3 Imaging examination of 52 patients reported in case series/reports.

Parameter		Value
Electrocardiogram (52)[a]	ST elevation	23 (44.2%)
	T wave inversions	9 (17.3%)
	Sinus tachycardia	7 (13.5%)
	Non-specific ST-T wave changes	5 (9.6%)
	PR interval decrease	3 (5.8%)
	Biphasic T waves	3 (5.8%)
	Normal	3 (5.8%)
	Flattened T-waves	2 (3.8%)
	Atrioventricular block	1 (1.9%)
	Q-waves increase	1 (1.9%)
	Trifascicular block	1 (1.9%)
Coronary angiography (12)[a]	Normal	11 (91.7%)
	Atherosclerosis	1 (8.3%)
Cardiac magnetic resonance imaging (28)[a]	Myocardial necrosis	12 (42.8%)
	Myocardial edema	7 (25.0%)
	Minimal pericardial effusion	2 (7.1%)
	Myocardial fibrosis	2 (7.1%)
	Interatrial septal hypertrophy	1 (3.6%)
	Benign pericardial cyst	1 (3.6%)
	Diffuse hypokinesis	1 (3.6%)
	Anterior and septal hypertrophy	1 (3.6%)
	Normal	1 (3.6%)
Ultrasound cardiogram (50)[a]	Pericardial effusion	15 (30.0%)
	Ventricular dysfunction	12 (24.0%)
	Decreased ejection fraction	7 (14.0%)
	Normal	7 (14.0%)
	Abnormal wall motion	6 (12.0%)
	Ventricular wall thickening	3 (6.0%)

[a]Represents the number of patients out of 52 in whom information regarding this particular parameter was provided.

that the onset time is not correlated with the dose (Kaiser et al., 1997; Perez-Colon et al., 2011). Almost 80% of patients with heart injury were men, suggesting that sex may be an independent risk factor for mesalazine-caused myocarditis. Most patients did not have cardiovascular diseases such as hypertension and nonischemic cardiomyopathy. Therefore, the cardiovascular disease may not be an independent risk factor for mesalazine cardiotoxicity. Ciprofloxacin, levofloxacin, and infliximab have been reported to be cardiotoxic (Lezcano-Gort et al., 2015; Dipasquale et al., 2018; Meng et al., 2019). Concomitant use of other cardiotoxic agents may be a risk factor for myocarditis caused by mesalazine.

Identifying the primary cause of mesalazine-induced cardiotoxicity is challenging because cardiotoxicity is a rare manifestation of IBD (Sorensen and Fonager, 1997; Oh et al., 2012). Cardiotoxicity from IBD typically manifests itself as pericarditis, myocarditis, myocardial infarction, and heart failure (Ibrahim et al., 2019; Rogler et al., 2021). Cardiac adverse reactions in patients with IBD treated with mesalazine are rare. The specific mechanisms by which mesalazine causes heart damage are unclear. Several possible mechanisms have been proposed. Mesalazine inhibits COX1 and accelerates the arachidonic acid metabolism into lipoxygenase products. Excess lipooxygenase products induce pro-inflammatory signaling, causing allergic myocarditis by releasing eosinophil-stimulating cytokines (Merceron et al., 2010). Mesalazine-induced pericarditis may be a humoral-mediated hypersensitivity response in which antibodies produced against mesalazine cross-react with heart tissues, leading to inflammation (Sentongo and Piccoli, 1998; Waite and Malinowski, 2002). Other possible mechanisms include the direct cardiotoxic effects of mesalazine and lgE- or cell-mediated hypersensitivity (Kaiser et al., 1997). In addition, mesalazine induces reactive oxygen species formation and the mitochondrial membrane potential collapse in rat cardiac mitochondria. This causes mitochondrial dysfunction and cytochrome c release, eventually leading to cardiomyocyte apoptosis and cardiovascular dysfunction (Salimi et al., 2020).

Clinical manifestations of mesalazine-induced cardiotoxicity are diverse and nonspecific, including fever, chest pain, and

TABLE 4 Treatment and prognosis of 52 patients reported in case series/reports.

Parameter		Value
Therapy (52)[a]	Discontinued	48 (92.3%)
	Continued	4 (7.7%)
	Treatment for IBD	25 (48.1%)
	Corticosteroids	5 (9.6%)
	Azathioprine	1 (1.9%)
	Balsalazide	1 (1.9%)
	Infliximab	1 (1.9%)
	Cyclosporine	
	Treatment for cardiotoxicity	18 (34.6%)
	NSAIDs	12 (23.1%)
	Antibiotics	6 (11.5%)
	Surgery: subtotal pericardectomy, pericardiocentesis, cardiac pacemaker implantation	6 (11.5%)
	Hypotensive drugs	4 (7.7%)
	Analgesics	1 (1.9%)
	Vasoactive drugs	1 (1.9%)
	Cardiac stimulants	1 (1.9%)
	Antianginal agents	
Symptom disappearance time (42)[a]	Immediately	14 (33.3%)
	0-7d	22 (52.4%)
	>7d	6 (14.3%)
Time of cardiotoxicity after mesalazine re-challenge (11)[a]	Immediately	3 (27.3%)
	0-7d	7 (63.6%)
	>7d	1 (9.1%)
Prognosis (52)[a]	Recover	51 (98.1%)
	Death	1 (1.9%)

[a]Represents the number of patients out of 52 in whom information regarding this particular parameter was provided.
IBD, inflammatory bowel disease; NSAIDs, Non-steroidal anti-inflammatory drugs.

dyspnea. cTnT and CK are specific markers of myocardial injury (Janardhanan, 2016). Most patients with cardiotoxicity had significantly increased biochemical markers such as cTnT, CK, and CRP. Leukocyte count and ESR also increased significantly. Once clinicians suspect a patient has mesalazine-induced cardiotoxicity, they should confirm the diagnosis by promptly examining the cardiac biochemical markers and initiating symptomatic treatment.

Imaging is an essential method for evaluating drug-induced cardiotoxicity. It can effectively assess structural and functional changes secondary to myocarditis or pericarditis. Imaging includes ECG, echocardiography, and CMRI (Nieminen et al., 1984; Valbuena-Lopez et al., 2016). Patients with cardiotoxicity usually show nonspecific ST-segment elevation or T-wave inversion on the ECG. Echocardiography can identify pericardial effusion or left cardiac insufficiency. CMRI can assess myocardial necrosis or myocardial edema. Cardiac imaging is recommended at the beginning of mesalazine treatment and follow-ups for early prevention or intervention of possible cardiotoxicity.

Treatment for cardiotoxicity is the immediate discontinuation of mesalazine. Most patients usually have symptoms that disappear within a few days. The toxic effects of the drug can lead to the development of immune-mediated acute myocarditis. The first-line treatment option for such immune-mediated acute myocarditis is discontinuing the medication and starting corticosteroids (Ammirati et al., 2020). Alemtuzumab and anti-thymocyte globulin are available as second-line treatments (Jain et al., 2018; Esfahani et al., 2019). The benefits of corticosteroids have not been compared in patients who received the therapy with those who did not (Brown, 2016). Patients usually wish to resume IBD treatment with mesalazine. However, rechallenging can lead to cardiotoxicity symptoms reappearing within hours or days (Agnholt et al., 1989; Gujral et al., 1996; Coman et al., 2014). Therefore, mesalazine rechallenge or switching to an alternative drug that does not contain 5-ASA should be carefully considered.

The study has the following limitations. First, the quality of the included studies was poor. More high-quality prospective cohort studies are needed. Second, only electronic databases were searched, and full texts of some studies could not be obtained, which can lead to selection and information bias.

5 Conclusion

Cardiotoxicity is a rare and serious adverse effect of mesalazine. Clinicians should consider the possibility of cardiotoxicity in patients with fever, chest pain, dyspnea, and other symptoms, especially within 4 weeks of treatment. Immediate discontinuation of mesalazine is necessary. Corticosteroids can improve patient symptoms, leading to a good prognosis. Laboratory tests (cTnT, serum CK) and imaging (ECG, echocardiogram, and cardiac MRI) should be performed.

6 Future prospects

As the number of patients diagnosed with IBD increases worldwide, more patients are exposed to mesalamine. Further rigorous experiments are needed to clarify the specific mechanism of mesalamine-induced cardiotoxicity. Multicenter prospective cohort studies with more rigorous designs, larger sample sizes, and higher qualities are necessary to identify high-risk groups and explore optimal treatment options. In patients who developed cardiotoxicity, follow-ups should be provided to observe the long-term prognosis of patients.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

JC, SL, and CW drafted and edited the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of commercial or financial relationships that could be construed as a potential conflict of interest.

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Cardiovascular safety of febuxostat and allopurinol in patients with gout: A meta-analysis

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Background: Gout is a common disease and is usually treated with uric acid-lowering drugs (the most commonly used of which are febuxostat and allopurinol). However, the cardiovascular safety of febuxostat and allopurinol is still controversial. The purpose of our study is to evaluate the cardiovascular safety of the two drugs in patients with gout using one-stage and two-stage meta-analysis.

Methods: PubMed, Embase, CBM, CNKI, WanFang, Central, and VIP were searched from inception to 30 January 2022. Randomized controlled trials which evaluated the cardiovascular safety of febuxostat or allopurinol for treating patients with gout were included. Based on the Kaplan–Meier curves of the two studies, individual patient data (IPD) were extracted and reconstructed. We used time-varying risk ratios (RRs) to summarize time-to-event outcomes, and the RRs of MACE incidence, cardiovascular mortality, and all-cause mortality were calculated by a multi-level flexible hazard regression model in 1-stage meta-analyses. *p* values were calculated using a log-rank test. At the same time, using the reconstructed IPD, we performed 2-stage meta-analyses to inform the quantitative estimates of time-specific relative risks at the six time points (1 , 2, 3, 4, 5, and 6 years) based on a random-effects model.

Results: Two RCTs with 12,318 participants were included. In the incidence of major adverse cardiovascular events between the two regimens, there was no significant difference [RR = 0.99 (95% CI, 0.89–1.11), $p = 0.87$]; at the same time, there was no significant difference in cardiovascular mortality [RR = 1.17 (95% CI, 0.98–1.40),$p = 0.08$] or all-cause mortality [RR = 1.03 (95% CI, 0.91–1.17),$p = 0.62$]. In terms of 2-stage meta-analyses, there was no significant difference in any outcomes at any time point (moderate-to low-certainty evidence).

Conclusion: In patients without atherosclerotic disease, febuxostat likely has a similar cardiovascular profile to allopurinol. However, in patients with a history of cardiovascular disease, allopurinol treatment is associated with less cardiovascular mortality as compared with febuxostat.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/#loginpage, identifier PROSPERO, CRD42022325656.

KEYWORDS

gout, allopurinol, febuxostat, cardiovascular safety, an individual-patient data level META analysis

1 Introduction

Gout is a metabolic disease, caused by elevation of serum urate level (Scuiller et al., 2020). The prevalence of gout in the world ranges from 0.68%–3.90% and is still increasing steadily (Dalbeth et al., 2021). Previous evidence showed that gout is a risk factor which can lead to cardiovascular disease (Krishnan et al., 2006; Kuo et al., 2010; Clarson et al., 2015a; Clarson et al., 2015b; Mouradjian et al., 2020). It is common that patients with gout also suffer from cardiovascular disease, and about 74% of patients have hypertension, 10% had a history of stroke, and 14% have a history of myocardial infarction (Zhu et al., 2012). In addition, the risk of death in patients with gout may be increased because of cardiovascular disease (Choi and Curhan, 2007). According to clinical guidelines in many countries, febuxostat and allopurinol are recommended as first-line drugs for treatment of gout (Yamanaka, 2011; Hui et al., 2017; Richette et al., 2017; FitzGerald et al., 2020). Allopurinol, a xanthine oxidase inhibitor, is considered one of the most effective uric acid-lowering drugs and is often used to treat chronic gout (Seth et al., 2014). Febuxostat reduces uric acid production by effectively and selectively inhibiting two forms of xanthine oxidase. With the approval of febuxostat in 2009, clinicians have a wider selection of drugs to treat gout (Bardin and Richette, 2019).

According to published randomized controlled trials, febuxostat is a more effective option than allopurinol (Becker et al., 2005). However, in 2017 and 2019, the U.S. Food and Drug Administration (FDA) issued two warnings, indicating that febuxostat might increase cardiovascular mortality and all-cause mortality compared with allopurinol in patients with gout (FDA, 2017; FDA, 2019). In addition, two randomized controlled trials with large sample size and long follow-up that focused on the cardiovascular safety of febuxostat and allopurinol received inconsistent conclusions (White et al., 2018; Mackenzie et al., 2020). Previous meta-analysis indicated that allopurinol prevents cardiovascular disease in patients with gout (van der Pol et al., 2021); however, any potential difference in cardiovascular safety between febuxostat and allopurinol should be interpreted. So, in this meta-analysis, we focused on time-event data which evaluated the cardiovascular safety of febuxostat and allopurinol using reconstructed individual-patient data.

2 Methods

We followed the PRISMA-IPD (Preferred Reporting Items for Systematic reviews and Meta-Analyses of individual participant data) when carrying out this research and reported the results (Stewart et al., 2015). We registered this study in PROSPERO (CRD42022325656).

2.1 Literature search and eligible criteria

With a combination of keywords (gout; allopurinol; febuxostat; drug therapy; randomized controlled trials), we searched PubMed, Embase, CBM, CNKI, WanFang, Central, and VIP comprehensively from inception to 30 January 2022 for relevant studies. In addition, we also searched ClinicalTrials.gov from inception to 30 January 2022 for unpublished data and screened reference lists of eligible studies to identify potential eligible studies.

The inclusion criteria: 1) participants: adult patients (>18 years) with gout. 2) Interventions: febuxostat. 3) Comparison: allopurinol. 4) Outcomes: MACE (major adverse cardiovascular events; a composite endpoint of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, and urgent revascularization for unstable angina), cardiovascular death, and all-cause death. 5) Study design: randomized controlled trials with Kaplan–Meier curves and had a follow-up of at least 52 weeks.

The exclusion criteria: 1) asymptomatic hyperuricemia, acute gout, and secondary gout. 2) Studies published in a language which is not Chinese or English. 3) Studies with missing data and studies with outcomes other than MACE incidence, cardiovascular mortality, and all-cause mortality. 4) Patients with moderate or severe hepatic impairment (value, ascites, lower limb edema, icterus, and alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 3× reference or increased prothrombin time >2× reference value). 5) Patients with severe renal impairment (eGFR <15 ml/min). 6) Patients with diseases that seriously affect the outcome indicators (such as immune diseases, hematological diseases, malignant tumors, etc.).

2.2 Screening process, data extraction, and risk of bias

First, two researchers (XG and SZ) searched databases according to keywords and imported literature into EndNote and then browsed titles and abstracts roughly according to the inclusion and exclusion criteria. For potentially relevant studies, we downloaded the full text of the literature and then read it carefully to decide whether to include it or not. After all the remaining literatures were screened, the entire process is drawn into a flowchart and displayed in the results. Any discrepancies in

the screening process will be resolved through the intervention of the third researcher (NS).

Two reviewers (XG and SZ) used R 4.1.3 to extract data from Kaplan–Meier curves in two randomized controlled trials (RCTs) and then reconstructed individual patient-based data (IPD) using an R package *IPDfromKM* (Guyot et al., 2012; Lee et al., 2020).

The study used the revised Risk of Bias 2.0 to evaluate the risk of bias (Sterne et al., 2019). Two members (XG and SZ) independently assessed the risk of bias according to the evaluation method in the tool. After assessment, they cross-checked and made a three-line table to display the results. Any disagreements were resolved by consultation with the third investigator (NS).

2.3 Certainty of evidence assessment

Using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) framework, two authors assessed the certainty of evidence based on five domains (risk of bias, inconsistency, imprecision, publication bias, and indirectness) and then rated the certainty for each outcome as high, moderate, low, or very low (Guyatt et al., 2008; Zeng et al., 2021).

2.4 Statistical analysis

First, we performed 1-stage meta-analyses by the reconstructed IPD to evaluate the qualitative trend of the relative effects over time. Risk ratios (RRs) were used to summarize time-to-event outcomes (that is, MACE (major adverse cardiovascular events; a composite endpoint of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, and urgent revascularization for unstable angina), cardiovascular death, and all-cause death] and calculated by using the multi-level flexible hazard regression model (Tierney et al., 2007). p values were calculated using the log-rank test (Bland JM, 2004). The result will be presented as Kaplan–Meier curves.

In addition, using the reconstructed IPD, we also performed 2-stage meta-analyses to evaluate the quantitative estimates of time-specific relative risks at the six time points (1, 2, 3, 4, 5, and 6 years) and robustness of the results. All analyses were completed using the R 4.1.3 (meta-package), and the results will be presented as forest plots.

2.5 Role of the funding source

The study design, data collection, data synthesis, and analysis or interpretation were not influenced by funding sources.

3 Results

3.1 Characteristics of eligible studies

According to the inclusion criteria, we found two eligible randomized controlled trials totaling 12,318 participants in our systematic review (Figure 1). The two inclusion trials were the febuxostat versus allopurinol streamlined trial (FAST) and the cardiovascular safety of febuxostat and allopurinol in patients with gout and cardiovascular morbidities (CARES) trial. The patients in the two trials were all gout patients with cardiovascular comorbidities.

In FAST, 6,128 patients were left in an intention-to-treat analysis (3,063 in the febuxostat group and 3,065 in the allopurinol group) and were followed for a median of 1,467 days (IQR1029-2052). The primary composite endpoint was the first occurrence of hospitalization for non-fatal myocardial infarction or biomarker-positive acute coronary syndrome; non-fatal stroke (whether reported to have led to hospitalization or not or to have occurred during a hospitalization); or death due to a cardiovascular event. The conclusion is that the cardiovascular safety of the two drugs has no statistical difference.

In CARES, 6,190 patients were assigned randomly to receive febuxostat (n = 3,098) or allopurinol (n = 3,092), and median follow-up time was 32 months (maximum, 85 months). The primary outcome was a composite of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, or urgent revascularization for unstable angina. The conclusion is that the cardiovascular safety of febuxostat is better than that of allopurinol.

The baseline characteristics of the included studies are summarized in Table 1.

3.2 Risk of bias of included studies

According to ROB 2, one study (FAST) was evaluated at high risk of bias in the domain of the randomization process, and the other study (CARES) was evaluated at low risk of bias in all domains (Table 2).

3.3 Results of 1-stage meta-analysis

Two randomized controlled trials (including 12,318 patients) provided Kaplan–Meier curves in the study. In the incidence of major adverse cardiovascular events between the two regimens, there was no significant difference [RR = 0.99 (95% CI, 0.89–1.11), p = 0.87]; at the same time, there was no significant difference in cardiovascular mortality [RR = 1.17 (95% CI, 0.98–1.40),p = 0.08] or all-cause mortality [RR = 1.03 (95% CI, 0.91–1.17),p = 0.62]. The curve fitting results are shown in Figure 2.

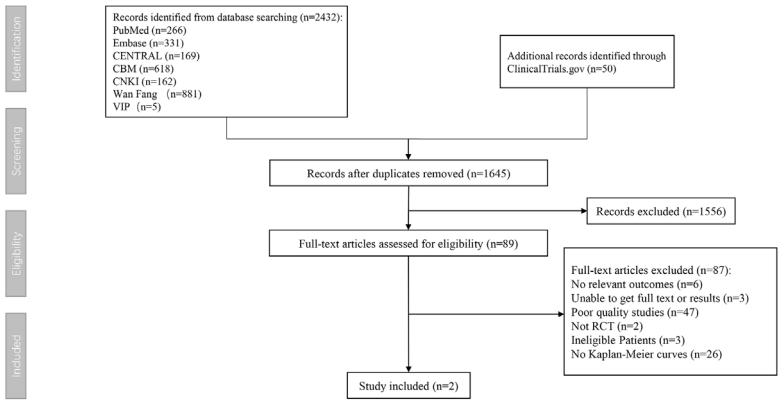

FIGURE 1
Flow diagram of literature search and selection.

TABLE 1 Baseline characteristics of each included study (n = 2).

Author (year)	Number (F/A)	Patient	Male proportion (%)	Age		Intervention		Follow-up time	Baseline serum uric acid	Outcome
				F	A	F	A			
White 2018 (CARES)	3,098/3,092	Patients with gout and cardiovascular disease	83.94	64.0, (58.0, and 71.0)	65.0, (58.0, and 71.0)	40 mg/day–80 mg/day	300 mg/day–600 mg/day	Median 136 weeks; maximum 364 weeks	0.518 mmol/L	①②③
Mackenzie 2020 (FAST)	3,063/3,065	Patients with gout	85.26	71.0 ± 6.4	70.9 ± 6.5	80 mg/day–120 mg/day	100 mg/day –900 mg/day	Median follow-up time was 1,467 days	0.297 mmol/L	①②③

F, febuxostat; A, allopurinol; ①, all adverse cardiovascular events during follow-up and treatment (a composite endpoint of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, and urgent revascularization for unstable angina). ② Cardiovascular death (death due to cardiovascular causes during follow-up and treatment). ③ All-cause death (death due to any cause during follow-up and treatment).

TABLE 2 Risk of bias assessment results.

Study	R	D	Mi	Me	S	O
Low risk of bias White (2018)	+	+	+	+	+	+
High risk of bias Mackenzie (2020)	+	+	+	+	+	+

R: bias arising from the randomization process; D: bias due to deviations from intended interventions; Mi: bias due to missing outcome data; Me: bias in measurement of the outcome; S: bias in selection of the reported result; O: overall risk of bias. +: Low risk of bias; +: High risk of bias.

3.4 Results of 2-stage meta-analysis

The results suggested that febuxostat was not associated with a statistically significant increase at all times in the risk of MACE, cardiovascular mortality, and all-cause mortality (moderate-to low-certainty evidence). In cardiovascular mortality, we found significant heterogeneity at 5 years (I2 = 53%, p = 0.14) and 6 years (I2 = 70%, p = 0.07). In all-cause mortality, we found significant heterogeneity at 3 years (I2 = 61%, p = 0.11), 4 years (I2 = 80%, p = 0.02), 5 years (I2 = 84%, p = 0.01), and 6 years (I2 = 88%, p < 0.01). Because of heterogeneity between two RCTs, we used random-effects models. (Table 3 and Appendix Figure 3).

4 Discussion

To compare the cardiovascular safety of febuxostat and allopurinol in patients with gout, we conducted 1-stage meta-analysis based on reconstructed individual patient data and 2-stage analysis at different time points. The result indicates that,

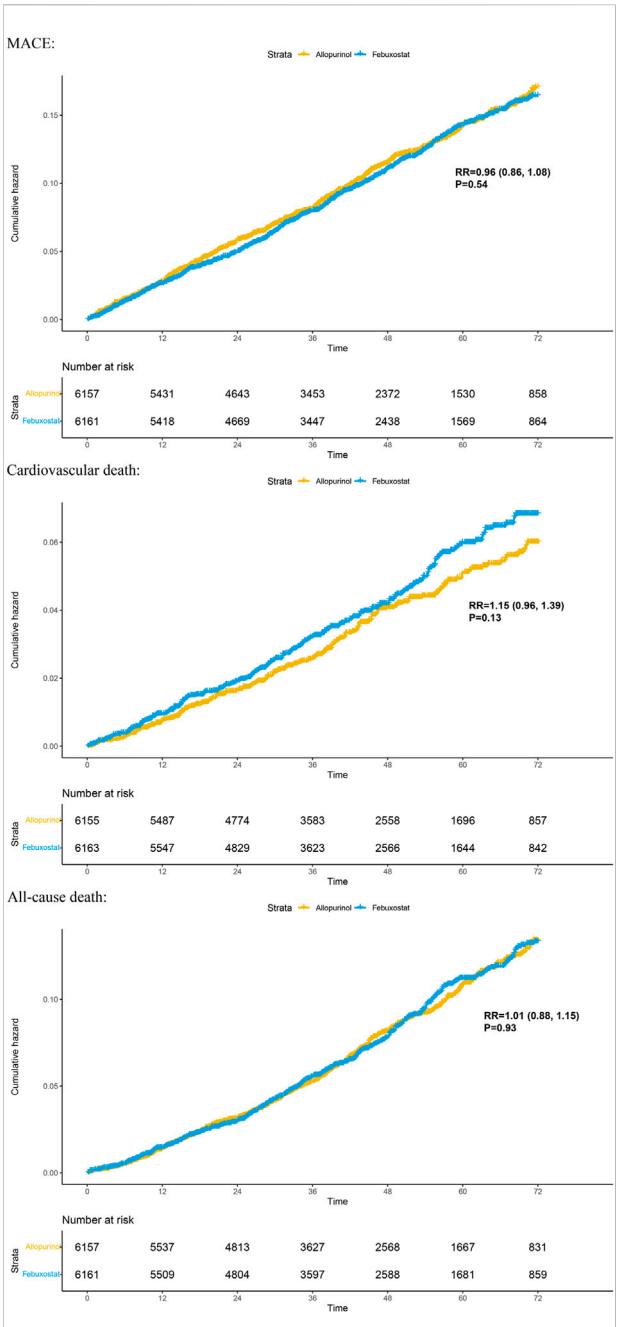

FIGURE 2
Kaplan–Meier plots for benefit outcomes in 1-stage meta-analyses. In the Kaplan–Meier curves, the ordinate represents the incidence of adverse events, and the abscissa represents time. Two curves with different colors represent different groups; blue represents the febuxostat group, and yellow represents the allopurinol group. The numbers below the curves represent the numbers at risk in different groups at different time points. MACE, major adverse cardiovascular events.

compared to allopurinol, febuxostat does not increase the incidence of MACE, cardiovascular death, or all-cause death in the treatment of patients with gout.

For heterogeneity between the two studies in two-stage meta-analysis, we speculate the following reasons: 1) the baseline characteristics are different in two trials, such as the proportion of patients with cardiovascular disease (in CARES, almost 40% of the study population has a history of myocardial infarction, 14% a history of stroke, and around 12% a history of peripheral artery disease, while these percentages were considerably lower in the FAST trial: 10%, 5%, and 5%, respectively). Because the reconstructed IPD may not completely represent the indeed IPD, these differences in baseline prevalence of cardiovascular disease between FAST and CARES may potentially affect the cardiovascular outcomes; 2) doses of medicines are different. In CARES, the dose of allopurinol is 200–600 mg/day, and the dose of febuxostat is 40–80 mg/day, and in FAST, the dose of allopurinol is 100–900 mg/day, and the dose of febuxostat is 80–120 mg/day. It is worth considering that the risk of adverse drug events usually increases with increasing drug dose; however, the lower dose of febuxostat in CARES increases all-cause mortality and cardiovascular mortality than that in FAST. Therefore, we believe that the result of FAST, which is consistent with our conclusion, is more reliable; 3) the loss rate of CARES is higher than that of FAST; 4) differences in sponsors, practitioners, and trial procedures may also lead to differences in final conclusions. However, considering that the two RCTs both met the inclusion and exclusion criteria, the sample sizes were both sufficient, and the follow-up time met the requirements. Hence, we do not think that the stability of the results will be affected. As a method which evaluates the robustness of 1-stage meta-analysis, our results of 2-stage meta-analysis showed consistent results.

In addition to the two randomized controlled trials, there exist other studies about the cardiovascular safety of febuxostat and allopurinol, and the conclusions are also inconsistent. Above all, our conclusion is consistent with that of one network meta-analysis (Zhang et al., 2021), three systematic meta-analyses (Liu et al., 2019; Barrientos-Regala et al., 2020; Gao et al., 2021), and two cohort studies (Chen et al., 2019; Kang et al., 2019). However, our findings are inconsistent with those of one cohort study in the real world (Su et al., 2019). Considering that even if the study used relevant statistical methods to minimize the impact of covariates on outcome indicators, it still cannot be considered that all possible covariates have been dealt with, and the research results still need to be corroborated by randomized controlled trials with high data quality or real-world data. Therefore, we believe that our research results are still reliable, which can provide specific reference significance for clinical practice and provide a certain basis for the selection of XOI drugs for clinical treatment of gout.

Our research not only enriches the content of related fields but also provides a certain reference for the selection of uric acid-lowering drugs for the clinical treatment of gout. Our meta-analysis has the following advantages: 1) to the best of our knowledge, individual-patient data level meta-analysis was not

TABLE 3 GRADE profiles: febuxostat compared to allopurinol for gout.

Outcome	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participant (studies)	Quality assessment						Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk			Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other consideration	
	Allopurinol	Febuxostat									
All adverse cardiovascular events (1 year)	Study population 26 per 1,000	25 per 1,000 (19 to 32)	RR 0.95 (0.73–1.23)	12,318 (two studies)	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
All adverse cardiovascular events (2 years)	Study population 51 per 1,000	44 per 1,000 (37 to 51)	RR 0.86 (0.73–1)	12,318 (two studies)	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
All adverse cardiovascular events (3 years)	Study population 66 per 1,000	63 per 1,000 (55 to 72)	RR 0.96 (0.84–1.1)	12,318	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
All adverse cardiovascular events (4 years)	Study population 82 per 1,000	78 per 1,000 (67 to 90)	RR 0.95 (0.82–1.1)	12,318	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
All adverse cardiovascular events (5 years)	Study population 91 per 1,000	95 per 1,000 (83 to 110)	RR 0.97 (0.87–1.09)	12,318 (two studies)	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
All adverse cardiovascular events (6 years)	Study population 98 per 1,000	95 per 1,000 (83 to 110)	RR 0.97 (0.84–1.12)	12,318 (two studies)	Randomized trials	Serious[1]	Serious[2]	No serious indirectness	No serious imprecision	None	⊕⊕⊖⊖ **Low**[1,2]
Cardiovascular death (1 year)	Study population 7 per 1,000	9 per 1,000(6 to 13)	RR 1.25 (0.84–1.85)	12,318 (two studies)	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
Cardiovascular death (2 years)	Study population 11 per 1,000	26 per 1,000 (21 to 33)	RR 1.13 (0.82–1.55)	12,318 (two studies)	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
Cardiovascular death (3 years)	Study population 21 per 1,000	26 per 1,000 (21 to 33)	RR 1.25 (0.99–1.57)	12,318	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
Cardiovascular death (4 years)	Study population 28 per 1,000	31 per 1,000 (25 to 38)	RR 1.09 (0.87–1.36)	12,318 (two studies)	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
Cardiovascular death (5 years)	Study population 32 per 1,000	37 per 1,000 (28 to 48)	RR 1.17 (0.89–1.53)	12,318 (two studies)	Randomized trials	Serious[1]	No serious inconsistency[3]	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1,3]
Cardiovascular death (6 years)	Study population 36 per 1,000	41 per 1,000 (30 to 56)	RR 1.13 (0.82–1.55)	12,291 (two studies)	Randomized trials	Serious[1]	No serious inconsistency[4]	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1,4]
All-cause death (1 year)	Study population 13 per 1,000	14 per 1,000 (10 to 18)	RR 1.02 (0.76–1.38)	12,318	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]

(Continued on following page)

10.3389/fphar.2022.998441

TABLE 3 (*Continued*) GRADE profiles: febuxostat compared to allopurinol for gout.

Outcome	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participant (studies)	Quality assessment						Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk			Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other consideration	
	Allopurinol	Febuxostat									
All-cause death (2 years)	**Study population 28 per 1,000**	26 per 1,000 (21 to 33)	**RR 0.94** (0.75–1.2)	12,318 (two studies)	Randomized trials	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision	None	⊕⊕⊕⊖ **Moderate**[1]
All-cause death (3 years)	**Study population 42 per 1,000**	44 per 1,000 (33 to 57)	**RR 1.03** (0.79–1.35)	12,318 (two studies)	Randomized trials	Serious[1]	Serious[5]	No serious indirectness	No serious imprecision	None	⊕⊕⊖⊖ **Low**[1,5]
All-cause death (4 years)	**Study population 51 per 1,000**	50 per 1,000 (35 to 72)	**RR 0.98** (0.69–1.4)	12,318 (two studies)	Randomized trials	Serious[1]	Serious[6]	No serious indirectness	No serious imprecision	None	⊕⊕⊖⊖ **Low**[1,6]
All-cause death (5 years)	**Study population 66 per 1,000**	76 per 1,000 (53 to 109)	**RR 1.02** (0.73–1.42)	12,318 (two studies)	Randomized trials	Serious[1]	Serious[3]	No serious indirectness	No serious imprecision	None	⊕⊕⊖⊖ **Low**[1,3]
All-cause death (6 years)	**Study population 75 per 1,000**	76 per 1,000 (53 to 109)	**RR 1.01** (0.71–1.45)	12,318 (two studies)	Randomized trials	Serious[1]	Serious[4]	No serious indirectness	No serious imprecision	None	⊕⊕⊖⊖ **Low**[1,4]

*The basis for the **assumed risk** (e.g., the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI); **CI:** confidence interval; **RR:** risk ratio; **moderate quality(⊕⊕⊕⊖):** further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; and **low quality (⊕⊕⊖⊖):** further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

[1]Downgraded one level for risk of bias (Mackenize et al., 2020: high risk of bias for blinding).

[2]Downgraded one level for inconsistency (substantial heterogeneity was present among the studies (I2 = 44%, $p = 0.18$). One study's conclusion contradicted another's).

[3]Downgraded one level for inconsistency (substantial heterogeneity was present among the studies (I2 = 84%, $p = 0.01$). One study's conclusion contradicted another's).

[4]Downgraded one level for inconsistency (substantial heterogeneity was present among the studies (I2 = 88%, $p < 0.01$). One study's conclusion contradicted another's).

[5]Downgraded one level for inconsistency (substantial heterogeneity was present among the studies (I2 = 61%, $p = 0.11$). One study's conclusion contradicted another's).

[6]Downgraded one level for inconsistency (substantial heterogeneity was present among the studies (I2 = 80%, $p = 0.02$). One study's conclusion contradicted another's).

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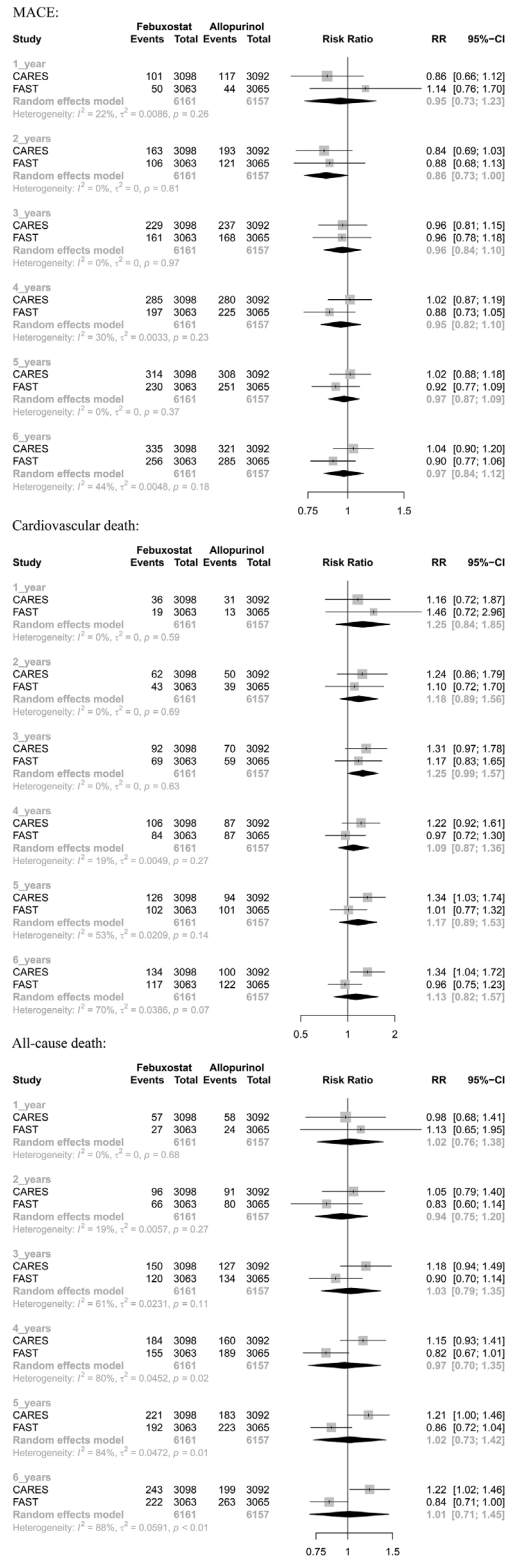

FIGURE 3
Forest plots of time-specific relative risks in 2-stage meta-analyses.

used to compare the cardiovascular safety of febuxostat and allopurinol in patients with gout before, and our study is the first to adopt this approach. 2) This 1-stage meta-analysis presents the results as Kaplan–Meier curves, which can reflect the time-event more intuitively and can visually observe the comparison of cardiovascular safety at various time points.

The main limitations of our study are the following: 1) the inclusion criteria were not so strict, so some patients with various diseases were included in this study, which may have resulted in some heterogeneity or bias. However, this study can still give clinical references for treatment of gout because gout patients in the real world often have comorbid diseases. 2) Because the language is limited to Chinese and English, some studies may be omitted. 3) Only two studies were included, and this problem may be solved by more published relevant randomized controlled trials or real-world studies.

5 Conclusion

Febuxostat likely has a similar cardiovascular profile to allopurinol in patients without atherosclerotic disease based on the reconstructed IPD. However, in patients with a history of cardiovascular disease, allopurinol treatment is associated with less cardiovascular mortality as compared with febuxostat. Because their results are inconclusive, febuxostat still needs to be used cautiously for patients with gout and cardiovascular diseases.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials; further inquiries can be directed to the corresponding author.

Author contributions

XG and SZ were in charge of study design, data collection and interpretation, quality assessment of evidence, and manuscript preparation; NS and JL critically reviewed the manuscript and provided revisions; and LZ, YL, and FW were involved in data collection, data interpretation, and quality assessment of evidence.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.998441/full#supplementary-material

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The pathogenesis, diagnosis, prevention, and treatment of CAR-T cell therapy-related adverse reactions

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Chimeric antigen receptor (CAR)-T cell therapy is effective in the treatment of refractory/relapsed (r/r) hematological malignancies (r/r B-cell lymphoblastic leukemia, B-cell lymphoma, and multiple myeloma). In addition, it is being explored as a treatment option for solid tumors. As of 31 March 2022, seven CAR-T therapies for hematological malignancies have been approved worldwide. Although CAR-T therapy is an effective treatment for many malignancies, it also causes adverse effects. The incidence of cytokine release syndrome (CRS), the most common adverse reaction after infusion of CAR-T cells, is as high as 93%.CRS, is the leading risk factor of immune effector cell-associated neurotoxicity syndrome (ICANS), as well as cardiovascular, hematological, hepatorenal, skin, pulmonary, and gastrointestinal toxicity. Severe adverse reactions complicated by CRS severely impede the widespread application of CAR-T therapy. The CAR-T product was initially approved in 2017; however, only limited studies have investigated the adverse reactions owing to CAR-T therapy compared to that of clinically approved drugs. Thus, we aimed to elucidate the mechanisms, risk factors, diagnostic criteria, and treatment of toxicities concurrent with CRS, thereby providing a valuable reference for the safe, effective, and widespread application of CAR-T therapy.

KEYWORDS

CAR-T cell therapy, cytokine release syndrome, ICANS, consensus grading, organ system toxicity, treatment strategies

Introduction

Chimeric antigen receptor (CAR)-T cell therapy has gained attention as an effective treatment for related tumors owing to the unsatisfactory efficacy of conventional chemoimmunotherapy and radiotherapy for most relapsed/refractory (r/r) hematological malignancies. Seven CAR-T therapies approved including tisagenlecleucel, axicabtagene ciloleucel, lisocabtagene maraleucel, brexucabtagene autoleucel and relmacabtagene autoleucel (these five target CD-19), idecabtagene vicleucel and ciltacabtagene autoleucel (these two target B cell maturation antigen

[BCMA]), have been approved globally (Table 1) (Neelapu et al., 2017; Maude et al., 2018; Schuster et al., 2019; Abramson et al., 2020; Berdeja et al., 2021; Munshi et al., 2021; Shah et al., 2021; Westin et al., 2021; Ying et al., 2021). Currently, relmacabtagene autoleucel has been approved in China only. CAR-T therapies have currently been approved for the treatment of hematological malignancies including r/r B-lymphoblastic leukemia (r/r B-ALL), r/r B-cell non-Hodgkin lymphoma (NHL), and r/r multiple myeloma (r/r MM) (Maude et al., 2018; Abbasi et al., 2020; Abramson et al., 2020; Berdeja et al., 2021; Jain et al., 2021; Munshi et al., 2021; Ying et al., 2021).

Compared with established radiotherapy and chemotherapy, the mechanism of adverse reactions related to CAR-T therapy is more complex and difficult to clarify. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are the most common adverse events during CAR-T cell therapy (Fried et al., 2019; Dolladille et al., 2021). Previous clinical trials have suggested that during CAR-T cell treatment, the incidence of CRS was 57–93%, such that the severe form of CRS (≥ grade 3) had an incidence of 13–32%, the incidence of ICANS was 39–69%, and that of the severe form (≥ grade 3) was 11–41.5% (Neelapu et al., 2017; Schuster et al., 2017; Maude et al., 2018; Park et al., 2018; Santomasso et al., 2018; Cohen et al., 2019; Schuster et al., 2019; Nastoupil et al., 2020; Shalabi et al., 2020; Holtzman et al., 2021). In comparing two clinical studies, we observed that the incidence of CRS and ICANS after treatment with tisagenlecleucel was 58% and 12%, respectively, and was significantly lower than that of CRS (93%) and ICANS (64%) after the treatment with axicabtagene ciloleucel (Neelapu et al., 2017; Schuster et al., 2019). Severe CRS can lead to organ dysfunction; however, we lack options for excluding the influence of other mechanisms on these organ toxicities. Therefore, compared to conventional radiotherapy and chemotherapy, the mechanism of adverse reactions related to CAR-T therapy is more complex and challenging to elucidate.

In a retrospective pharmacovigilance study, Goldman et al. (2021) analyzed reports of 2,657 patients treated with axicabtagene-ciloleucel and tisagenlecleucel and suggested that the mortality rate of cardiovascular and pulmonary adverse events (CPAE) was 30.9%, which was significantly higher than that of CRS (17.4%). Moreover, among

TABLE 1 Approved CAR-T cell therapy.

Name (trade name)	Company	Target antigen	CAR construct (Crees and Ghobadi, 2021; Anderson, 2022)	Listing date	Indication
Tisagenlecleucel (Kymriah)	Novartis	CD19	Second generation, CD3ζ+4-1BB Lentiviral vector	FDA 2017.08.30 EMA 2018.08.27	Paediatric and young adult patients (age 3–25 years) with r/r B-ALL; adult (≥18 years) patients with r/r DLBCL (Braendstrup et al., 2020)
				FDA 2022.05.27	Adult patients with r/r FL (Fowler et al., 2022)
Axicabtagene ciloleucel (Yescarta)	Kite pharma	CD19	Second generation, CD3ζ+CD28 Retroviral vector	FDA 2017.10.18 EMA 2018.08.27	Adult patients with LBCL failing at least two other kinds of treatment (including r/r DLBCL, r/r PMBCL, high-grade BCL and DLBCL arising from FL) (Jacobson et al., 2020)
Brexucabtagene autoleucel (Tecartus)	Kite pharma	CD19	Second generation, CD3ζ+CD28 Retroviral vector	FDA 2020.07.24 EMA 2020.12.17	Adult patients with r/r MCL Adults with r/r B-ALL (Tbakhi and Reagan, 2022)
Lisocabtagene maraleucel (Breyanzi)	Juno Therapeutics/ Bristol Myers Squibb	CD19	Second generation, CD3ζ+4-1BB Lentiviral vector	FDA 2021.02.05	Adult patients with r/r LBCL failing at least two other kinds of treatment (including r/r DLBCL, r/r PMBCL, high-grade BCL, Grade 3B FL) (Crees and Ghobadi, 2021)
Idecabtagene Vicleucel (Abecma)	Bristol Myers Squibb	BCMA	Second generation, CD3ζ+4-1BB Lentiviral vector	FDA 2021.03.26 EMA 2021.08.19	Adult patients with r/r MM (Sharma et al., 2022)
Relmacabtagene autoleucel (relma-cel)	JW Therapeutics	CD19	Second generation, CD3ζ+4-1BB Lentiviral vector	NMPA 2021.09.03	Adult patients with r/r DLBCL (Ying et al., 2021)
Ciltacabtagene autoleucel (Carvykti)	Legend Biotech/ Janssen Biotech	BCMA (consisting of two BCMA-binding domains)	Second generation, CD3ζ+4-1BB Lentiviral vector	FDA 2022.02.28	Adult patients with r/r MM (Berdeja et al., 2021)

FDA, Food and Drug Administration; EMA, European Medicines Agency; NMPA, National Medical Products Administration; r/r B-ALL, relapsed or refractory B-cell acute lymphoblastic leukaemia; r/r DLBCL, relapsed or refractory diffuse large B-cell lymphoma; LBCL, large B-cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; BCL, B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma.

TABLE 2 Adverse reactions related to CAR-T cell therapy.

Adverse reaction	Main symptoms	Relationship with CRS	Characteristic
CRS	Fever; Hypotension; Hypoxia; DIC; Multi organ system toxicities	—	• Systemic inflammatory reaction caused by a large number of inflammatory factors
ICANS	Aphasia; Headache; Mild encephalopathy; Focal neurological Deficit; Tremor; Seizures; brain edema	CRS is one of the main inducers of ICANS, ICANS and CRS may occur simultaneously or not	• The breakdown of the BBB and capillary leakage lead to the entry of pro-inflammatory cytokines and CAR-T cells into the CSF to damage the CNS.
Cardiovascular toxicity	Hypotension; Sinus tachycardia; Increased serum troponin levels; Arrhythmia; Reduced LVEF; Cardiogenic shock; QT prolongation; Heart failure	CRS is one of the main inducers of cardiovascular toxicity, which can lead to serious direct and indirect cardiovascular complications	• Abnormal elevation of inflammatory cytokines IL-6, VWF, Ang-2, TNF-α and off-target cross-reaction of CAR-T cells to actin can lead to cardiovascular toxicity
Hematologic toxicity	Neutropenia; Thrombocytopenia; Leucopenia; Anemia; B-cell aplasia; Coagulopathy	Patients with severe CRS were more likely to develop late hematologic toxicity	• Neutropenia is closely related to infectious complications • B-cell aplasia is a common toxicity of anti-CD19 CAR-T therapy
HLH/MAS	Ferritin is extremely elevated; High fever; Hepatosplenomegaly; Hemocytopenia; Coagulopathy	HLH/MAS is a severe manifestation of CRS, so it is difficult to distinguish diagnosis of them	• The incidence of HLH/MAS is low, but its mortality is high and prognosis is poor
Skin toxicity	Rash; Dry skin; Purpura; Papules; Maculopapular; Urticarial rash; Bullous eruptions; Oral mucositis	CRS is one of the inducers of skin toxicity, and the reduced immune function induced by CRS may lead to skin infections in patients	• The clinical manifestations and mechanisms of skin toxicities are still poorly understood • Currently, there are no guidelines to diagnose and treat skin toxicity
Pulmonary toxicity	Respiratory failure	CRS is one of the main inducers of pulmonary toxicity	• The incidence of pulmonary toxicity is lower than that of CRS and ICANS. • There are definite clinical diagnostic indicators about pulmonary toxicity
Renal toxicity	Adrenal insufficiency; Electrolyte disorders; Kidney failure; Acidosis	CRS is one of the main inducers of renal toxicity	• The incidence of renal toxicity is lower than that of CRS and ICANS. • There are definite clinical diagnostic indicators about renal toxicity • Usually symptomatic treatment
Hepatotoxicity	Liver injury	CRS is one of the main inducers of hepatotoxicity	• The incidence of hepatotoxicity is lower than that of CRS and ICANS. • There are definite clinical diagnostic indicators about hepatotoxicity
Gastrointestinal toxicity	Diarrhea; Vomiting; Bleeding; Nausea	CRS is one of the main inducers of gastrointestinal toxicity	• The incidence of gastrointestinal toxicity is lower than that of CRS and ICANS. • There are definite clinical diagnostic indicators about gastrointestinal toxicity • Usually symptomatic treatment

CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; DIC, disseminated intravascular coagulation; BBB, blood brain barrier; CSF, cerebrospinal fluid; CNS, central nervous system; LVEF, left ventricular ejection fraction; IL, interleukin; Ang-2, angiopoietin-2; VWF, von willebrand factor; TNF-α, tumor necrosis factor alpha; HLH/MAS, Hemophagocytic Lymphohistiocytosis/Macrophage Activation Syndrome.

546 patients with CPAE, 68.3% had concurrent CRS (Goldman et al., 2021). A combination of CRS with other organ system toxicity is common, and most CAR-T cell-induced adverse reactions (Table 2) could be managed if diagnosed early., However, the organ system toxicity of concurrent CRS is not easily recognized, thereby hindering the timely diagnosis and treatment. Thus, a comprehensive understanding of these adverse reactions their risk factors, and the management strategies for related adverse reactions are crucial in reducing mortality and improving recovery rates.

CAR-T cell therapy

The primary process of autologous CAR-T therapy is to first collect T cells, then genetically modify them to identify tumor antigens and amplify CAR-T cells, and finally introduce lymphodepletion chemotherapy prior to infusion of CAR-T cells back into the patient (Subklewe et al., 2019; Hong et al., 2020). Notably, lymphodepletion chemotherapy causes events such as infection and cytopenia. Currently, the marketed target antigens of CAR-T products include CD19 and BCMA. Numerous target antigens, including CD22, CD33, CD70,

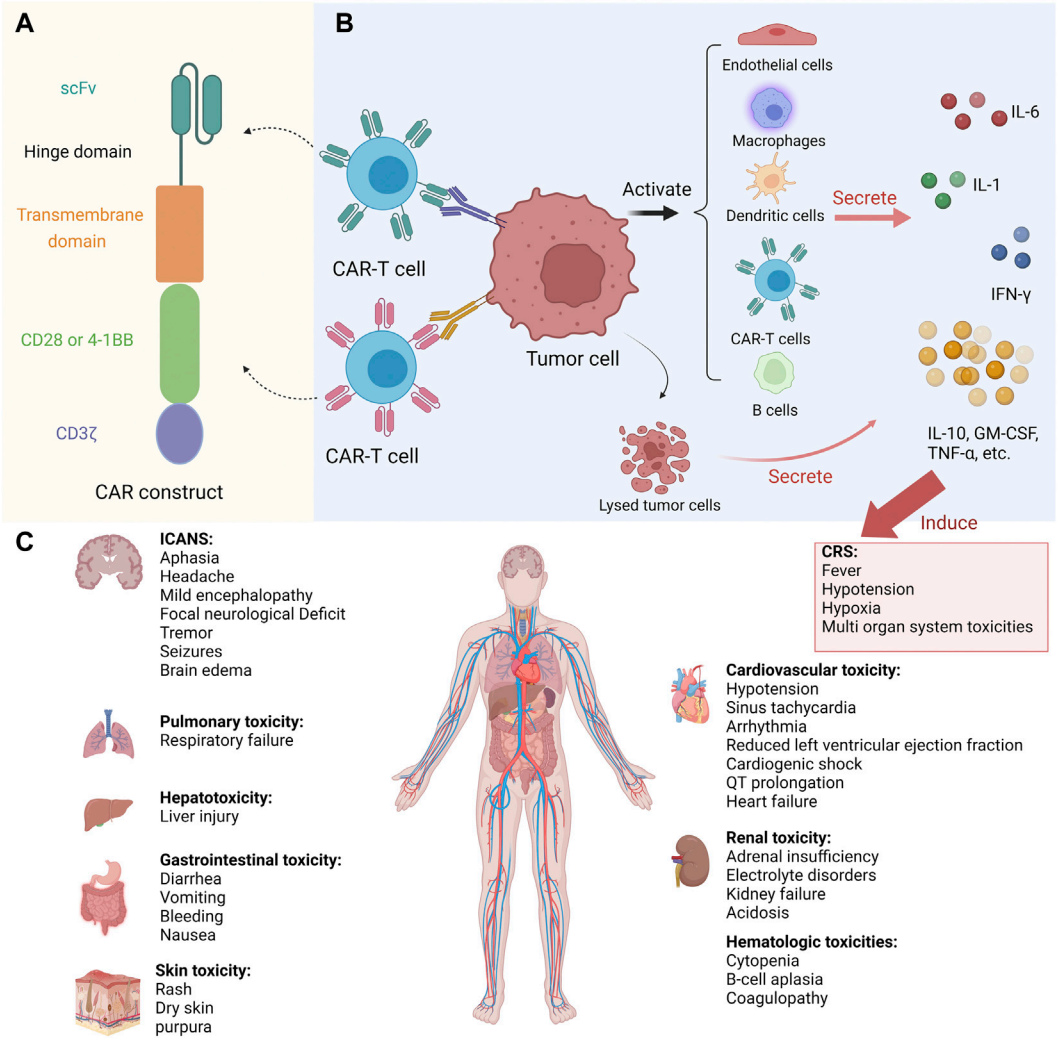

FIGURE 1
Toxicities during CAR-T therapy. **(A)** The structure of CAR. **(B)** Pathogenesis of CRS. **(C)** Organ systemic toxicities induced by CRS. Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; scFv, single-chain variable fragment; IL, interleukin; IFN-γ, interferon gamma; GM-CSF, granulocyte macrophage colony-stimulating factor; TNF-α, tumor necrosis factor alpha. This figure created with BioRender.com.

CD123, CD138, CD171, HER2, EGFR, B7-H3, claudin 6, gp120, GPRC5D, PSMA, and mesothelin, have been studied (Larson, Maus; Johnson and June, 2017; Smith et al., 2019; Sauer et al., 2021). Clinical studies on these targets are promising for CAR-T cell therapy in treating of r/r advanced solid tumors, autoimmune diseases, and acquired immunodeficiency syndrome (AIDS) (Rust et al., 2020; Mougiakakos et al., 2021; Totzeck et al., 2022). CAR comprises four domains (Figure 1A); extracellular antigen recognition, hinge, transmembrane connecting, and intracellular activating domains (Neelapu et al., 2018a; Hong et al., 2020; Larson, Maus; Nusbaum et al., 2021). The extracellular part consists of a single-chain variable fragment (scFv) of a monoclonal antibody (responsible for recognizing and binding tumor antigens) and a hinge region that acts as a linker, whereas the intracellular part consists of signal transduction domains and single or multiple T cell costimulatory domains (Badieyan and Hoseini, 2018; Stoiber et al., 2019). The intracellular domain of the first-generation CAR is composed of CD3ζ, whereas that of the second-generation CAR is composed of CD3ζ and a costimulatory domain (CD28 or 4-1BB), and that of the third-generation CAR is composed of CD3ζ and two costimulatory domains (CD28 and 4-1BB) (Kosti et al., 2018; Mochel et al., 2019). The expansion and persistence of second- and third-generation CAR-T cells with costimulatory domains are significantly improved compared to that of first-generation CAR-T cells (Imai et al., 2004; Savoldo et al., 2011).

The marketed CAR-T therapies all involve second-generation CARs, where the intracellular domains of axicabtagene ciloleucel and brexucabtagene autoleucel consist of CD3ζ and the costimulatory domain CD28 (Reagan, Friedberg). The intracellular domain of the other five CAR-T therapies consist of CD3ζ and the costimulatory domain 4-1BB (Table 1).

CAR-T manufacturing generally takes 2–4 weeks and may extend to 3–6 weeks due to the turnaround and transportation time to the final infusion into the patient (Freyer and Porter, 2020). The turnaround time for CAR-T manufacturing/delivery varies with the products and the physical condition of patients. The primary sources of T cells in CAR-T cell immunotherapy are allogeneic and autologous. Allogeneic CAR-T cell therapy has a higher incidence of graft *versus* host disease (GVHD) than autologous CAR-T cell therapy; however, it is more beneficial in mass production, timely supply to cancer patients, and low production cost. It is currently the most promising method for the clinical application of CAR-T batches. However, the current CAR-T cell therapy mainly employs autologous T cells, which have a long production cycle and are expensive (Mahadeo et al., 2019).

The main adverse reactions of CAR-T cell therapy

Cytokine release syndrome – Diagnosis and treatment

CRS is the most common adverse reaction to CAR-T cell infusion. It is mainly a systemic inflammatory reaction caused by a large number of inflammatory factors released by activated immune cells (T cells, macrophages, B cells, monocytes, natural killer cells, and dendritic cells) and endothelial cells (Figure 1B) (Dal'bo et al., 2020; Neelapu et al., 2018a; Giavridis et al., 2018; Ganatra et al., 2019b). Following CAR-T cell infusion, the onset of CRS ranges from hours to days as T cells expand (Maude et al., 2014). The timing of CRS occurrence is closely related to the structure of CAR. For example, patients treated with anti-CD19-CD28-CD3ζ CAR typically develop CRS earlier than those treated with anti-CD19-4-1BB-CD3ζ CAR (Neelapu et al., 2018a).

Common signs of CRS are fever (≥38°C), hypotension (systolic blood pressure <90 mmHg), and hypoxia (oxygen saturation <90%) (Neelapu et al., 2018a). Severe CRS can induce disseminated intravascular coagulation (DIC), multiple organ toxicity (Figure 1C) (adult respiratory distress syndrome, ICANS, cardiac dysfunction, cytopenia), and even death (Neelapu et al., 2018a; Ganatra et al., 2019b; Sterner and Sterner, 2021). Risk factors for severe CRS include patient-related factors (B-ALL diagnosis, high tumor burden, baseline thrombocytopenia, and endothelial activation), tumor-related factors (B-ALL diagnosis), and treatment-related factors (high number of infused CAR-T cells, high peak of CAR-T cell expansion, CD28 co-stimulatory, high-intensity lymphodepletion regimens) (Jin et al., 2018; Ganatra et al., 2019b; Zheng et al., 2020; Schubert et al., 2021).

IL-6 produced by human circulating monocytes is a key cytokine that leads to CRS in CAR-T therapy (Norelli et al., 2018). The FDA approved tocilizumab (an IL-6 receptor antagonist) for treating CAR-T cell-induced CRS in 2017. The recommended therapeutic dose of tocilizumab is 4–8 mg/kg (maximum 800 mg) (Brudno and Kochenderfer, 2016; Le et al., 2018). Thus, tocilizumab is not recommended for inflammation induced by infection, neutropenic sepsis, or tumor lysis syndrome (TLS). CRS must be confirmed before tocilizumab treatment. ZUMA-1 cohort 4 and 6 studies have demonstrated that prior use of corticosteroids and/or tocilizumab and prophylactic corticosteroids may reduce the incidence of ≥ grade 3 CRS and ICANS (Oluwole et al., 2021; Topp et al., 2021). Regarding the early identification and prediction of CRS, Teachey et al. (2016) further screened three specific markers from 24 biomarkers for predicting severe CRS. Differences by age were found, with predicted biomarkers gp130, IFN-γ, and IL-1RA for adults and IFN-γ, IL-13, and MIP-1α in children (Teachey et al., 2016). It is expensive to predict whether a patient is likely to develop severe CRS based on the measurement of multiple cytokines. Pennisi et al. (2021) found that the modified endothelial activation and stress index (EASIX) score (lactate dehydrogenase [LDH; U/L] × C-reactive protein [CRP; mg/dl]/platelets [PLTs; 10^9 cells/L]) is the most clinically relevant formula for predicting severe CRS and ICANS. Hay et al. (2017) designed a simple two-step algorithm to predict grade ≥4 CRS; they first checked whether the patient had a fever ≥38.9°C within 36 h of CAR-T infusion and then performed serum MCP-1. These methods facilitate the prediction of severe toxicity during CAR-T therapy.

The consensus criteria for grading (Table 3) and management (Table 4) of CRS are invaluable for treating this particular toxicity. Historically, there are numerous grading systems for CRS, but clinicians typically use the consensus American Society of Transplantation and Cellular Therapy (ASTCT) guidelines (Pennisi et al., 2020). ASTCT (Lee et al., 2019) defines CRS as "a supraphysiological response following any immune therapy that results in the activation or engagement of endogenous or infused T-cells and/or other immune effector cells. The onset of symptoms can be progressive, must include fever at the onset, and may include hypotension, capillary leak (hypoxia), and end-organ dysfunction." In addition, Santomasso et al. (2021) systematically reviewed the evidence of immune-related adverse events in patients treated with CAR-T cells published from 2017 to 2021 and developed ASCO guidelines in conjunction with a multidisciplinary team (consisting of medical oncology, neurology, hematology, emergency medicine, nursing). However, owing to the lack of high-

TABLE 3 Grading of CRS.

CRS grading system	CTCAE version 5.0 (National Cancer Institute, 2017)	Lee criteria (Lee et al., 2014)	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Lee et al., 2019)	ASCO guideline (Santomasso et al., 2021)
Grade 1	• Fever (≥38.0°C) • And/or constitutional symptoms	Symptoms are not life-threatening and require symptomatic treatment only (e.g., fever, nausea, fatigue, headache, myalgias, malaise)	• Fever (≥38.0°C) • No hypotension • No hypoxia • And/or grade 1 organ toxicities (CTCAEv4.03)	• Fever (≥38.0°C) • No hypotension • No hypoxia	• Fever (≥38.0°C) not attributable to any other cause • No hypotension • No hypoxia
Grade 2	• Fever (≥38.0°C) • Hypotension (responds to fluids) • hypoxia (FiO2 <40%)	Symptoms require and respond to moderate intervention • Hypotension (responds to IV fluids or low dose of one vasopressor) • Hypoxia (FiO2 <40%) • Grade 2 organ toxicity (CTCAEv4.03)	• Fever (≥38.0°C) • Hypotension (Responds to IV fluids or low-dose vasopressors) • Or hypoxia (FiO2 <40%) • Or grade 2 organ toxicities (CTCAEv4.03)	• Fever (≥38.0°C) • And hypotension not requiring vasopressors • And/or hypoxia requiring low-flow nasal cannula (≤6 L/min)	• Fever (≥38.0°C) not attributable to any other cause • And hypotension not requiring vasopressors • And/or hypoxia requiring low-flow nasal cannula (≤6 L/min) or blowby
Grade 3	• Fever (≥38.0°C) • Hypotension (needs one vasopressors) • hypoxia (FiO2 ≥40%)	Symptoms require and respond to aggressive intervention • Hypotension (responds to high-dose or multiple vasopressors) • hypoxia (FiO2 ≥40%) • Grade 3 organ toxicity or grade 4 transaminitis (CTCAEv4.03)	• Fever (≥38.0°C) • Hypotension (needs high-dose or multiple vasopressors) • Or hypoxia (FiO2 ≥40%) • Or grade 3 organ toxicity or grade 4 transaminitis (CTCAEv4.03)	• fever (≥38.0°C) • And hypotension requiring one vasopressor ± vasopressin • And/or hypoxia requiring high-flow nasal cannula (>6 L/min), facemask, non-rebreather mask, or venturi mask	• Fever (≥38.0°C) not attributable to any other cause • And hypotension requiring one vasopressor ± vasopressin • And/or hypoxia requiring high-flow nasal cannula, facemask, non-rebreather mask, or venturi mask
Grade 4	• Fever (≥38.0°C) • Life-threatening consequences; urgent intervention needed	Life-threatening symptoms • Hypoxia (needing ventilator support)	• Fever (≥38.0°C) • Hypotension (Life-threatening)	• fever (≥38.0°C) • And hypotension requiring multiple vasopressors (excluding vasopressin)	• Fever (≥38.0°C) not attributable to any other cause • And hypotension requiring multiple vasopressors (excluding vasopressin)

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TABLE 3 (*Continued*) Grading of CRS.

CRS grading system	CTCAE version 5.0 (National Cancer Institute, 2017)	Lee criteria (Lee et al., 2014)	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Lee et al., 2019)	ASCO guideline (Santomasso et al., 2021)
		• Grade 4 organ toxicity except grade 4 transaminitis (CTCAEv4.03)	• Or hypoxia (needing ventilator support) • Or grade 4 organ toxicity except grade 4 transaminitis (CTCAEv4.03)	• And/or hypoxia requiring positive pressure (e.g., CPAP, BiPAP, intubation and mechanical ventilation)	• And/or hypoxia requiring positive pressure (e.g., CPAP, BiPAP, intubation and mechanical ventilation)
Grade 5	Death	Death	—	death due to CRS	—

Hypotension, Systolic blood pressure <90 mmHg; Hypoxia, Needing oxygen for SaO2 >90%.
High-dose vasopressors (all doses are required for ≥3 h) (Lee et al., 2014) are defined as any of the following: noradrenaline ≥20 μg/kg/min; dopamine ≥10 μg/kg/min; phenylephrine ≥200 μg/kg/min; adrenaline ≥10 μg/kg/min; if on vasopressin, vasopressin + noradrenaline equivalent of ≥10 μg/kg/min; if on combination vasopressors (not including vasopressin), noradrenaline equivalent of ≥20 μg/kg/min. VASST Trial vasopressor equivalent equation: norepinephrine equivalent dose = [norepinephrine (μg/min)] + [dopamine (μg/kg/min) ÷ 2] + [epinephrine (μg/min)] + [phenylephrine (μg/min) ÷10].
CRS, cytokine release syndrome; CTCAE, Common Terminology Criteria for Adverse Events; FiO2, fraction of inspired oxygen; IV, intravenous; CTCAE, Common Terminology Criteria for Adverse Events; CARTOX, CAR-T cell therapy associated toxicity; ASTCT, American Society for Transplantation and Cellular Therapy; CPAP, continuous positive airway pressure; BiPAP, Bilevel positive airway pressure; ASCO, American Society of Clinical Oncology.

quality evidence, recommendations are based only on the consensus of experts. The CRS grading criteria in the ASCO guidelines were formulated based on the ASTCT consensus, as shown in Table 3. Regarding CRS treatment (Table 4), all guidelines involve supportive care (treatment of fever, hypotension, and hypoxia). Tocilizumab is the first-line drug used for the treatment of CRS. Corticosteroids (dexamethasone or methylprednisolone) should be added when tocilizumab fails to effectively control CRS or when CRS worsens. However, consensuses differ on varying dose regimens in treating patients with CRS Furthermore, in order to reduce the harm of severe CRS to patients, some prophylactic drugs (such as dexamethasone, anakinra, itacitinib, tocilizumab) are used in patients receiving CAR-T therapy. The specific clinical trials are shown in Table 5.

ICANS

ICANS, also known as neurotoxicity or CAR-T-cell-related encephalopathy syndrome (CRES), is another unique toxicity of CAR-T therapy, but is less likely to occur than CRS (Neelapu et al., 2018a; Miao et al., 2021). It was defined as any new and well-defined neurological symptom that occurred within 60 days of CAR-T cell infusion and was attributable to the infusion (Gofshteyn et al., 2018). Common signs and symptoms of ICANS include aphasia, headache, mild encephalopathy, focal neurological deficits, tremors, seizures, and rarely, fatal cerebral edema (Hirayama and Turtle, 2019; Ragoonanan et al., 2021). In addition, Van Oekelen et al. (2021) reported rare neurocognitive and hypokinetic movement disorders with Parkinsonian tendencies in patients using anti-BCMA CAR-T therapy. The time to ICANS onset during CAR-T therapy ranged from 0 to 19 days (Gust et al., 2017; Santomasso et al., 2018; Schuster et al., 2019; Holtzman et al., 2021). ICANS and CRS can occur simultaneously or not. In most cases, ICANS occurs after the complete resolution of CRS; if CRS does not occur before ICANS, ICANS is usually mild (Santomasso et al., 2018; Freyer and Porter, 2020; Neill et al., 2020). Santomasso et al. (2018) studied 53 patients treated with 19-28z CAR-T cells and found that all patients with neurological symptoms developed at least grade 1 CRS with fever. Gust et al. (2017) studied neurological adverse reactions in 133 patients infused with CAR-T cells and observed that ≥ grade 3 neurotoxicity was often accompanied by more severe CRS and endothelial dysfunction (diffuse intravascular coagulation, capillary leakage, and blood-brain barrier disruption). CRS is closely related to severe ICANS, but not all cases of ICANS are accompanied by CRS. Most pathophysiological studies of ICANS are based on imaging findings in patients with severe or fatal neurotoxicity, such as cerebral edema. Tumor-associated non-occlusive thrombosis, petechial hemorrhages, pontine infarcts, and non-specific white matter changes were found in 28% of patients with ICANS compared to baseline brain MRI before CAR-T cell treatment (Holtzman et al., 2021). However, further investigation is required to ascertain whether these pathophysiological mechanisms apply to mild reversible neurotoxicity.

TABLE 4 Management of CRS.

CRS management system	Lee criteria (Lee et al., 2014)	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Neelapu, 2019)	ASCO guideline (Santomasso et al., 2021)
Grade 1	• Vigilant supportive care (treat fever and neutropenia if present, antipyretics, analgesics as needed)	• Fever: Acetaminophen and hypothermia blanket; Consider tocilizumab 8 mg/kg IV or siltuximab 11 mg/kg IV for persistent (lasting >3 days) and refractory fever	•Antipyretics and IV hydration	• Supportive care with antipyretics, IV hydration, and symptomatic management of organ toxicities and constitutional symptoms
	• Assess for infection	• Organ toxicity: Symptomatic management	• Diagnostic work-up to rule out infection	• Consider empiric broad-spectrum antibiotics if neutropenic
	• Monitor fluid balance	• Empiric broad-spectrum antibiotics and filgrastim if neutropenic	• Consider growth factors and antibiotics if neutropenic	• If neutropenia, consider empiric broad-spectrum antibiotics and G-CSF (GM-CSF is not recommended)
		• Maintenance IV fluids for hydration		• In patients with persistent (>3 days) or refractory fever, consider managing as per grade 2
Grade 2	• Maintenance of adequate hydration and blood pressure	• Fever: manage fever as in grade 1 CRS	• Supportive care as in grade 1	• Supportive care as per grade 1
	• Vigilant supportive care (monitor cardiac and other organ function closely), if the patient doesn't have extensive co-morbidities or older age	• Hypotension: IV fluid bolus of 500–1,000 ml of normal saline; Second IV fluid bolus if pressure remains <90 mmHg; Tocilizumab or siltuximab for the hypotension refractory to fluid boluses (tocilizumab can be repeated after 6 h); If hypotension persists, start vasopressors, consider transfer to ICU), dexamethasone (10 mg q6h, IV)	• IV fluid boluses and/or supplemental oxygen	• Administer tocilizumab (8 mg/kg, IV); Repeat q8h if no improvement in signs and symptoms of CRS; Limit to a maximum of three doses in a 24 h period, with a maximum of four doses total
	• Tocilizumab (adults 4 mg/kg, children 8 mg/kg) ± corticosteroids (methylprednisolone 2 mg/kg/day, dexamethasone 0.5 mg/kg maximum 10 mg/dose), if the patient has extensive co-morbidities or older age	• Hypoxia: supplemental oxygen; Tocilizumab or siltuximab ± corticosteroids and supportive care	• Tocilizumab ± dexamethasone or its equivalent of methylprednisolone	• In patients with hypotension that persists after two fluid boluses and after one to two doses of tocilizumab, may consider dexamethasone (10 mg q12h, IV) for one to two doses and then reassess
		• Organ toxicity: symptomatic management of organ toxicities, as per standard guidelines; Tocilizumab or siltuximab ± corticosteroids and supportive care		• Manage per grade 3 if no improvement within 24 h of starting tocilizumab
Grade 3	• Maintenance of adequate hydration and blood pressure	• Fever: manage fever as in grade 1 CRS	• Supportive care as in grade 1	• Supportive care as per grade 2 and include vasopressors as needed
	• Vigilant supportive care	• Hypotension: IV fluid bolus, tocilizumab and siltuximab as recommended for grade 2 CRS; Increase dexamethasone to 20 mg q6h IV, if refractory; Transfer to ICU, obtain echocardiogram, and perform haemodynamic monitoring	• Consider monitoring in intensive care unit	• Tocilizumab as per grade 2 if maximum dose is not reached within 24 h period plus dexamethasone (10 mg q6h, IV) and taper once symptoms improve
	• Tocilizumab (adults 4 mg/kg, children 8 mg/kg) ± corticosteroids	• Hypoxia: supplemental oxygen including high-flow oxygen delivery and non-invasive positive	• Vasopressor support and/or supplemental oxygen	• If echocardiogram was not already performed, obtain ECHO to assess cardiac function and

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TABLE 4 (*Continued*) Management of CRS.

CRS management system	Lee criteria (Lee et al., 2014)	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Neelapu, 2019)	ASCO guideline (Santomasso et al., 2021)
	(methylprednisolone 2 mg/kg/day, dexamethasone 0.5 mg/kg maximum 10 mg/dose)	pressure ventilation; Tocilizumab or siltuximab + corticosteroids		conduct hemodynamic monitoring
		• Organ toxicity: symptomatic management of organ toxicities, as per standard guidelines; Tocilizumab or siltuximab + corticosteroids	• Tocilizumab + dexamethasone (10–20 mg q6h, IV) or its equivalent of methylprednisolone	• If refractory, manage as per grade 4
				• Admit patient to ICU
Grade 4	• maintenance of adequate hydration and blood pressure	• Fever: manage fever as in grade 1 CRS	• Supportive care as in grade 1	• Supportive care as per grade 3 plus mechanical ventilation as needed
	• Vigilant supportive care	• Hypotension: manage hypotension as in grade 3 CRS; Methylprednisolone (1 g/day, IV)	• Monitoring in intensive care unit	• Tocilizumab as per grade 2 if maximum dose is not reached within 24 h period; Initiate high-dose methylprednisolone (500 mg q12h, IV) for 3 days, followed by 250 mg IV q12h for 2 days, 125 mg IV q12h for 2 days, and 60 mg IV q12h until CRS improvement to grade 1
	• Tocilizumab (adults 4 mg/kg, children 8 mg/kg) ± corticosteroids (methylprednisolone 2 mg/kg/day, dexamethasone 0.5 mg/kg maximum 10 mg/dose)	• Hypoxia: mechanical ventilation; Tocilizumab or siltuximab + corticosteroids	• Vasopressor support and/or supplemental oxygen *via* positive pressure ventilation	• If not improving, consider methylprednisolone (1g, IV) 2 times a day or alternate therapy
		• Organ toxicity: symptomatic management of organ toxicities, as per standard guidelines; Tocilizumab or siltuximab + corticosteroids	• Tocilizumab + methylprednisolone 1 g/day	

Tocilizumab IV over 1 h, Maximum amount of tocilizumab per dose is 800 mg.
CRS, cytokine release syndrome; CARTOX, CAR-T cell therapy associated toxicity; IV, intravenous; ICU, intensive-care unit; q6h, every 6 hours; q8h, every 8 hours; q12h, every 12 hours; ASTCT, American Society for Transplantation and Cellular Therapy; ASCO, American Society of Clinical Oncology; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ECHO, echocardiography.

Pathogenesis of ICANS

CRS is one of the main inducers of ICANS, and the pathogenesis of both is similar but not entirely consistent. The potential pathogenesis of ICANS may be related to the following mechanisms (Figure 2): *1*) blood-brain barrier breakdown, increased central nervous system (CNS) vascular permeability, followed by diffusion of inflammatory cytokines (IL-6, IL-15, and IFN-γ) into the CNS, ultimately exacerbating neurotoxicity (Gust et al., 2017). *2*) Direct CNS toxicity of CAR-T cells in cerebrospinal fluid (CSF). Studies have shown that patients with neurotoxicity have a significantly higher number of CAR-T cells in the CSF than those without neurotoxicity (Lee et al., 2015). *3*) CRS-induced hypoxemia can cause ICANS. *4*) CNS resident cells (endothelial, pericyte, microglia, astrocytes) secrete ICANS-related cytokines after CAR-T infusion. CSF levels of S100 calcium-binding protein B and glial fibrillary acidic protein increase during neurotoxicity, resulting in astrocyte damage (Gust et al., 2019; Gust et al., 2020).

Risk factors for ICANS

Similar but not identical to the risk factors of CRS, the risk factors of ICANS mainly include: *1*) patients with neurologic comorbidities or other high disease burden before CAR-T therapy (Gust et al., 2017; Santomasso et al., 2018). *2*) Fever ≥38.9°C within 36 h after CAR-T cell infusion (Gust et al., 2017). *3*) Lymphocyte depletion therapy with

TABLE 5 Current interventional clinical trials aiming to reduce CAR-T specific toxicities.

Name	Clinical trials	Specific toxicities	Prophylactic drug	Recruitment status
Axicabtagene ciloleucel	NCT05459571	CRS ICANS	Dexamethasone: dexamethasone (10 mg, orally or IV) before CAR-T cell infusion	Recruiting
Axicabtagene ciloleucel	NCT04314843	ICANS	Lenzilumab: sequenced therapy of lenzilumab and axicabtagene ciloleucel on Day 0	Terminated (Development program terminated.)
Axicabtagene ciloleucel	NCT04150913	CRS ICANS	Anakinra: anakinra (dosage per protocol, SC) on days 0–6	Recruiting
Axicabtagene ciloleucel	NCT04514029	ICANS	Simvastatin: simvastatin (40 mg/day, orally) will be started at least 5 days prior to apheresis and will be continued until day +30 after infusion. Dexamethasone: intrathecal dexamethasone 8 mg on days −1, +6, +13 (± 2 days)	Recruiting
Axicabtagene ciloleucel	NCT04432506	CRS ICANS	Anakinra: anakinra SC on days 0–6	Active, not recruiting
Axicabtagene ciloleucel	NCT03954106	ICANS	Defibrotide: defibrotide 6.25 mg/kg/dose once daily as a single dose on CAR-T Day −5, −4, and −3 before lymphodepletion, then every 6 h daily for 8 days (CAR-T Day 0 to Day 7)	Terminated (Primary endpoint would unlikely to be met based on the unplanned interim assessment on the first 20 efficacy evaluable patients.)
Axicabtagene ciloleucel	NCT04205838	ICANS	Anakinra: anakinra SC every 6–12 h for 12–36 doses over 9 days	Suspended (funding)
Axicabtagene ciloleucel	NCT04071366	CRS	Itacitinib: itacitinib (200 mg/day, orally) for 30 days or itacitinib (200 mg bid, orally) for 30 days	Recruiting
Axicabtagene ciloleucel	NCT02348216	CRS ICANS	Cohort 3 Levetiracetam: levetiracetam (750 mg orally or IV, BID) starting on Day 0 Tocilizumab: tocilizumab (8 mg/kg IV over 1 h [not to exceed 800 mg]) on Day 2 Cohort 4 Corticosteroids: dexamethasone or methylprednisolone. Tocilizumab: tocilizumab (8 mg/kg IV over 1 h [not to exceed 800 mg] at lower grades of toxicity) Levetiracetam: levetiracetam (750 mg orally or IV, BID) starting on Day 0 Cohort 5 Levetiracetam: levetiracetam (750 mg orally or IV, BID) starting on Day 0 Cohort 6 Corticosteroids: dexamethasone prior to axicabtagene ciloleucel infusion on Day 0, Day 1 and Day 2 Tocilizumab: tocilizumab at lower grades of toxicity Levetiracetam: levetiracetam (750 mg orally or IV, BID) starting on Day 0	Active, not recruiting
Lisocabtagene maraleucel	NCT04359784	CRS ICANS	Anakinra: anakinra SC daily on days 0–13	Recruiting

CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; IV, intravenous; SC, Subcutaneous Injections.

fludarabine and cyclophosphamide, high tumor burden during CAR-T infusion, high-dose infusion of CAR-T cells, the high peak of CAR-T cell expansion, pretreatment thrombocytopenia, and endothelial activation (Kochenderfer et al., 2017; Nastoupil et al., 2020; Holtzman et al., 2021; Schubert et al., 2021). *4*) Comparing the two studies, it was found that the incidence of epilepsy (8%) of CAR-T cells containing the 4-1BB costimulatory domain was lower than that of CAR-T cells containing the CD28 costimulatory domain (48%) (Gust et al., 2017; Santomasso et al., 2018).

Possible cytokine predictors of ICANS

Different from the definite role of IL-6 in CRS, no single cytokine is know to affect ICANS; therefore, predicting severe ICANS with many cytokines may be a new promising direction.

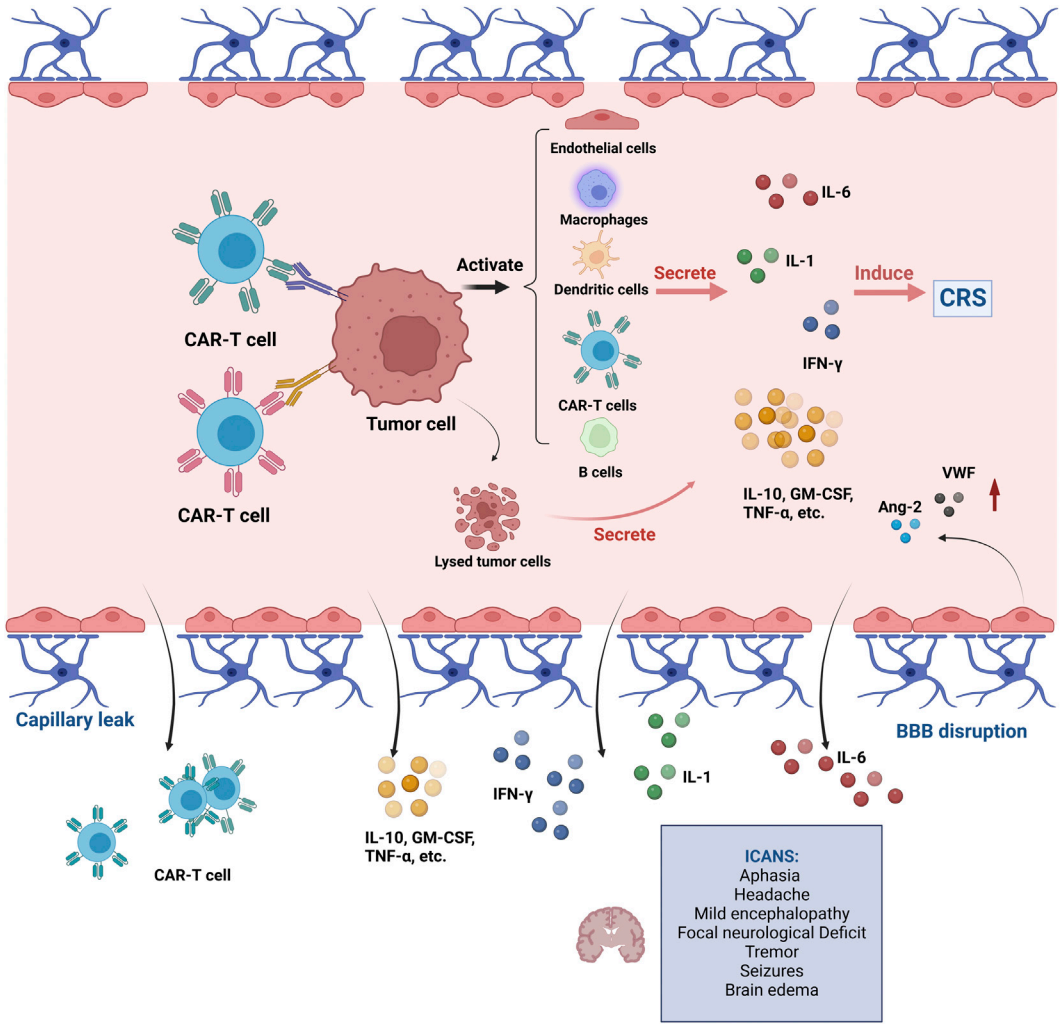

FIGURE 2
Pathogenesis of ICANS during CAR-T therapy. Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; IL, interleukin; IFN-γ, interferon gamma; GM-CSF, granulocyte macrophage colony-stimulating factor; TNF-α, tumor necrosis factor alpha; BBB, blood brain barrier. This figure created with BioRender.com.

Santomasso et al. (2018) predicted severe neurotoxicity through changes in multiple cytokines and found that patients with low IL-15 (<50 pg/ml) or high EGF (>120 pg/ml) had a lower risk of severe neurotoxicity; patients with high IL-15, low EGF, and low IL-10 (<200 pg/ml) were at moderate risk; and patients with high IL-15, low EGF, and high IL-10 levels were at high risk of severe neurotoxicity. Kochenderfer et al. (2017) also found that patients with ICANS (≥ grade 3) had higher peak levels of serum IL-10 and IL-15. Other studies have revealed that elevated fibrinogen and ferritin levels during early CAR-T cell infusion, or serum IL-6 ≥ 16 pg/ml and MCP-1 ≥ 1343.5 pg/ml within 36 h after CAR-T cell infusion, may predict high-risk patients with ICANS (Gust et al., 2017; Holtzman et al., 2021).

Gust et al. (2020) analyzed eight studies and reported that serum concentrations of IL-6, IL-10, GM-CSF, IFN-γ, IL-15, IL-2, GzB, IL-2Rα, IL-1RA, and CXCL10 positive correlated with the onset of ICANS. IFN-γ, GM-CSF, IL-6, IL-10, and IL-15 are closely related to ICANS and are potential predictors of ICANS. This paper also summarized 10 studies (Table 6) and found that ICANS was closely related to increased cytokines such as IL-15, IL-10, IFN-γ, and IL-6. However, confirming the relationship between most cell biomarkers and ICANS is challenging owing to the limited reports on their association and several interference factors. For example, Faramand et al. (2020) and Gust et al. (2017) found that ICANS was associated with an increase in the serum biomarkers, angiopoietin-2 (Ang-2) and von Willebrand factor (VWF) related to endothelial activation. However, another study demonstrated that serum VWF, VEGF-A,

TABLE 6 Cytokines related to ICANS.

Researchers	CAR-T products	Number of patients	Cancer type	Relative cytokines
Gofshteyn et al. (2018)	Tisagenlecleucel (CTL09)	51	B-cell ALL (n = 50) T-cell ALL (n = 1)	IL-2, sIL-4R, HGF, IL-15, sTNFR-1
Cohen et al. (2019)	CART-BCMA	25	r/r MM	IL-1RA, IFN-γ, MIP-1α, IL-6
Gust et al. (2019)	SCRI-CAR19v1	43	r/r B-ALL	GM-CSF, TNF-α, MIP-1α, IFN-γ, IL-6, IL-10, GzB
Santomasso et al. (2018)	19-28z CAR-T	53	r/r B-ALL	IL-1α, IL-2, IL-3, IL-5, IL-6, IL-10, IL-15, IP-10, INF-γ, G-CSF, GM-CSF, MCP-1, EGF (decrease)
Shalabi et al. (2018)	Anti-CD22 CAR-T	22	r/r B-ALL (n = 21) r/r DLBCL (n = 1)	TNF-α, IL-6, IL-8, IL-15, IL-2, IL-10, IL-13, GM-CSF, IFN-γ, MIP-α
Hay et al. (2017)	Anti-CD19 CAR-T	133	r/r B-ALL (n = 47) r/r CLL (n = 24) r/r NHL (n = 62)	IL-8, IL-6, IL-10, IL-15, IFN-γ, TNFRp55, MCP-1, MIP-1β
Neelapu et al. (2017)	axicabtagene ciloleucel	111	DLBCL (n = 81) PMBCL or TFL (n = 30)	IL-1RA, IL-2Rα, IL-2, IL-6, IL-8, IL-10, IL-15, IFN-g, GzB, GM-CSF, ferritin
Faramand et al. (2020)	axicabtagene ciloleucel	75	LBCL or indolent lymphoma	IL-6, Ang-2/Ang-1 ratio, Ang-2, IL-15, IFN-γ, Ang-1 (decrease), ferritin
Gust et al. (2017)	Anti-CD19 CAR-T	133	B-ALL (n = 47) NHL (n = 62) CLL (n = 24)	IL-6, IFN-γ, TNF-α, Ang-2, VWF
Kochenderfer et al. (2017)	Anti-CD19 CAR-T	22	DLBCL (n = 19) FL (n = 2) MCL (n = 1)	GzB, IL-10, IL-15, IFN-γ
Park et al. (2017)	19-28z CAR-T	51	r/r B-ALL	GM-CSF, IFN-γ, IL-15, IL-5, IL-10, IL-2, ferritin

ALL, acute lymphoblastic leukaemia; r/r MM, relapsed/refractory multiple myeloma; r/r B-ALL, relapsed or refractory B-cell acute lymphoblastic leukaemia; r/r DLBCL, relapsed or refractory diffuse large B-cell lymphoma; r/r CLL, relapsed or refractory chronic lymphocytic leukemia; r/r NHL, relapsed or refractory non-hodgkin lymphoma; PMBCL, primary mediastinal B-cell lymphoma; TFL, transformed follicular lymphoma; LBCL, Large B Cell Lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; IL, interleukin; sIL, soluble interleukin; HGF, hepatocyte growth factor; sTNFR-1, soluble tumor necrosis factor 1; IFN-γ, interferon gamma; MIP-1α, macrophage inflammatory protein 1 alpha; GzB, granzyme B; GM-CSF, granulocyte macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1β; TNFRp55, tumor necrosis factor receptor p55; Ang, angiopoietin; TNF-α, tumor necrosis factor alpha.

Ang-1, and Ang-2 levels are not associated with neurotoxicity (Gust et al., 2019).

ICANS grading standards

Before the development of the ASTCT consensus criteria, the CTCAE and CARTOX criteria were used to grade CAR-T cell-related neurotoxicity (Table 7). The ASTCT consensus guidelines were modified based on the CARTOX criteria, and the CARTOX-10 score was slightly modified to the immune effector cell-associated encephalopathy (ICE) score (Table 8), forming the ASTCT neurotoxicity (called ICANS) grading standard (Lee et al., 2019). To assess the mental status after CAR-T cell therapy, the ASTCT consensus group recommends the ICE score for adults and the Cornell Assessment of Pediatric Delirium (CAPD) for children (<12 years old) (Traube et al., 2014; Silver et al., 2015; Maus et al., 2020). The ASCO guidelines published in 2021 refer to the ASTCT consensus to classify ICANS and formulate management strategies for ICANS based on a multidisciplinary approach and relevant published evidence (Santomasso et al., 2021).

Therapeutic measures for ICANS

One study found that the prophylactic use of tocilizumab reduced the incidence of severe CRS and did not increase the risk of ICANS when infused with anti-CD19 CAR-T cells containing CD3ζ/4-1BB costimulatory signaling to treat NHL patients (Caimi et al., 2021). However, some studies reveal that the prophylactic use of tocilizumab increases the incidence of severe ICANS (Totzeck et al., 2022). Therefore, the prophylactic use of tocilizumab requires more evaluations and trials for verification. Ragoonanan et al. (2021) revealed that if ICANS and CRS coexist, tocilizumab is recommended for any grade of ICANS, and dexamethasone or methylprednisolone can be given if tocilizumab is ineffective. However, Norelli et al. (2018) found that blocking IL-6 receptors with tocilizumab could treat CRS in mouse models. However, this was ineffective against delayed fatal neurotoxicity. Santomasso et al. (2018) also reported that administering tocilizumab was ineffective in most patients with neurotoxicity. A possible reason is that tocilizumab does not easily cross the blood-brain barrier, and its administration leads to a compensatory increase in IL-6 in the

TABLE 7 Grading of ICANS.

ICANS grading system	CTCAEv5.0	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Lee et al., 2019)	ASCO guideline (Santomasso et al., 2021)
Grade 1	• Encephalopathy: mild symptoms • Seizure: brief partial seizure and no loss of consciousness • Dysphasia: awareness of receptive or expressive characteristics; Not impairing ability to communicate • Tremor: mild symptoms • Headache: mild pain • Confusion: mild disorientation • Depressed level of consciousness: decreased level of alertness	• CARTOX-10 score 7–9 (mild impairment) • No raised intracranial pressure • No seizures or motor weakness	• ICE score 7–9 • CAPD score 1–8 • And/or depressed level of consciousness but awakens spontaneously • No seizures • No motor weakness • No elevated ICP/cerebral edema	• ICE score 7–9 with no depressed level of consciousness
Grade 2	• Encephalopathy: moderate symptoms; Limiting instrumental ADL • Seizure: brief generalized seizure • Dysphasia: moderate receptive or expressive characteristics; Impairing ability to communicate spontaneously • Tremor: moderate symptoms; Limiting instrumental ADL • Headache: moderate pain; Limiting instrumental ADL • Confusion: moderate disorientation; Limiting instrumental ADL • Depressed level of consciousness: sedation; Slow response to stimuli; Limiting instrumental ADL	• CARTOX-10 score 3–6 (moderate impairment) • No raised intracranial pressure • No seizures or motor weakness	• ICE score 3–6 • CAPD score 1–8 •And/or depressed level of consciousness but awakens to voice • No seizures • No motor weakness • No elevated ICP/cerebral edema	• ICE score 3–6 • And/or Mild somnolence awaking to voice
Grade 3	• Encephalopathy: severe symptoms; Limiting self-care ADL • Seizure: new-onset seizures (partial or generalized); Multiple seizures despite medical intervention • Dysphasia: severe receptive or expressive characteristics; Impairing ability to read, write, communicate intelligibly • Tremor: severe symptoms; limiting self-care ADL • Headache: severe pain; Limiting self-care ADL	• CARTOX-10 score 0–2 (severe impairment) • Stage 1–2 papilloedema, or CSF opening pressure <20 mmHg • Partial seizure, or non-convulsive seizures on EEG with response to benzodiazepine	• ICE score 0–2 • CAPD score ≥9 • And/or depressed level of consciousness but awakens to tactile stimulus • Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention • No motor weakness	• ICE score 0–2 • And/or depressed level of consciousness awakening only to tactile stimulus • And/or any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention • And/or focal or local edema on neuroimaging

(Continued on following page)

TABLE 7 (*Continued*) Grading of ICANS.

ICANS grading system	CTCAEv5.0	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Lee et al., 2019)	ASCO guideline (Santomasso et al., 2021)
	• Confusion: severe disorientation; Limiting self-care ADL • Depressed level of consciousness: difficult to arouse • Cerebral edema: new onset; Worsening from baseline		• Focal/local edema on neuroimaging	
Grade 4	Life-threatening consequences; urgent intervention indicated	• Unable to perform CARTOX-10 •Stage 3–5 papilloedema, or CSF opening pressure ≥20 mmHg, or cerebral oedema •Generalized seizures, or convulsive or non-convulsive status epilepticus, or new motor weakness	• ICE score 0 (unable to perform ICE) • unable to perform CAPD • patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse (stupor or coma) • Life-threatening prolonged seizure (>5 min); Or repetitive clinical or electrical seizures without return to baseline in between • Deep focal motor weakness such as hemiparesis or paraparesis • Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad	• ICE score 0 (unable to perform ICE) •And/or stupor or coma •And/or life-threatening prolonged seizure (> 5 min) or repetitive clinical or electrical seizures without return to baseline in between •And/or diffuse cerebral edema on neuroimaging, decerebrate or decorticate posturing or papilledema, cranial nerve VI palsy, or Cushing's triad
Grade 5	Death	—	Death due to ICANS	—

Papilloedema grading is performed according to the modified Frisén scale (Frisen, 1982).
ICANS, immune effector cell-associated neurotoxicity syndrome; CTCAE, Common Terminology Criteria for Adverse Events; ADL, indicates activities of daily living; CARTOX, CAR-T cell therapy associated toxicity; CARTOX-10, CAR-T cell therapy associated toxicity 10-point neurological assessment; CSF, cerebrospinal fluid; EEG, electroencephalogram; ASTCT, American Society for Transplantation and Cellular Therapy; ICE, Immune Effector Cell-Associated Encephalopathy score; CAPD, Cornell Assessment of Pediatric Delirium; ICP, intracranial pressure; ASCO, American Society of Clinical Oncology.

CNS, which eventually aggravates ICANS (Nishimoto et al., 2008; Nellan et al., 2018). Therefore, systemic corticosteroid dexamethasone is recommended as a first-line treatment for grade 2–3 ICANS, and dexamethasone (10 mg, IV) is recommended every 6–8 h (Brudno and Kochenderfer, 2016; Neelapu, 2019; Schubert et al., 2021). High-dose methylprednisolone (1000 mg, IV) is recommended for 3 days when dexamethasone is ineffective or if ICANS is grade 4 (Gust et al., 2017; Schubert et al., 2021). Studies have shown that the prompt use of corticosteroids prevents severe ICANS without influencing the efficacy of CAR-T cell therapy (Topp et al., 2020). Holtzman et al. (2021) also confirmed that corticosteroids could be used to treat ICANS without compromising CAR-T efficacy. However, they mentioned that the rapid reduction in corticosteroids could trigger the onset of ICANS. Therefore, high doses of corticosteroids should be slowly reduced and

TABLE 8 Encephalopathy assessment tools for grading of ICANS.

CARTOX-10 (Neelapu et al., 2018a)	ICE (Lee et al., 2019)
• Orientation (5 points): orientation to year, month, city, hospital, president/prime minister of country of residence	• Orientation (4 points): orientation to year, month, city, hospital
• Naming (3 points): name three objects (e.g., point to clock, pen, button)	• Naming (3 points): name three objects (e.g., point to clock, pen, button)
• Writing (1 points): write a standard sentence (e.g., "Our national bird is the bald eagle")	• Writing (1 points): write a standard sentence (e.g., "Our national bird is the bald eagle")
• Attention (1 points): count backwards from 100 in 10	• Attention (1 points): ability to count backwards from 100 by 10
	• Following commands (1 points): follow simple commands (e.g., "Show me 2 fingers" or "Close your eyes and stick out your tongue")
Grade 1 ICANS: 7–9 points	
Grade 2 ICANS: 3–6 points	
Grade 3 ICANS: 0–2 points	
Grade 4 ICANS: unarousable, unable to complete assessment	

CARTOX-10, CAR-T cell therapy associated toxicity 10-point neurological assessment; ICE, immune Effector Cell-Associated Encephalopathy score; ICANS, immune effector cell-associated neurotoxicity syndrome.

closely monitored for recurrence of ICANS. The optimal dose and duration of corticosteroid administration remain uncertain, and further research is needed to determine whether long-term high-dose corticosteroids affect the therapeutic effect of CAR-T therapy.

Unlike tocilizumab, siltuximab binds to circulating IL-6 and further reduces active IL-6 in the CNS; therefore, it could be an effective drug for treating patients who did not respond to tocilizumab or corticosteroids (Riegler et al., 2019). However, further clinical trials are needed to confirm this finding. In addition, the IL-1 receptor antagonist, anakinra, showed an excellent therapeutic effect on CRS and ICANS and can be an effective drug for treating steroid-refractory ICANS with or without CRS (Norelli et al., 2018; Wehrli et al., 2022). Relevant clinical trials on the early prophylactic use of anakinra are also underway (Table 5). Sachdeva et al. (2019) found that when CAR-T cells were knocked out for GM-CSF, CRS-related inflammatory cytokines released by monocytes decreased. In another *in vivo* study, Sterner et al. (2019) found that blocking GM-CSF improved CRS and ICANS while enhancing the antitumor activity of CAR-T cells in mouse models. Blocking GM-CSF is a possible mechanism for treating CRS; however, more trials are needed to support its application in humans. For high-risk patients with ICANS, levetiracetam 750 mg orally or intravenously every 12 h on the day of CAR-T cell infusion is recommended to prevent seizures (Neelapu et al., 2018a). Antiepileptic drugs, such as levetiracetam, phenobarbital and benzodiazepines, are known for treating epilepsy (Neelapu, 2019). In conclusion, no substantial evidence exists that blocking a single cytokine can prevent or improve ICANS. Moreover, since the pathogenesis of ICANS is still unclear, the treatment of ICANS is mostly symptomatic and not causative.

According to the management opinions (Table 9) put forward by each guideline, management is mainly performed for ICANS without concurrent CRS and ICANS with concurrent CRS. Tocilizumab (8 mg/kg, IV) is recommended for treating ICANS in patients with concurrent CRS. Dexamethasone (10 mg q6h, IV) and methylprednisolone (1 mg/kg q12h, IV) were administered to treat ICANS without concurrent CRS or ICANS with concurrent CRS that did not respond to anti-IL-6 therapy. For life-threatening grade 4 ICANS, high-dose methylprednisolone (1 g, IV) could be used as maintenance therapy until it improves to grade 1 and then slowly tapered (Table 9). The therapeutic dose of dexamethasone and time interval for different grades of ICANS were slightly different (Table 9). In addition to the administration of drugs, daily supportive care and neurological examinations should be performed for patients with ICANS.

Organ system toxicities of CAR-T cell therapy

Cardiovascular toxicity concurrent cytokine release syndrome

CRS is also a crucial factor that induces adverse cardiovascular events that can lead to severe cardiovascular complications. Similar to other systemic inflammatory response syndromes, sinus tachycardia and hypotension are the most common clinical signs (Totzeck et al., 2022). Fever caused by CRS is the inducement of sinus tachycardia (Ghosh et al., 2020). Other cardiovascular complications associated with CRS include increased serum troponin levels, decreased left ventricular ejection fraction (LVEF), cardiogenic shock, arrhythmias, corrected QT prolongation, decompensated heart failure, and cardiovascular death (Asnani, 2018; Burstein et al., 2018; Ganatra et al., 2019a; Alvi et al., 2019).

TABLE 9 Management of ICANS.

ICANS management system	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Neelapu, 2019; Castaneda-Puglianini and Chavez, 2021)	ASCO guideline (Santomasso et al., 2021)
Grade 1	• Supportive care, aspiration precautions, IV hydration	• Aspiration precautions and IV hydration	• No concurrent CRS: offer supportive care with IV hydration and aspiration precautions
	• Low doses of lorazepam (0.25–0.5 mg q8h, IV) or haloperidol (0.5 mg q6h, IV) can be used, for agitated patients	• Seizure prophylaxis with levetiracetam	• With concurrent CRS: administer tocilizumab (8 mg/kg, IV); Repeat q8h as needed; Limit to a maximum of three doses in a 24 h period; Maximum total of four doses; Caution with repeated tocilizumab doses in patients with ICANS; Consider adding corticosteroids to tocilizumab past the first dose
	• MRI of the brain with and without contrast, CT scan of the brain can be performed if MRI of the brain is not feasible	• EEG	
	• Daily 30 min EEG until toxicity symptoms resolve	• Imaging of brain (MRI preferred if no contraindication)	
	• Levetiracetam (750 mg, q12h) to prevent epilepsy	• Consider tocilizumab if there is concurrent CRS	
	• Tocilizumab (8 mg/kg, IV) or siltuximab (11 mg/kg, IV), if ICANS is associated with concurrent CRS	• Neurocognitive assessment q6h using ICE scoring system	
Grade 2	• Supportive care and neurological work-up as described for grade 1 ICANS	• Supportive care as in grade 1	• No concurrent CRS: offer supportive care as per grade 1; For high-risk products or patients, consider dexamethasone (10 mg, IV) two doses (or equivalent) and reassess. Repeat q 6–12 h if no improvement; Taper steroids as clinically appropriate once symptoms improve to grade 1
	• Tocilizumab (8 mg/kg, IV) or siltuximab (11 mg/kg, IV) if associated with concurrent CRS	• Consider dexamethasone (10 mg q6h, IV) or its equivalent of methylprednisolone	• With concurrent CRS: consider ICU transfer if ICANS associated with ≥ grade 2 CRS; Administer tocilizumab as per grade 1; If refractory to tocilizumab past the first dose, initiate dexamethasone (10 mg q6-12h, IV) or methylprednisolone equivalent (1 mg/kg q12h, IV) until improvement to grade 1, and then taper
	• Dexamethasone (10 mg q6h, IV) or methylprednisolone (1 mg/kg q12h, IV) if refractory to anti-IL-6 therapy or for ICANS without concurrent CRS	• Tocilizumab if concurrent CRS	
	• Consider transferring patient to ICU if ICANS associated with grade ≥2 CRS		
Grade 3	• Supportive care and neurological work-up as described for grade 1 ICANS	• Supportive care as in grade 1	• Transfer patient to ICU
	• Tocilizumab (8 mg/kg, IV) or siltuximab (11 mg/kg, IV) if associated with concurrent CRS	• Dexamethasone (10–20 mg q6h, IV) or its equivalent of methylprednisolone	• No concurrent CRS: administer dexamethasone (10 mg q6h, IV) or methylprednisolone equivalent (1 mg/kg q12h, IV)
	• Corticosteroids as outlined for grade 2 ICANS if symptoms worsen despite anti-IL-6 therapy, or for ICANS without concurrent CRS	• Control seizures with benzodiazepines (for short-term control) and levetiracetam ± phenobarbital and/or lacosamide	• With concurrent CRS: administer tocilizumab as per grade 1; If refractory to tocilizumab past the first dose, initiate dexamethasone (10 mg q6h, IV) or methylprednisolone equivalent (1 mg/kg q12h, IV) until improvement to grade 1, and then taper
	• ICU transfer and repeat neuroimaging (CT or MRI every 2–3 days) are recommended	• High-dose methylprednisolone (1 g/day) for focal/local edema	
		• Transfer to ICU	

(Continued on following page)

TABLE 9 (*Continued*) Management of ICANS.

ICANS management system	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Neelapu, 2019; Castaneda-Puglianini and Chavez, 2021)	ASCO guideline (Santomasso et al., 2021)
Grade 4	• Supportive care and neurological work-up as described for grade 1 ICANS •Anti-IL-6 therapy and repeat neuroimaging as described for grade 3 ICANS • High-dose corticosteroids methylprednisolone (1 g/day, IV) for 3 days continued until improvement to grade 1 ICANS and then taper •ICU monitoring, consider mechanical ventilation for airway protection	• Supportive care as in grade 1 • High-dose methylprednisolone (1 g/day) for 3 days followed by taper • Control seizures with benzodiazepines (for short-term control) and levetiracetam ± phenobarbital and/or lacosamide • Imaging of spine for focal motor weakness • Lower ICP by hyperventilation, hyperosmolar therapy with mannitol/hypertonic saline, and/or neurosurgery consultation for ventriculoperitoneal shunt in patients with cerebral edema • Transfer to ICU	• Admit patient to ICU • No concurrent CRS: administer high-dose methylprednisolone (1g, IV) one to two times per day for 3 days; If not improving, consider 1g of methylprednisolone two to three times per day or alternate therapy; Continue corticosteroids until improvement to grade 1, and then taper; Status epilepticus to be treated as per institutional guidelines • With concurrent CRS: administer tocilizumab as per grade 1 in addition to methylprednisolone (1g, IV) one to two times per day for 3 days; If not improving, consider methylprednisolone (1g, IV) two to three times a day or alternate therapy; Continue corticosteroids until improvement to grade 1, and then taper

Tocilizumab IV over 1 h, Maximum amount of tocilizumab per dose is 800 mg.
ICANS, immune effector cell-associated neurotoxicity syndrome; CARTOX, CAR-T cell therapy associated toxicity; IV, intravenous; q6h, every 6 hours; q8h, every 8 hours; q12h, every 12 hours; MRI, magnetic resonance imaging; CT, computed tomography; EEG, electroencephalogram; CRS, cytokine release syndrome; ICU, intensive-care unit; ASTCT, American Society for Transplantation and Cellular Therapy; ICE, immune Effector Cell-Associated Encephalopathy score; ICP, intracranial pressure; ASCO, American Society of Clinical Oncology.

Cardiotoxicity occurs between 2 and 24 days after CAR-T cell infusion (Ganatra et al., 2020; Lefebvre et al., 2020). Alvi et al. (2019) found in a retrospective cohort study of 137 patients treated with CAR-T that cardiovascular events occurred only in cases of CRS ≥ grade 2, with an incidence of 12–28%. Ganatra et al. (2020) performed echocardiographic follow-up in 116 patients with CRS ≥ grade 2 and found that 10.3% developed new or worsening cardiomyopathy. Elevated troponin and inappropriate administration of tocilizumab in CRS patients after CAR-T infusion are associated with an increased risk of subsequent cardiovascular events (Alvi et al., 2019; Ghosh et al., 2020).

Children with hematological cancers have a higher incidence of adverse cardiovascular events after CAR-T therapy. Fitzgerald et al. (2017) analyzed 39 pediatric patients and found that 14 (36%) developed cardiovascular dysfunction after CAR-T therapy. Another study of 98 pediatric patients revealed hypotension in 24 patients (24%) and life-threatening hypotension in 21 patients (21%) (Burstein et al., 2018). In an adult CAR-T-related cardiovascular adverse event study, Alvi et al. (2019) found that 17 (12%) patients experienced cardiovascular events such as cardiovascular death, decompensated heart failure, and arrhythmias. In another study, Lefebvre et al. (2020) found that 31 adult patients (21%) developed major adverse cardiovascular events (MACE), including heart failure and arrhythmia. Although CRS is the leading cause of cardiovascular toxicity during CAR-T therapy, other factors, such as tumor lysis syndrome (TLS), infection, and primary cardiovascular events, need to be excluded. In addition to treating hematological cancers, CAR-T targeting fibroblast activating protein (FAP) is effective in mice with cardiac fibrosis (Aghajanian et al., 2019). CAR-T cell therapy exhibits cardiovascular toxicity and the potential to treat heart diseases.

Pathogenesis of cardiovascular toxicity

The specific mechanism of cardiovascular adverse reactions in CAR-T treatment is not precise, and the potential mechanisms (Figure 3) include: *1*) severe CRS results in hemodynamic instability, capillary leakage, and DIC, and increased serum concentrations of VWF and Ang-2 (Hay et al., 2017); *2*) IL-6 is a crucial cytokine leading to CAR-T therapy-related CRS, and a significant increase in IL-6 is closely related to adverse cardiovascular reactions (Stein-Merlob et al., 2021). Pathan et al. (2011) found that IL-6 (serum endothelial activating cytokine)

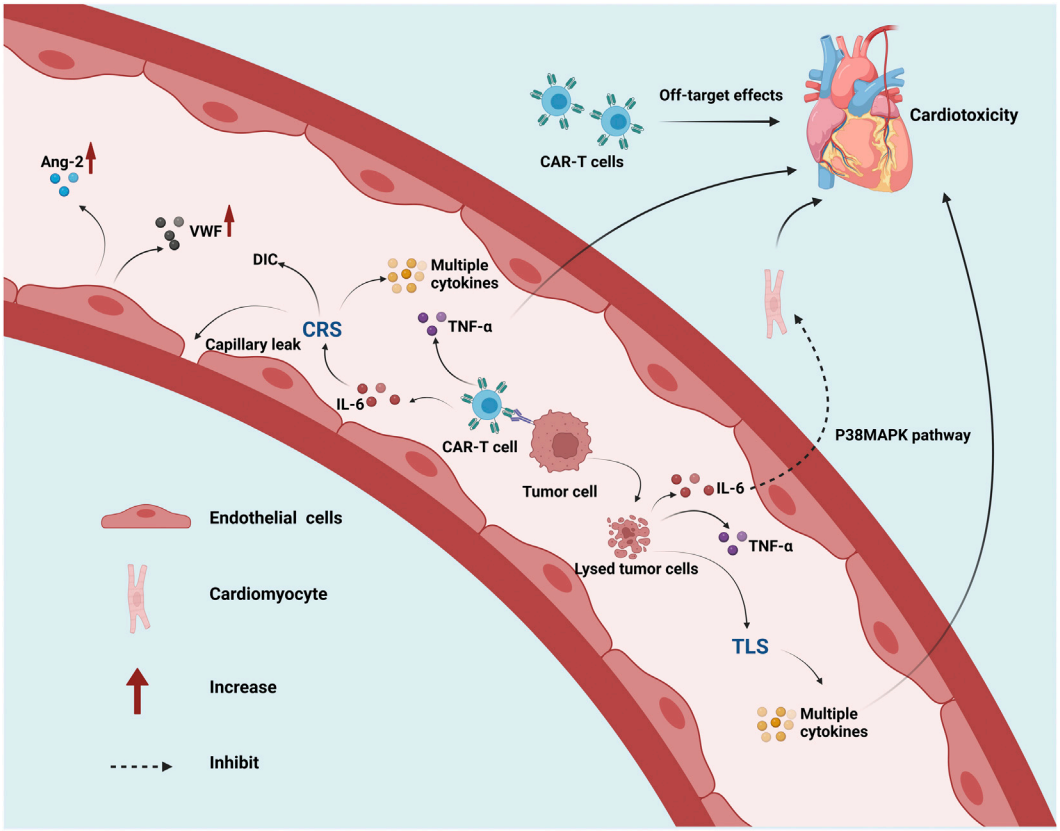

FIGURE 3
Pathogenesis of cardiovascular toxicity during CAR-T therapy. Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome; IL, interleukin; TNF-α, tumor necrosis factor alpha; MAPK, mitogen-activated protein kinase; TLS, tumor lysis syndrome; DIC, disseminated intravascular coagulation; Ang-2, angiopoietin-2; VWF, von willebrand factor. This figure created with BioRender.com.

inhibits the contractile function of myocardium through the p38MAPK signaling pathway; *3*) Increased expression of TNF-α in the myocardium enhances cardiotoxicity (Michel et al., 2022); *4*) Direct cardiotoxicity caused by off-target cross-reaction of CAR-T cells to actin (Linette et al., 2013); *5*) Arrhythmias induced by TLS-related metabolic disorders (Ganatra et al., 2019b).

Risk factors of cardiovascular toxicity

Currently, there is no exact cardiovascular risk assessment method, and formulating relevant rules requires multidisciplinary cooperation. High-risk factors or predictors of severe cardiovascular toxicity include *1*) cardiotoxic therapy such as anthracyclines and chest radiotherapy (Ghosh et al., 2020); *2*) Patients with cardiovascular complications such as hypertension, atrial fibrillation/flutter, coronary ischemia, and structural heart disease (Ganatra et al., 2019a); *3*) Higher age, CRS grade ≥2 and hyperlipidemia are all risk factors for inducing cardiovascular toxicity in CAR-T cell therapy (Alvi et al., 2019; Ganatra et al., 2020); *4*) Higher baseline creatinine levels were independently associated with MACE, and the use of statins, insulin, and aspirin was associated with adverse cardiovascular reactions (Lefebvre et al., 2020).

Monitoring and treatment of cardiovascular toxicity

Although cardiovascular adverse events may be transient and reversible in patients with sufficient cardiovascular reserve, they are particularly challenging for high-risk patients (Ganatra et al., 2019a). It is important to identify and predict patients at risk of fatal cardiotoxicity is crucial for initiating early interventions and reducing the risk of CAR-T therapy. Exercise tolerance should be evaluated in patients with a history of cardiovascular disease and cardiovascular abnormalities detected in the initial examination, and further tests should be performed to rule out potential occult coronary ischemia or other structural heart diseases to assess tolerance to hemodynamic changes induced by CRS after CAR-T therapy (Ganatra et al., 2019a). Shalabi et al. (2020) proposed early intervention for possible severe vascular toxicity through monitoring and analysis of echocardiography, baseline LV global

longitudinal strand (GLS), and cardiac biomarkers (troponin and pro-B-type native peptide). In addition, Totzeck et al. (2022) suggested that electrocardiogram (ECG), echocardiography, high-sensitivity cardiac troponin (hscTn), N-terminal pro-brain natriuretic peptide (NT-proBNP), and other examinations should be performed on the seventh day of CAR-T treatment. High-risk patients should be followed up for 3 months, to determine early and late cardiovascular toxicity. Cardiovascular assessment before and during CAR-T therapy is helpful for the early identification of patients with insufficient cardiovascular reserve. Before treatment, the disease status of the patient, medication, and treatment histories should be investigated in detail. In addition, the cardiac function of the patient should be monitored using 12-lead ECG, echocardiography, cardiac biomarkers and other methods.

First, patients with existing cardiovascular disease should be actively treated with drugs to control the disease. Second, the occurrence of CRS and cardiovascular toxicity in the early stage of CAR-T immunotherapy should be assessed for severity (Totzeck et al., 2022). Tocilizumab should be a priority when CRS is combined with cardiotoxicity, followed by glucocorticoids if the condition cannot be controlled (Schuster et al., 2017). Abnormal levels of cardiac biomarkers before and after CAR-T therapy and left ventricular systolic dysfunction (LVSD) are found on transthoracic echocardiography (TTE) or cardiac magnetic resonance (CMR) imaging, prompting β-blockers, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers to be used as cardiac protection (Ghosh et al., 2020). A retrospective study found that 94% of patients with elevated troponin and CRS ≥2 grade had cardiovascular toxicity, and elevated troponin levels are a possible indication for treatment with tocilizumab (Alvi et al., 2019). The results showed that patients could benefit from early tocilizumab treatment when troponin levels are elevated (Alvi et al., 2019).

Hematologic and infectious toxicity

CAR-T cell therapy-related hematologic toxicities include cytopenia (neutropenia, thrombocytopenia, leucopenia, anemia, or any combination of these), B-cell aplasia, and coagulation disorders. Among them, the incidence of neutropenia is 33–94%, thrombocytopenia is 30–80%, leucopenia is 31–47%, and anemia is 30–68% (Zhao et al., 2018; Fried et al., 2019; Locke et al., 2019; Schuster et al., 2019). Lymphodepleting chemotherapy is considered the leading cause of early cytopenia, but the cause of late cytopenias remains unclear (Fried et al., 2019). Close attention to possible herpes zoster and *Pneumocystis jirovecii* pneumonia and drug prophylaxis for at least 1 year is recommended if fludarabine is used for lymphodepletion chemotherapy (Neelapu, 2019). Persistent cytopenia is associated with infectious complications such as late fatal encephalitis and systemic mycosis (Kansagra et al., 2019). CAR-T cell infusion is not recommended for patients with bacterial infection if the fever is not well controlled and the bacterial culture is not negative for 48 h (Maus et al., 2020). Furthermore, patients whose viral and fungal infections have not been effectively controlled are not recommended to continue CAR-T therapy (Maus et al., 2020). Prophylaxis against infections with antibacterial and fungal agents should be considered in patients with prolonged grade 4 neutropenia (Neelapu, 2019). Fried et al. (2019) showed that patients with severe CRS and recent stem cell transplantation (<1 year) were more likely to develop late hematologic toxicity and that serum SDF-1 levels were associated with neutropenia. In the ASCO guidelines, granulocyte-colony stimulating factor (G-CSF) rather than GM-CSF is considered to treat CAR-T-induced neutropenia (Santomasso et al., 2021). Furthermore, G-CSF is strongly recommended for the treatment of long-term neutropenia; however, to avoid interaction with the peak CRS risk and CAR-T expansion period, only use G-CSF after 14 days of CAR-T cell infusion or CRS resolution (Maus et al., 2020; Schubert et al., 2021). GM-CSF is not recommended because it can aggravate CRS (Maus et al., 2020). Patients with thrombocytopenia are at an increased risk of gastrointestinal, genitourinary, intracranial, and pulmonary bleeding and should be closely monitored for 1 month after CAR-T therapy (Johnsrud et al., 2021).

CD19 is expressed in normal B-cells and B-cell malignancies; therefore, B-cell aplasia is a common toxicity of anti-CD19 CAR-T therapy (Townsend et al., 2018; Roddie et al., 2021). The significant signs were low B-cell counts and immunoglobulin levels (Santomasso et al., 2021). Fortunately, B-cell aplasia is clinically tolerated because hypogammaglobulinemia resulting from CD19 ablation of B cells can be managed with intravenous immunoglobulin (Maude et al., 2018; Miao et al., 2021). Approximately 51–56.6% of patients with hematologic malignancies develop coagulopathy after CAR-T cell therapy (Miao et al., 2021). Further deterioration of coagulopathy can cause DIC, and patients with severe CRS have a higher incidence of coagulopathy and DIC (Miao et al., 2021).

HLH/MAS

In severe CRS, ferritin is considerably elevated, accompanied by high fever, hepatosplenomegaly, hemocytopenia, and coagulopathy, revealing the possible occurrence of hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS) (Pehlivan et al., 2018; Frigault and Maus, 2020; Spiegel et al., 2021). The incidence of HLH/MAS in CAR-T cell therapy was approximately 3.48%, but the mortality rate was up to 80%, and the prognosis was poor (Alblooshi et al., 2020; Sandler et al., 2020a; Sandler et al., 2020b).

HLH/MAS should be considered in CRS patients with peak serum ferritin levels >10,000 ng/ml within 5 days after CAR-T

infusion and any two of the following: grade ≥3 organ toxicity involving the liver, kidney, or lung (according to CTCAEv5.0), or hemophagocytosis in the bone marrow or other organs (Neelapu et al., 2018a). In patients with suspected secondary HLH/MAS, testing for fasting triglycerides and soluble IL-2R is recommended (Ragoonanan et al., 2021). Currently, no targeted therapies are available for patients with HLH/MAS. In principle, more aggressive immunosuppressive therapy should be administered at an early stage, with glucocorticoids and tocilizumab as the mainstay of treatment (Neelapu et al., 2018a; Miao et al., 2021). Etoposide should only be used in patients with late-onset HLH/MAS who are refractory to tocilizumab (Maus et al., 2020). Moreover, for the treatment of late-onset HLH/MAS, third-line CRS agents such as anakinra at starting doses of 5–8 mg/kg/day should be considered (Maus et al., 2020; Shah et al., 2020). Other treatments for late-onset HLH/MAS include intrathecal methotrexate and cytarabine, but these are controversial and lack formal assessment (Horne et al., 2017; Neelapu et al., 2018b; Hashmi et al., 2019).

Skin toxicity

The incidence of rash in FDA-approved anti-CD19 CAR-T therapies (axicabtagene ciloleucel, tisagenlecleucel, and brexucabtagene autoleucel) is 9–22% (Nusbaum et al., 2021). However, attention should be paid to distinguishing an allergic skin reaction caused by chemotherapy, antibacterial agents, DMSO, or other drugs. CAR-T skin toxicities usually manifest as papules, maculopapular eruptions, purpura, urticarial rash, bullous eruptions, dry skin, and oral mucositis (Rubin et al., 2016; Wang et al., 2017; Hu et al., 2020; Ramos et al., 2020; Nusbaum et al., 2021). Skin toxicity occurs 5 days to 19 months after CAR-T infusion (Rubin et al., 2016; Hu et al., 2020). However, there are few reports on cases, literature reviews, and clinical trials of skin toxicity caused by CAR-T cell therapy; thereby providing a gap for research.

Wang et al. (2017) found that 11.1% of patients developed urticarial-like rashes after infusion of anti-CD30 CAR-T cells. Another study elaborated that 48% of patients developed maculopapular rashes after infusion of anti-CD30 CAR-T cells; these rashes were transient and did not require specific treatment (Ramos et al., 2020). Rubin et al. (2016) reported adverse skin reactions, including secondary cutaneous malignancies, disseminated infection, eruptions with unusual mononuclear cell dermal infiltrate, and transient eruptions suggestive of the "eruption of lymphocyte recovery" after anti-CD19 CAR-T treatment in five patients. In phase I clinical trials of anti-EGFR CAR-T cells in the treatment of metastatic pancreatic cancer, dry skin, dermatitis herpetiformis, oral mucositis, and other skin toxicities were found (Liu et al., 2020). In addition, one case reported that the patient developed a diffuse maculopapular rash 5 days after CAR-T infusion, which then evolved into tension bullae (Hu et al., 2020). The main cells in the bullous fluid are CAR-T cells, and the concentrations of IL-6 and IFN-γ in the bullous fluid are significantly higher than those in serum (Hu et al., 2020). IL-6 is a critical cytokine in CRS, and reduced immune function induced by CRS lead to skin infections in such patients. Moreover, the secretion of other pro-inflammatory cytokines during CAR-T therapy exacerbates the severity of skin reactions. Skin toxicity can cause psychological and physical harm to patients, and severe skin toxicity can lead to death. As CAR-T therapy becomes more widely used in cancer treatment, doctors should pay attention to the possible adverse skin reactions and manage patients accordingly.

Other organ toxicities concurrent cytokine release syndrome

CRS is associated with various clinical findings, including fever and multiple organ dysfunction (pulmonary, renal, hepatotoxicity, and gastrointestinal toxicity) (Ghosh et al., 2020). In a pharmacovigilance and meta-analysis study, it was found that the incidence of CAR-T cell-related pulmonary toxicity (respiratory failure) was 9.0%, nephrotoxicity (acidosis, adrenal insufficiency, electrolyte disturbances, and renal failure) was 6.0%, and hepatotoxicity (liver injury) was 1.5% (Dolladille et al., 2021). The results of phase I/II clinical studies by Hay et al. (2017) showed that 3 (30.0%) patients with grade ≥4 CRS developed grade ≥3 acute kidney injury, one patient required hemodialysis for 15 days, and nine patients developed liver dysfunction (elevated aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin). In addition to causing pulmonary, hepatic, and renal toxicity, gastrointestinal toxicity also occurred after the infusion of CAR-T cells. The most common gastrointestinal toxicities were diarrhea, vomiting, bleeding, and nausea. (Zeng et al., 2020). Furthermore, Zeng et al. (2020) showed that damage to the mucosal barrier leads to the spread of bacteria from the gastrointestinal tract into the blood, resulting in bacteremia and sepsis if patients present with simultaneous gastrointestinal bleeding and diarrhea. Severe CRS can easily lead to severe multi-organ system toxicity, which is one of the main reasons limiting the safe application of CAR-T therapy.

Potential cytokine release syndrome therapeutic drugs and methods

Tocilizumab is an FDA-approved treatment of CAR-T treatment-induced CRS. Corticosteroids are also used in the treatment of CRS as first- and second-line therapies. As a tocilizumab congener, siltuximab blocks IL-6 signaling by

binding to IL-6. As siltuximab has a higher affinity for IL-6 than tocilizumab for IL-6R, it is considered another potential drug for the treatment of CRS. If the patient does not respond to tocilizumab and corticosteroids, siltuximab may be administered at a dose of 11 mg/kg BW/dose (Riegler et al., 2019; Schubert et al., 2021). However, the FDA has not approved siltuximab for the treatment of CAR-T cell-induced CRS and ICANS. In addition to anti-IL-6 therapies, ongoing trials are exploring the use of the JAK1 selective inhibitor itacitinib to prevent CRS induced by tisagenlecleucel or axicabtagene ciloleucel (NCT04071366) and the JAK 1/2 inhibitor ruxolitinib for the treatment of HLH/MAS (Ahmed et al., 2019; Ragoonanan et al., 2021). In mouse models of CRS, studies have shown that administering a short course of the tyrosine kinase inhibitor dasatinib early after CAR-T cell infusion can avoid fatal CRS by inhibiting CAR-T cell functions, such as proliferation and cytokine secretion (Mestermann et al., 2019; Weber et al., 2019). In addition, ibrutinib was found to attenuate CRS while increasing antitumor efficacy in mice treated with CAR-T (Fraietta et al., 2016; Ruella et al., 2017). Patients who received ibrutinib 2 weeks before surgery and 3 months after CAR-T cell infusion had lower CRS severity than those who did not receive ibrutinib (Sheth and Gauthier, 2021). In addition to the above drugs, TNF-α inhibitors, etanercept, and infliximab; IL-1 inhibitor, anakinra; IFN-γ inhibitor fontolizumab, and others are promising drugs for the treatment of CRS (Riegler et al., 2019).

Similar to the treatment of uremia, removing harmful substances and pro-inflammatory cytokines from blood is an effective treatment for severe CRS that is ineffective with current drug therapy. A 23-year-old man with a typical CRS response was effectively controlled with dexamethasone (10 mg, q6h) and plasmapheresis after failing to control his condition with glucocorticoids and tocilizumab (Xiao et al., 2019). Another study showed that when tocilizumab and glucocorticoid therapy were ineffective in controlling CAR-T cell-induced adverse effects, hemofiltration immediately ameliorated severe CRS and induced multiple organ dysfunction (Liu et al., 2018). In addition, the treatment of a 65-year-old man with grade 4 CRS with *in vitro* cytokine adsorption showed a more than 50% reduction in multiple pro-inflammatory cytokines levels (Stahl et al., 2020).

Another way to reduce the adverse reactions of CAR-T cells from the root and improve their safety is to set a "suicide" switch on CAR-T cells. When CAR-T cells are infused, this switch activates and consumes CAR-T cells on demand at the desired time. Commonly used "suicide switches" include inducible caspase 9 (iC9), herpes simplex virus thymidine kinase (HSV-TK), CD20, and truncated epidermal growth factor receptor (EGFRt) (Gargett and Brown, 2014; Greco et al., 2015; Yu et al., 2019; Klopp et al., 2021; Warda et al., 2021). Among these, HSV-TK and iC9 have been integrated into CAR-T cells and tested clinically (Andrea et al., 2020). Because HSV-TK is a cell cycle-dependent suicide gene that needs to function based on ganciclovir, iC9 is recommended for CAR-T cell therapy rather than HSV-TK (Tiberghien et al., 2001). Existing clinical trials using iC9 CAR-T cells include NCT03016377, NCT03594162, NCT03696784, and NCT03579927 (Andrea et al., 2020).

Summary and prospects

CAR-T cell therapy is one of the most attractive treatment options for patients with r/r hematological malignancy. It also has considerable potential for treating other malignancies. However, CRS and severe adverse reactions in the organ system after CAR-T cell infusion can be fatal to patients, and are also essential factors preventing the early application of CAR-T in hematological malignancies. It is especially urgent to clarify the symptoms, pathophysiology, grading criteria, and therapeutic measures of related adverse reactions to ensure the safety and efficacy of CAR-T immune cell therapy.

For severe CAR-T cell-related adverse reactions, rapid progression of toxicity causes irreversible harm to the body. Therefore, early identification and intervention effectively reduce the incidence and mortality of severe adverse reactions. In addition to considering preventive and therapeutic measures for CRS-related severe adverse reactions after CAR-T infusion, the design of the structure of CAR-T (such as multiple target antigens, adding "suicide" genes), manufacturing methods of CAR-T cell products, and optimizing the composition and infusion dose of CAR-T cell products curbs the incidence of severe adverse reactions concurrent with CRS from the cause. The target antigen of CAR-T therapy is key to treating cancer and is the main reason for target or off-target effects. Designing dual-target antigens are beneficial for reducing toxicities caused by insufficient target expression specificity. Multiple CAR-T cell clinical trials (NCT02903810, NCT03098355, NCT03241940) targeting CD19 and CD22 are currently ongoing (Annesley et al., 2018). With the development of relevant research and the advancement of gene screening technology, understanding the specific mutations in tumors and selecting tumor-specific antigens for precise, personalized treatment will substantially benefit the patients. In addition, using CRISPR/Cas9 technology to control the production of pro-inflammatory mediators in CAR-T cells is another effective way to improve severe CRS-related adverse reactions (Salas-Mckee et al., 2019). However, these methods are still in the research phase and need to be tested in clinical trials, which is a lengthy and costly process.

Unlike surgery, chemoradiotherapy, and immune checkpoint inhibitors, T-cells in CAR-T therapy can exist in the body for up

to 10 years (Scholler et al., 2012). However, CAR-T therapy first approved in the market only 5 years; therefore, understanding the mechanisms of related adverse reactions and effectively treating them is currently ongoing. Unresolved issues in CAR-T therapy include the following:

- Siltuximab, etanercept, infliximab, fontolizumab, anakinra, itacitinib, ruxolitinib, dasatinib, and ibrutinib may be effective in the treatment of CRS; however, clinical trials on these are sparse.
- Although the ZUMA-1 cohort 6 study showed that prophylactic use of corticosteroids in patients receiving axicabtagene ciloleucel for LBCL could reduce the incidence of grade ≥3 CRS and ICANS, there is no clear consensus on the requirement of the prophylactic use of corticosteroids for all patients receiving CAR-T cell infusion (Oluwole et al., 2021).
- Some progress has been made in research on biomarkers for predicting the toxicity of CAR-T therapy, but there are no precise biomarkers to predict its efficacy. The main difficulty is that different CAR-T products, patient ages, and measurement times after infusion may require distinct cell biomarkers.
- Whether the long-term high-dose use of corticosteroids affects the effect of CAR-T therapy requires further research.

In summary, close clinical monitoring and early prevention, diagnosis, and treatment of adverse reactions are vital to reducing adverse events. With the concerted efforts of researchers, doctors, pharmacists, and nurses, CAR-T therapy will eventually become a safer and more effective conventional treatment for cancers.

Author contributions

YL (11th author) conceived and supervised the project. YL (1st author) summarized the literature and drafted the manuscript. YM collected and organized the adverse reaction management guidelines and revised the manuscript. All authors have contributed to the manuscript and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Potential cardiotoxicity induced by Euodiae Fructus: *In vivo* and *in vitro* experiments and untargeted metabolomics research

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Background: Euodiae Fructus, a well-known herbal medicine, is widely used in Asia and has also gained in popularity in Western countries over the last decades. It has known side effects, which have been observed in clinical settings, but few studies have reported on its cardiotoxicity.

Methods: In the present study, experiments using techniques of untargeted metabolomics clarify the hazardous effects of Euodiae Fructus on cardiac function and metabolism in rats in situations of overdosage and unsuitable syndrome differentiation. *In vitro* assays are conducted to observe the toxic effects of evodiamine and rutaecarpine, two main chemical constituents of Euodiae Fructus, in H9c2 and neonatal rat cardiomyocytes (NRCMs), with their signaling mechanisms analyzed accordingly.

Results: The cardiac cytotoxicity of evodiamine and rutaecarpine in *in vivo* experiments is associated with remarkable alterations in lactate dehydrogenase, creatine kinase, and mitochondrial membrane potential; also with increased intensity of calcium fluorescence, decreased protein expression of the cGMP-PKG pathway in H9c2 cells, and frequency of spontaneous beat in NRCMs. Additionally, the results in rats with Yin deficiency receiving a high-dosage of Euodiae Fructus suggest obvious cardiac physiological dysfunction, abnormal electrocardiogram, pathological injuries, and decreased expression of PKG protein. At the level of endogenous metabolites, the cardiac side effects of overdose and irrational usage of Euodiae Fructus relate to 34 differential metabolites and 10 metabolic pathways involving among others, the purine metabolism, the glycerophospholipid metabolism, the glycerolipid metabolism, and the sphingolipid metabolism.

Conclusion: These findings shed new light on the cardiotoxicity induced by Euodiae Fructus, which might be associated with overdose and unsuitable syndrome differentiation, that comes from modulating the cGMP-PKG pathway and disturbing the metabolic pathways of purine, lipid, and amino

acid. Continuing research is needed to ensure pharmacovigilance for the safe administration of Chinese herbs in the future.

KEYWORDS

Euodiae Fructus, cardiotoxicity, H9c2, neonatal rat cardiomyocytes, molecular mechanism, untargeted metabolomics

1 Introduction

Euodiae Fructus, commonly known as "Wuzhuyu" in Chinese, is a potent internal-warming traditional herbal medicine, and has been extensively used in clinical treatment due to its analgesic, antiemetic, anti-inflammatory, antidiarrheal, neuroprotective, and cardioprotective activities (Lee et al., 2011; Liao et al., 2011; Cai et al., 2014; Li and Wang, 2020). Although Euodiae Fructus has demonstrated promising therapeutic effects for headaches, abdominal pain, diarrhea, and vomiting induced by pathogenic cold, its potential cardiotoxicity has also been recently recognized (Zeng and Jiang, 2010; Yang et al., 2017). With regard to the herb itself, potential cardiotoxicity might be related to bioactive substances with the dual characteristics of efficacy and toxicity, such as evodiamine and rutaecarpine. On the one hand, evodiamine and rutaecarpine can produce beneficial pharmacodynamic and pharmacological effects for anti-arrhythmia, myocardial protection and recovery, as evidenced by previous research based on experiments around isolated atria in guinea pigs, cardiac fibrosis in mice, and myocardial ischemia-reperfusion injury and cardiac hypertrophy in rats (Kobayashi et al., 2001; Rang et al., 2004; Jiang et al., 2017; Tian et al., 2019; Li et al., 2021; Zhan et al., 2021). On the other hand, the toxicological effects of evodiamine on the heart, which might be associated with oxidative stress, have been observed through *in vivo* and *in vitro* experiments with primary neonatal rat cardiomyocytes and zebra fish (Yang et al., 2017). In addition, dehydroevodiamine and hortiamine might be responsible for potential proarrhythmic effects, because they have been identified from the extract of Euodiae Fructus as hERG inhibitors *via* the technologies of HPLC-microfractionation, patch clamp, and so on (Zhan et al., 2021).

It is worth noting that irrational use of TCM herbs, including overdose, self-medication, and so forth, can occasionally induce serious adverse reactions or even fatal poisoning (Zhang et al., 2012; Chan et al., 2015; Li et al., 2018a; Zhang et al., 2020). The distinct cardiovascular activity of Euodiae Fructus might thus be transformed into underlying cardiac toxicity under different physiological, pathological, and clinical conditions, with overdose and unsuitable syndrome differentiation contributing to the cardiac risk. Despite the large number of studies focusing on the herb-related adverse reactions and corresponding mechanisms of Euodiae Fructus, the current profiles of the cardiac toxicity of Euodiae Fructus are not well delineated (Cai et al., 2014; Zhang et al., 2015; Pan et al., 2020). There is overwhelming research evidence that the cGMP-PKG pathway in the heart plays a principal role in regulating myocardial function and electrophysiology through multiple downstream targets, involving the G-protein coupled receptor, the calcium signaling pathway, and so on (Inserte and Garcia-Dorado, 2015; Park et al., 2018; Nakamura and Tsujita, 2021). Given this, advanced and comprehensive methodologies were applied in *in vivo* and *in vitro* experiments and in untargeted metabolomics, such as electrocardiograms (ECGs), serum biomarkers, histopathology, and metabolomics, to better characterize the manifestations of cardiac toxicity in H9c2 cells, neonatal rat cardiomyocytes (NRCMs), and rats, and to further illustrate the signaling mechanisms and endogenous metabolites for the related poisoning.

2 Materials and methods

The present study, focusing on the cardiotoxicity induced by Euodiae Fructus, was conducted by cell experiments *in vitro* of H9c2 and NRCMs, by experiments *in vivo* of the model of rats with either Yang or Yin deficiency, and by untargeted metabolomics research on the serum of the group with significant cardiotoxicity (Figure 1).

2.1 *In vitro* experiments: Cardiotoxicity from evodiamine and rutaecarpine in H9c2 cells

2.1.1 Cell culture

Rat cardiomyocyte-derived H9c2 cells from the National Infrastructure of Cell Line Resources (Chinese Academy of Medical Sciences, Beijing, China) were cultivated in Dulbecco's Modified Eagle Medium (DMEM), high glucose (Biological Industries, Israel) supplemented with 10% fetal bovine serum (FBS, Biological Industries, Israel), 1% penicillin-streptomycin (Corning, United States) at 37°C in a 5% CO_2 atmosphere. *In vitro* experiments were performed using H9c2 cardiomyocytes between passages 15 and 20, which were subcultured at a confluence of approximately 80%.

2.1.2 Cell viability assay

H9c2 cardiomyocytes (5,000/well) were cultured into 96-well plates for 24 h, and were exposed to a series concentration of evodiamine and rutaecarpine (Shanghai Yuanye Bio-Technology Co., Ltd., China) for another 24 h. Cell viability was measured

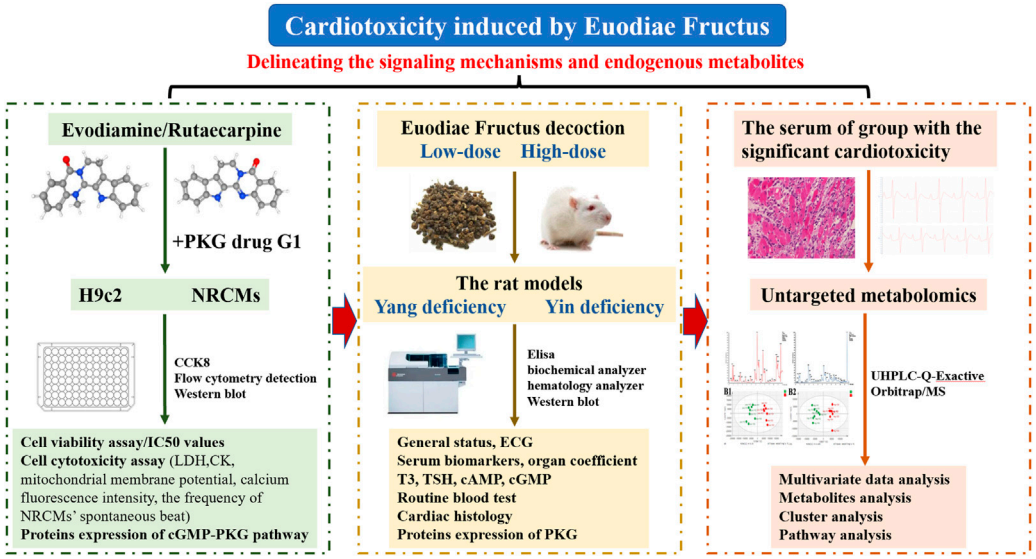

FIGURE 1
The flowchart of the technical strategy in the present study.

using the cell counting kit-8 (CCK-8) solution assay (Biorigin Inc., China) at 450 nm. Subsequently, the absorbance values were applied to calculate the half maximal inhibitory concentration (IC_{50} values) and select appropriate concentrations for further experiments. All experiments were performed independently in triplicate.

2.1.3 Cell cytotoxicity assay

After incubation with different concentrations of evodiamine (5, 10, 25 μM) and rutaecarpine (60, 80, 100 μM) for 24 h, according to the manufacturer's directions, the leakage of lactate dehydrogenase (LDH) and the activity of creatine kinase (CK) were determined using the commercial LDH and CK detection kits (Nanjing Jiancheng Bioengineering Institute, China), respectively. Additionally, the mitochondrial membrane potential and the intensity of calcium fluorescence were evaluated with the JC-1 and Fluo-3AM detection kit (Beyotime Biotechnology, China) through FACS Calibur flow cytometry detection (Becton, Dickinson and Company, United States).

2.1.4 Western blot analysis

The proteins of H9c2 cardiomyocytes from different groups were harvested and lysed with cold RIPA buffer (Beijing Solarbio Technology Co., Ltd., China), supplemented with a protease inhibitor cocktail for 15 min on ice, and the concentrations of the supernatant were measured with a BCA protein assay kit (Beijing Solarbio Technology Co., Ltd., China). Briefly, equal amounts (10 μg) of protein were separated *via* pre-cast 8% SDS-polyacrylamide gel and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore Inc., United States). After blocking with TBST containing 5% skim milk for 1 h at room temperature, the PVDF membranes were incubated overnight at 4°C with PRKG1 antibody (1:1,000, Proteintech Group, Inc., United States), cGMP antibody (1:1,000, Santa Cruz Biotechnology, United States), and GAPDH antibody (1:30,000, Proteintech Group, Inc., United States), followed by incubation with the appropriate secondary antibodies at room temperature for another 1 h. Ultimately, all the blots were visualized by SageCapture software (Beijing Sage Creation Science Compony, China), the levels of protein expression were normalized to that of GAPDH, and relative protein expression was quantified by utilizing Image-ProPlus 6.0 software (Media Cybernetics, United States). Western blots were performed at least three times.

2.2 *In vitro* experiments: Cardiotoxicity from evodiamine and rutaecarpine plus the PKG drug G1 in H9c2 cells

The PKG drug G1 (Selleck Chemicals LLC, United States), the activator of protein kinase G Iα (PKG Iα) was used as tool to further research the function of PKG protein for cardiac toxicity induced by evodiamine and rutaecarpine in H9c2 cells (Burgoyne et al., 2017; Maset et al., 2021). Based on the cell viability of H9c2 cardiomyocytes and the expression of PKG, the optimal concentration of the activator was detected for follow-up studies.

In the aforementioned process, the cell cytotoxicity assay was conducted to include LDH leakage, CK activity, mitochondrial

membrane potential, and the intensity of calcium fluorescence, while related protein expression was measured for H9c2 cells exposed to evodiamine and rutaecarpine plus the PKG drug G1.

2.3 *In vitro* experiments: Cardiotoxicity from evodiamine and rutaecarpine in NRCMs

2.3.1 Cell culture

Given the limitations of H9c2 cardiomyocytes, neonatal rat cardiomyocytes (NRCMs), considered common models for studying the morphological, biochemical, and electrophysiological characteristics of the heart (Chlopcikova et al., 2001), were obtained from 2-3 day-old Sprague–Dawley (SD) rats (Beijing Si Pei Fu Biotechnology, Certificate SCXK-2019-0010) after strict sterilization by the methodology used for isolation and cultivation in previous research, with some modifications (Sabri et al., 2003; Rafiq et al., 2006; Shukla et al., 2018). The apex of isolated heart tissue was digested repeatedly in the short term in a mixture of collagenase II (Biorigin Inc., China) and 0.25% trypsin (Gibco Life Technologies, China) with a magnetic stirrer at 37°C. The cells were incubated in DMEM, supplemented with 15% FBS and 1% penicillin-streptomycin for 1 h. There were fibroblasts adhering to the wall, and the supernatant was resuspended in 96-well plates at a density of 5×10^5 cells/ml, while 100 μM 5-bromo-2-deoxyuridine (BrdU, Biorigin Inc., China) was added to the culture medium to inhibit fibroblast proliferation. These non-adherent cells were incubated at 37°C under humidified conditions of 5% CO_2 for 24 h, and the medium was replaced. On days 4 to 5 of culture, confluent monolayers of NRCMs with regular spontaneous contractility were used for the observation of cardiac toxicity induced by evodiamine and rutaecarpine (Frolova et al., 2016; Frolova et al., 2019).

2.3.2 Cell cytotoxicity assay

To detect the influence of spontaneous contractility, the spontaneous beat frequency of the NRCMs was recorded after interventions with evodiamine (5, 10, 25 μM) and rutaecarpine (60, 80, 100 μM) for 15 min, 30 min, 1 h, 2 h, and 4 h, separately. Moreover, the cell viability and the LDH leakage of the NRCMs were detected using corresponding kits after 4 h of administration.

2.4 *In vivo* experiments: Cardiotoxicity from Euodiae Fructus in rats

2.4.1 Preparation of reagents and Euodiae Fructus decoction

A hydrocortisone sodium succinate for injection (Tianjin Biochem Pharmaceutical Co., Ltd., China) was diluted with saline to a 20 mg/ml solution for use. The preparation of the 1.5 mg/ml thyroid suspension was made by dissolving oral thyroid tablets (Shanghai Zhonghua Pharmaceutical Co., Ltd., China) in carboxymethylcellulose sodium (CMC-Na, BioRuler Company, United States). In addition, the herbal materials called Euodiae Fructus Praeparata were purchased from Beijing Sanhe Pharmaceutical Co. Ltd (Beijing, China, Lot 12410101), and authenticated by Prof. Chunsheng Liu, Beijing University of Chinese Medicine, as the fruit of *Tetradium ruticarpum* (A. Juss.) T. G. Hartley. The decoction of Euodiae Fructus was boiled twice; 1 kg decoction pieces were decocted with water (1:10 volume) for 45 min the first time, before eight times the amount of water was added for another 30 min. Finally, the supernatants were combined and concentrated into a 0.525 g/ml decoction of Euodiae Fructus.

2.4.2 Experimental design

Adult male SD rats weighing 180 ± 10 g (Beijing Si Pei Fu Biotechnology, Certificate SCXK-2020-0033) were acclimatized for 3 days in the animal facility at Beijing University of Chinese Medicine. The rat models of Yang deficiency and those of Yin deficiency were gavage administered the with the decoction of Euodiae Fructus, whose potential cardiotoxicity was investigated to delineate the signaling mechanisms *in vivo*. All the animal experiments were conducted in accordance with approved guidelines specified by the animal ethics committee of Beijing University of Traditional Chinese Medicine (Beijing, China; No. BUCM-4-2021090302-3052).

The manufacture of rat models with Yang deficiency was achieved by an intragluteal injection of 20 mg/ml hydrocortisone sodium succinate (1 ml/kg), continued for 15 days, as in the previous work of our team (Zhang, 2013). Meanwhile, the rat models with Yin deficiency received gavage administration of 1.5 mg/ml thyroid suspension (10 ml/kg) for 15 days (Zhang et al., 2019). Simultaneously, the medication group received intragastric administration of the decoction of Euodiae Fructus (the low dose was 0.0583 g/ml, the high dose was 0.525 g/ml), based on the modeling of Yang and Yin deficiencies.

All rats were randomly divided into eight groups (n = 8/group): 1) the Yang-K group (treated with intragluteal injection of an equal volume of the sterilized saline); 2) the Yang-X group (received intragluteal injection of hydrocortisone sodium succinate 1 ml/kg); 3) the Yang-D group (administered intragluteal injection of hydrocortisone sodium succinate 1 ml/kg + the decoction of Euodiae Fructus 0.0583 g/ml); 4) the Yang-G group (administered intragluteal injection of hydrocortisone sodium succinate 1 ml/kg + the decoction of Euodiae Fructus 0.525 g/ml); 5) the Yin-K group (received gavage administration of water); 6) the Yin-X group (given gavage administration of thyroid suspension 10 ml/kg); 7) the Yin-D group (received gavage administration of thyroid suspension 10 ml/kg + the decoction of Euodiae Fructus 0.0583 g/ml); and 8) the Yang-G group (received gavage

administration of thyroid suspension 10 ml/kg + the decoction of Euodiae Fructus 0.525 g/ml).

2.4.3 Observation of general status

The changes in the general status of different groups were observed immediately; the body weights and rectal temperatures of rats were measured 7 days and 14 days after treatment.

2.4.4 Measurement of ECG, serum biomarkers, and organ coefficients

All rats per group were sacrificed on day 15 by anesthetization with an intraperitoneal injection of 10% chloral hydrate (3 ml/kg). After anesthesia, the rats were fixed in a supine position, and the ECG was recorded through a BL-420S biological function experiment system (Chengdu Taimeng Software Co., Ltd., China) to inspect the cardiac function.

Blood was collected from the abdominal aorta for different detection indexes, for which the plasma, serum and whole blood were prepared separately. To explore the cardiac injury, serum biomarkers, including lactate dehydrogenase (LDH), creatine kinase (CK), α-hydroxybutyrate dehydrogenase (HBDH), and aspartate aminotransferase (AST), along with the glucose and lipid metabolism involving glucose (GLU), triacylglycerol (TG), and cholesterol (CHO), were detected using the AU5800 automatic biochemical analyzer (Beckman Coulter, Inc., United States). With regard to the organ coefficients, the organs (including liver, kidneys, heart, spleen, and lungs) of each rat were dissected and weighed, and the hearts were removed for subsequent experimentation.

2.4.5 Measurement of T3 and TSH content in serum, cAMP and cGMP in plasma, and routine blood tests

The content of triiodothyronine (T3) and the thyroid stimulating hormone (TSH) in serum, and cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in plasma, were determined using related enzyme-linked immunosorbent assay (ELISA) kits (Wuhan Cloud-Clone Corp., China) in accordance with the manufacturer's instructions. Using the hematology analyzer (Sysmex Corporation, Japan), the routine blood test was conducted on blood samples collected from rats in the different groups, measuring especially white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), platelets (PLT), neutrophil ratio (NEUT%), lymphocyte ratio (LYMPH %), and monocyte ratio (MONO%).

2.4.6 Cardiac histology

Normal saline was used for irrigating the hearts. Afterward, the hearts were fixed in 10% formalin for 24 h, embedded in paraffin, and sectioned transversely at 4 μm. The histopathological changes of myocardia for rats in different groups were investigated by haematoxylin-eosin (HE) staining.

2.4.7 Western blot analysis

Total proteins from the heart tissue of rats were homogenized and extracted, and the expression of PKG protein was examined through western blot analysis, according to related procedures of *in vitro* experimentation.

2.5 Untargeted metabolomics: Cardiotoxicity from Euodiae Fructus in rats

2.5.1 Sample preparation

For the group with significant cardiotoxicity, the endogenous metabolites in the serum were investigated using the approach of ultra-high performance liquid chromatography quadrupole-exactive Orbitrap/mass spectrum (UHPLC-Q-Exactive Orbitrap/MS), as described previously (Liu et al., 2019).

Briefly, aliquots (100 μl) of plasma samples were mixed with 300 μl chromatographic acetonitrile. After centrifugation (13,000 rpm, 15 min, 4°C), the supernatant was transferred to a clean tube for analysis. For methodological investigations, the quality control (QC) samples were prepared from mixtures of 10 μl plasma in each sample.

2.5.2 Sample detection

Aliquots (2 μl) of experimental samples were eluted through an ACQUITY UPLC BEH C18 chromatographic column (2.1 mm × 100 mm, 1.7 μm, Waters Corporation, United States) in a Vanquish Duo UHPLC chromatograph (Thermo Fisher Scientific Inc., United States), using the mobile phases of eluents A (acetonitrile) and B (0.1% formic acid in water) at a flow rate of 0.3 ml/min.

Electron spray ionization was employed for detecting both positive and negative ions in the abovementioned plasma samples *via* a hybrid quadrupole Orbitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific Inc., United States). The quadrupole scan range was set at mass-to-charge ratio (m/z) 100–1,200 Da, with the heated capillary temperature at 350°C, and the positive and negative spray voltages at 3.2 and 3.8 kV, respectively.

2.5.3 Multivariate data analysis

The raw data from the liquid chromatography-mass spectrometry (LC-MS) were manually phase-baseline corrected for peak area (PA) and retention time (RT) using the Mass Spectrometry-Data Independent Analysis software version 4 (MS-DIAL 4, http://prime.psc.riken.jp/compms/msdial/main.html) (Tsugawa et al., 2019; Tsugawa et al., 2020). Thereafter the multivariate data analysis was performed with SIMCA-P software (Version 14.1, Umetrics, Umea, Sweden), including principal component analysis (PCA) and the orthogonal partial least square-discriminate (OPLS-DA). Here PCA was a non-supervised approach to observe the distribution and outliers of the data set depicted in a scores plot based on orthogonal latent variables, which

TABLE 1 Cell viability assay and IC_{50} of evodiamine and rutaecarpine (n = 6, $\bar{x} \pm s$).

Groups	Concentration (μmol/L)	Cell viability (%)	IC_{50} (μmol/L)
Evodiamine	0	100.00 ± 0.014	42.82 ± 7.55
	2	74.43 ± 2.79	
	5	68.50 ± 4.25	
	10	71.03 ± 2.65	
	25	63.95 ± 8.60*	
	50	51.64 ± 12.39**	
Rutaecarpine	0	100.00 ± 7.87	117.97 ± 9.69
	20	112.57 ± 8.22	
	40	103.16 ± 10.75	
	60	106.68 ± 15.89	
	80	88.05 ± 15.86	
	100	63.17 ± 7.59	

Note: Compared with the control group, *$p < 0.05$; **$p < 0.01$.

were obtained from the overall direction of maximum variance (Duan et al., 2018). Furthermore, owing to supervised algorithms, OPLS-DA was employed to extract the underlying variability in behavior characterizing the endogenous metabolomics. The evaluation methods of the OPLS-DA model were described by the Q^2 and R^2 of the permutation respectively. The robustness of the model's prediction ability is directly proportional to the Q^2 ($0 < Q^2 < 1$), while the R^2 could represent the percentage of X and Y matrix information of the model interpretation (Triba et al., 2015; Li et al., 2018b; Jang et al., 2018; Plazas et al., 2019).

2.5.4 Metabolites analysis

The most discriminant variables were selected in terms of variable importance in the projection (VIP) with significant statistical difference in the corresponding PA. On the one hand, discriminant metabolites (VIP >1.0) were collected according to related results of OPLS-DA. On the other, the statistical tests were exhibited by SPSS software. The normality of data, considered as an adjusted p-value > 0.05, was determined by a Kolmogorov–Smirnov test for each group. With regard to normal and homoscedastic variables, statistical significance was determined using a one-way ANOVA. Otherwise, the differences between groups were determined using the Kruskal–Wallis test, and the significance was considered as a p-value below 0.05.

Subsequently, corresponding metabolites were identified according to the Human Metabolome Database (HMDB, http://www.hmdb.ca/) (Wishart et al., 2018). As directly displayed in heatmaps for the content and correlation of identified metabolites, the cluster analysis was constructed using MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/) (Chong et al., 2019), and the results of the pathway analysis for the differential metabolites in rats were visualized in a bubble chart, with the size of the bubble proportional to the importance of the pathway (Chong et al., 2018; Chong and Xia, 2020).

3 Results

3.1 Cardiotoxicity induced by evodiamine and rutaecarpine in H9c2 cells

3.1.1 Cell viability assay and IC_{50} of evodiamine and rutaecarpine

Compared with the control group, both evodiamine and rutaecarpine presented inhibitory effects in a dose-dependent manner for the cell viability of H9c2 cardiomyocytes. The IC_{50} values of evodiamine and rutaecarpine separately were 42.82 ± 7.55 and 117.97 ± 9.69 μmol/L, and the related details are summarized in Table 1.

3.1.2 Cell cytotoxicity assay of evodiamine and rutaecarpine in H9c2

According to the results of Figure 2; Table 2, the leakage of LDH and the activity of CK were notably more highly dose-dependent in the high-dose evodiamine and rutaecarpine group than in the control group ($p < 0.01$). Similarly, the intensity of calcium fluorescence for H9c2 cells in the high-dose evodiamine and rutaecarpine group was obviously higher ($p < 0.05$). However, significant differences were only observed in the evodiamine-induced H9c2 cells compared to the control group ($p < 0.01$). These results indicate that evodiamine and rutaecarpine might change the permeability of the myocardial cell, the activity of the myocardial enzyme, the energy supply, and the calcium concentration, thereby inducing cardiotoxicity of H9c2 cardiomyocytes.

3.1.3 Protein expression of the cGMP-PKG pathway of evodiamine and rutaecarpine

As presented in Figure 3, cGMP and PKG were downregulated in the H9c2 cardiomyocytes with evodiamine (5–25 μmol/L) and rutaecarpine (80–100 μmol/L), compared

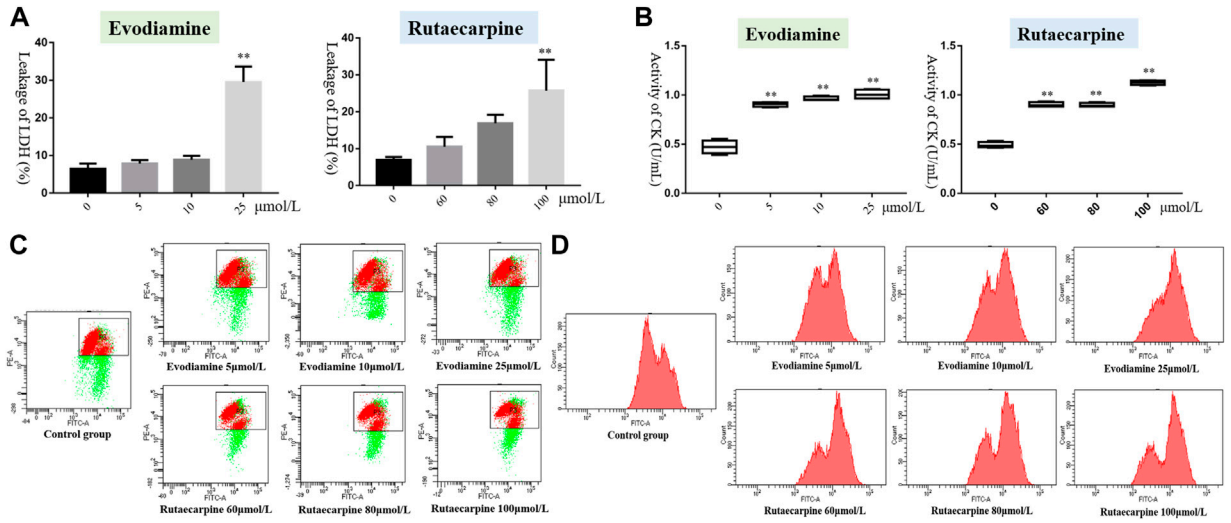

FIGURE 2
Cell cytotoxicity assay of evodiamine and rutaecarpine in H9c2. Note: **(A)** leakage of LDH; **(B)** activity of CK (U/ml); **(C)** mitochondrial membrane potential; **(D)** intensity of calcium fluorescence.

TABLE 2 Cell cytotoxicity assay of evodiamine and rutaecarpine in H9c2 (n = 4, $\bar{x} \pm s$).

Groups	Concentration (μmol/L)	Leakage of LDH (%) (n = 6)	Activity of CK (U/ml)	Mitochondrial membrane potential	Intensity of calcium fluorescence
Evodiamine	0	6.40 ± 1.21	0.47 ± 0.058	1.42 ± 0.025	9111.00 ± 693.42
	5	7.90 ± 0.73	0.90 ± 0.019**	1.44 ± 0.0090	9898.67 ± 677.27
	10	8.89 ± 0.90	0.96 ± 0.019**	1.26 ± 0.0088**	10612.33 ± 746.80
	25	29.54 ± 3.56**	1.01 ± 0.044**	1.11 ± 0.051**	12735.00 ± 594.64**
Rutaecarpine	0	6.91 ± 0.71	0.49 ± 0.025	1.42 ± 0.025	9111.00 ± 693.42
	60	10.54 ± 2.28	0.90 ± 0.022**	1.40 ± 0.028	10731.67 ± 666.35
	80	16.93 ± 1.99	0.90 ± 0.018**	1.39 ± 0.030	11113.00 ± 532.09*
	100	25.74 ± 7.23**	1.13 ± 0.021**	1.31 ± 0.074	12713.67 ± 339.22**

Note: Compared with the control group, *$p < 0.05$, **$p < 0.01$.

with the control group ($p < 0.05$), suggesting that the gene and protein expression levels of cGMP and PKG were significantly decreased in H9c2 cardiomyocytes under evodiamine and rutaecarpine (Supplementary Material).

3.2 Cardiotoxicity induced by evodiamine and rutaecarpine plus PKG drug G1 in H9c2 cells

3.2.1 Cell viability and cytotoxicity assay of evodiamine and rutaecarpine plus PKG drug G1

The cell viability of each group was apparently lower than in the non-medication group ($p < 0.05$). Additionally, compared with the PKG drug G1 group, only the 60 μmol/L rutaecarpine group was without significant inhibition of H9c2 cardiomyocytes, which means the combination of the PKG drug G1 with evodiamine or rutaecarpine could not have had an appreciable effect on the cell viability of H9c2 cardiomyocytes (Figure 4, Supplementary Material).

As shown in Figure 4, the PKG drug G1 could significantly reduce the leakage of LDH in the low-dose evodiamine and rutaecarpine groups of H9c2 cardiomyocytes, compared with the single agent group ($p < 0.05$). Meanwhile, treatment of the PKG drug G1 obviously improved the mitochondrial membrane potential in the group of 80 μmol/L rutaecarpine ($p < 0.05$), and there were no significant differences for the activity of CK and the intensity of calcium fluorescence between the combined

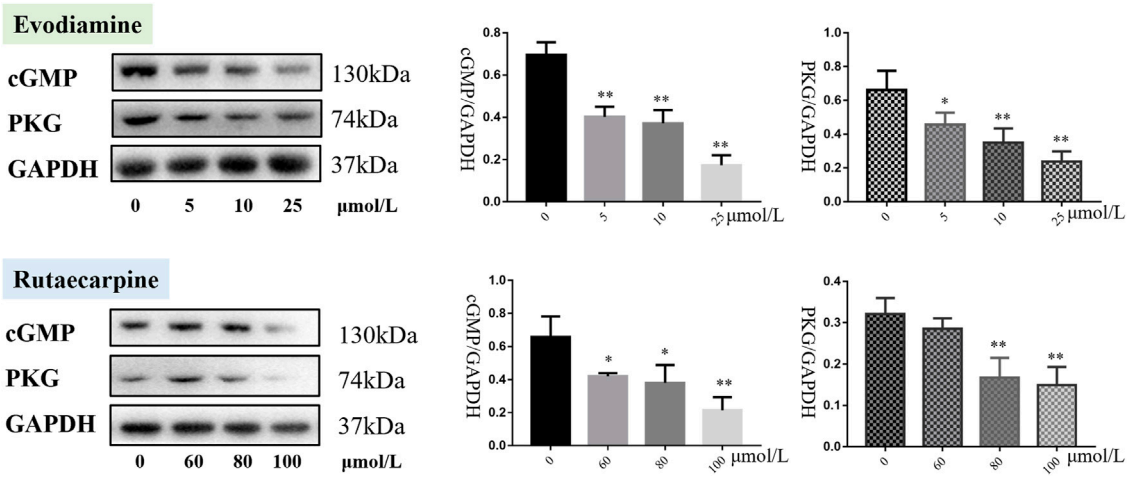

FIGURE 3
Protein expression of cGMP-PKG pathway of evodiamine and rutaecarpine.

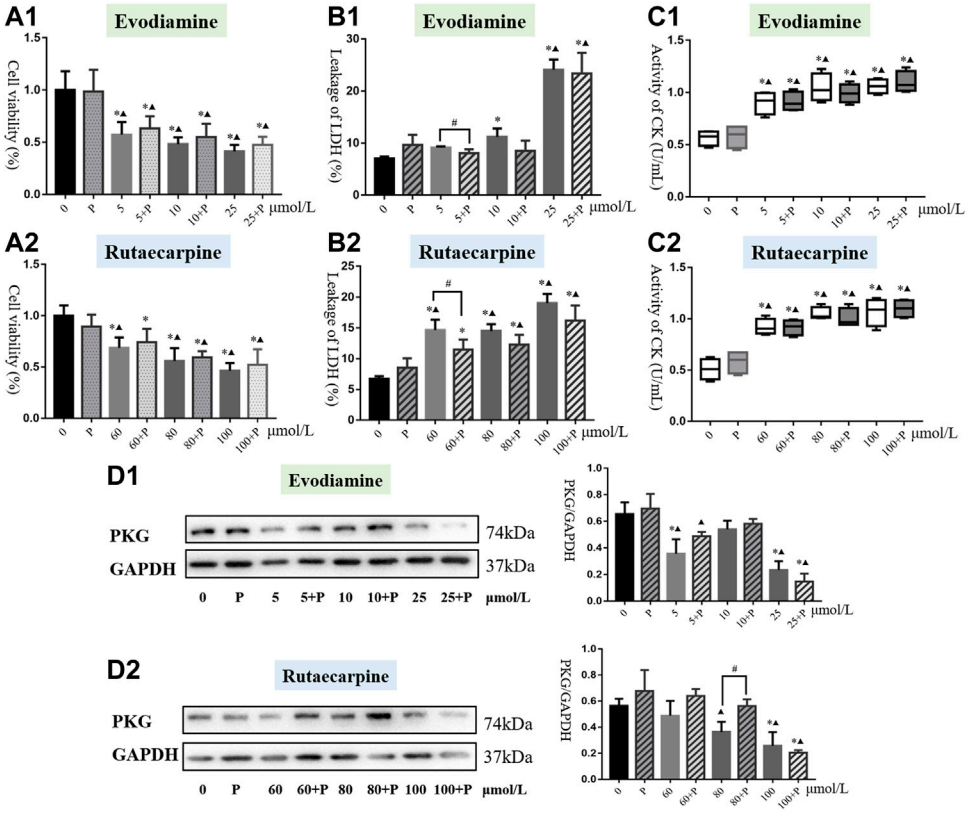

FIGURE 4
Results of cardiotoxicity induced by evodiamine and rutaecarpine plus PKG drug G1 in H9c2 cells. Note: **(A)** cell viability; **(B)** leakage of LDH; **(C)** activity of CK; **(D)** the protein expression of cGMP and PKG.

TABLE 3 Cell cytotoxicity of evodiamine and rutaecarpine plus PKG drug G1 (n = 4, $\bar{x} \pm s$).

Groups	Concentration (μmol/L)	Activity of CK (U/ml)	Mitochondrial membrane potential	Intensity of calcium fluorescence
Control	—	0.51 ± 0.088	1.48 ± 0.110	8852.33 ± 628.59
PKG drug G1	5	0.58 ± 0.097	1.49 ± 0.140	8334.00 ± 693.74
Evodiamine	5	0.90 ± 0.095*▲	1.27 ± 0.110	8674.67 ± 465.49
	5 + P	0.91 ± 0.079*▲	1.48 ± 0.110	8413.67 ± 510.99
	10	1.04 ± 0.120*▲	1.32 ± 0.150	9112.67 ± 501.60
	10 + P	0.99 ± 0.079*▲	1.46 ± 0.280	8915.67 ± 709.40
	25	1.06 ± 0.059*▲	1.25 ± 0.095	9875.00 ± 730.78
	25 + P	1.10 ± 0.088*▲	1.37 ± 0.079	8892.67 ± 472.79
Rutaecarpine	60	0.92 ± 0.068*▲	1.48 ± 0.140	9541.67 ± 678.66
	60 + P	0.92 ± 0.065*▲	1.45 ± 0.120	8758.00 ± 462.91
	80	1.05 ± 0.054*▲	1.28 ± 0.052	9703.67 ± 330.53
	80 + P	1.00 ± 0.082*▲	1.45 ± 0.049#	8576.33 ± 668.48
	100	1.15 ± 0.075*▲	1.23 ± 0.078	10,322.67 ± 428.60▲
	100 + P	1.17 ± 0.090*▲	1.31 ± 0.190	10,064.67 ± 377.77▲

Note: Compared with the control group (non-medication), *p < 0.05; compared with PKG, drug G1 group, ▲p < 0.05; compared with single compound group (corresponding dose), #p < 0.05: P represents 5 μmol/L PKG, drug G1.

group and the single agent group (Table 3). These results indicate that the PKG drug G1 might partially decelerate the cardiotoxicity of H9c2 cardiomyocytes caused by evodiamine and rutaecarpine.

3.2.2 Protein expression of PKG from evodiamine and rutaecarpine plus PKG drug G1

As demonstrated by western blot analysis (Figure 4, Supplementary Material), compared with single compound groups, there was an increasing trend of protein expression of PKG in compatibility groups. Remarkably, the PKG drug G1 could greatly enhance the expression of PKG for H9c2 cardiomyocytes incubating with 80 μmol/L rutaecarpine ($p < 0.05$). The inhibitory effects of rutaecarpine (80 μmol/L) were antagonized in concentration-dependent ways by treatment with the PKG drug G1 at concentrations of 5 mol/L.

3.3 Cardiotoxicity induced by evodiamine and rutaecarpine in NRCMs

Through observation of NRCMs, the frequency of spontaneous beat in the evodiamine and rutaecarpine groups underwent obvious changes compared with the control group ($p < 0.05$): notably, high-dosage and long-term intervention were associated with cardiomyocyte arrest (Table 4). A significant elevation of the LDH leakage of NRCMs was determined in the evodiamine and rutaecarpine groups compared with the control group over 4 h, whereas cell viability decreased, as listed in Table 5. Consequently, combined with the above results, evodiamine and rutaecarpine might affect the myocardial contractility and normal physiological state of NRCMs.

3.4 Cardiotoxicity induced by Euodiae Fructus in rats

3.4.1 General status

During the entire experiment *in vivo*, the weight of YANG-X, YANG-D, and YANG-G groups gradually decreased compared to the YANG-K group, while the YANG-D and YANG-G groups' rectal temperatures increased compared with YANG-K and YANG-X, as presented in Figures 5A,B ($p < 0.05$) (7 days, 14 days, Supplementary Material). In the model of rats with Yin deficiency, there were significant differences in weight and rectal temperature following oral administration of Euodiae Fructus compared with the control group. The changes in general status demonstrate that the treatment of Euodiae Fructus can affect the physical status of rats with Yang or Yin deficiencies, resulting in weight loss and temperature elevation.

3.4.2 ECGs, serum biomarkers, and organ coefficients

It was noticed that long-term exposure to Euodiae Fructus might induce changes in ECG for rats with Yang or Yin deficiency to different degrees. In particular, significant differences in heart rate, PR interval, QT interval, P-wave amplitude, R-wave amplitude, and ST-wave amplitude were observed in the high-dose groups compared with the

TABLE 4 Frequency of NRCM spontaneous beat of evodiamine and rutaecarpine (n = 3, $\bar{x} \pm s$).

Groups	Concentration (μmol/L)	15 min	30 min	1 h	2 h	4 h
Control	0	72.67 ± 1.70	72.33 ± 4.18	68.00 ± 1.63	67.67 ± 1.25	69.00 ± 2.94
Evodiamine	5	105.67 ± 4.19**	94.67 ± 1.25**	95.00 ± 1.63**	93.33 ± 1.70**	87.67 ± 2.05**
	10	95.33 ± 1.25**	93.33 ± 2.49**	93.67 ± 2.87**	85.67 ± 2.62**	77.33 ± 2.62**
	25	94.00 ± 2.94**	92.67 ± 2.05**	94.33 ± 2.49**	81.67 ± 4.11**	—
Rutaecarpine	60	106.33 ± 3.30**	94.33 ± 3.09**	82.33 ± 1.25**	81.67 ± 1.25**	76.67 ± 0.94**
	80	101.00 ± 3.56**	92.33 ± 2.49**	91.00 ± 1.63**	75.67 ± 1.25*	—
	100	100.67 ± 2.62**	83.67 ± 4.19*	85.33 ± 3.86**	56.00 ± 4.08**	—

Note: Compared with the control group, *$p < 0.05$, **$p < 0.01$; "/" represents cardiomyocyte arrest.

TABLE 5 Cardiotoxicity induced by evodiamine and rutaecarpine in NRCMs (n = 6, $\bar{x} \pm s$).

Groups	Concentration (μmol/L)	Cell viability (%)	Leakage of LDH (%)
Control	0	1.01 ± 0.056	8.70 ± 1.52
Evodiamine	5	0.61 ± 0.084**	22.67 ± 1.58**
	10	0.57 ± 0.055**	26.61 ± 3.74**
	25	0.58 ± 0.090**	26.16 ± 2.96**
Rutaecarpine	60	0.55 ± 0.092**	33.98 ± 4.81**
	80	0.52 ± 0.074**	35.54 ± 4.46**
	100	0.54 ± 0.054**	34.67 ± 2.96**

corresponding control group and model group (Figures 5C–H, Supplementary Material). Namely, long-term and overdose exposure to Euodiae Fructus could cause ECG abnormalities for rats with Yang or Yin deficiencies, including marked prolongation of the ventricular depolarization period and shortening of the effective refractory period, hence disturbing the atrioventricular conduction, which could lead to cardiac arrhythmia.

The results of the alteration in serum myocardial enzymes manifest that the levels of LDH, CK, HBDH, and AST increased in the YANG-G group over the corresponding control group, with a statistically significant difference ($p < 0.05$). Similarly, a remarkable rise of HBDH in the YIN-D group, and LDH, CK, HBDH, and AST in the YIN-G group were also observed over the corresponding control and model groups ($p < 0.05$) (Table 6). Therefore, overdosage and unsuitable syndrome differentiation are associated with the elevation of myocardial enzymes induced by Euodiae Fructus in rats.

To assess whether Euodiae Fructus involves changes to the glycolipid metabolism of rats with Yang or Yin deficiency, levels of GLU, TG, and CHO were measured in rats exposed to Euodiae Fructus decoction for 14 days. As summarized in Table 6, the high-dose gavage administration for rats with Yang deficiency resulted in significantly changed GLU, TG, and CHO levels compared to the related model groups ($p < 0.05$), while rats with Yin deficiency indicated obvious disorders in GLU, TG, and CHO levels compared to the related control groups ($p < 0.05$). Euodiae Fructus could thus contribute to clinical efficacy for rats with Yang deficiency and metabolic abnormality for those with Yin deficiency.

According to the results of the organ coefficients in Table 6, the obvious differences of heart and kidney were not observed among different groups; however, there was a higher level of liver coefficient in groups of high-dose Euodiae Fructus ($p < 0.05$). The results reveal that an overdose of Euodiae Fructus might contribute to hepatic damage in rats, whether with Yang deficiency or Yin deficiency.

3.4.3 T3 and TSH content in serum, cAMP and cGMP content in plasma, and routine blood test

Aside from changes in glycolipid metabolism, rats with Yang or Yin deficiency also possessed differing content of T3, TSH, cAMP, and cGMP. The level of T3 in the YIN-G group was significantly

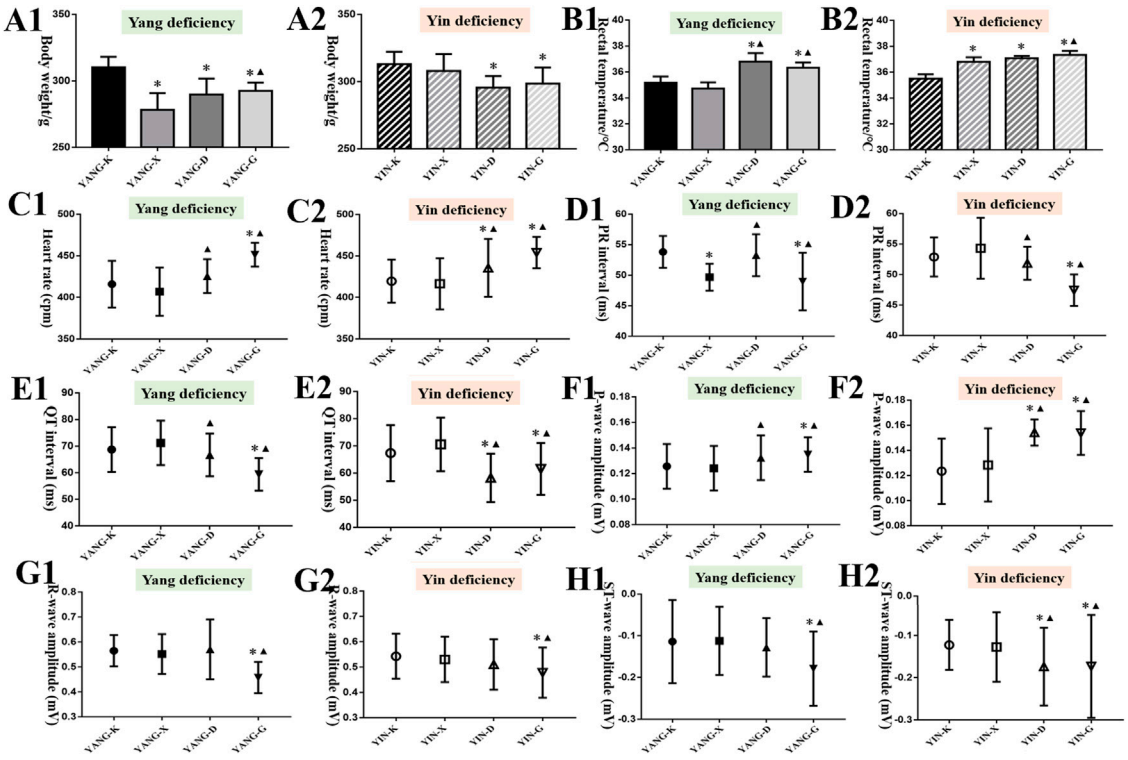

FIGURE 5
Results of general status and ECG of rats in different groups (14 d). Note: Compared with the corresponding control group, *$p < 0.05$; compared with the corresponding model group, ▲$p < 0.05$. **(A)** Body weight; **(B)** rectal temperature; **(C)** heart rate; **(D)** PR interval; **(E)** QT interval; **(F)** P-wave amplitude; **(G)** R-wave amplitude; **(H)** ST-wave amplitude.

higher than the corresponding control and model group. Hence, the intervention of Euodiae Fructus could increase cAMP/cGMP in rats with Yin deficiency significantly more than in the related control group ($p < 0.05$) (Table 7). These results suggest that an imbalance of hormone secretion and second messenger transcription might occur due to the irrational usage of Euodiae Fructus.

In addition, the levels of WBC, HGB, and NEUT% in the YANG-G group; of RBC, HGB, PLT, and NEUT% in the YIN-D group; and of WBC, RBC, HGB, PLT, NEUT%, and MONO% in the YIN-G group, were all different from the related model group with a statistically significant difference ($p < 0.05$), indicating continuous gavage with an overdose of Euodiae Fructus for 15 days could influence the blood routine levels of rats with Yang or Yin deficiencies.

3.4.4 Cardiac histology

As displayed in Figure 6A, obvious histological changes were not observed in the cardiac tissues of the YANG-K group, the YANG-X group, and the YANG-D group, as the cardiac muscle fibers were arranged neatly without inflammatory cell infiltration. In the YANG-G group, some myocardial fiber underwent hypertrophy and became uneven. In the YIN-D group, some cellular edema, break or necrosis, and obvious infiltration of inflammatory cells could be observed. Furthermore, pathological examination revealed that the myocardial fibers were in a disordered condition for the YIN-G group: the major lesions in the myocardial fibers were from degeneration and necrosis, inflammatory infiltration, and edema. These results establish that cardiac pathological injury in rats is associated with overdosage and unsuitable syndrome differentiation of Euodiae Fructus.

3.4.5 Protein expression of PKG in heart issue

The inhibitory effects of Euodiae Fructus for the protein expression of PKG were concentration-dependent in rats with Yin deficiency, while the protein expression of PKG in heart issue was also lower in the YANG-G group than in the corresponding control and model groups, and statistically significant differences were observed among these groups (Figure 6B, Supplementary Material).

3.5 Untargeted metabolomics of cardiotoxicity induced by Euodiae Fructus in rats

3.5.1 Multivariate data analysis

The untargeted metabolomics of cardiotoxicity induced by Euodiae Fructus in rats with Yin deficiency were evaluated; the

TABLE 6 Results of serum biomarkers and organ coefficients of rats in different groups (n = 8, $\bar{x} \pm s$).

Indexes	YANG-K	YANG-X	YANG-D	YANG-G	YIN-K	YIN-X	YIN-D	YIN-G
LDH (U/L)	457.00 ± 89.48	505.88 ± 129.04	490.13 ± 108.98	811.75 ± 164.57*▲	466.13 ± 162.89	519.13 ± 107.25	658.25 ± 115.93*	884.50 ± 165.16*▲
CK (U/L)	557.90 ± 66.44	571.59 ± 74.52	555.76 ± 74.83	663.79 ± 52.48*▲	532.39 ± 79.44	553.26 ± 70.31	606.85 ± 90.18	708.20 ± 127.66*▲
HBDH (U/L)	126.74 ± 23.74	138.79 ± 21.86	110.31 ± 13.09	165.03 ± 42.30*	122.59 ± 26.10	122.24 ± 20.18	188.90 ± 43.49*▲	203.99 ± 38.23*▲
AST (U/L)	113.45 ± 9.47	106.96 ± 5.75	107.91 ± 6.25	130.38 ± 15.93*▲	113.33 ± 7.68	116.56 ± 18.24	131.69 ± 17.25	138.66 ± 17.63*▲
GLU (mmol/L)	10.28 ± 1.05	7.37 ± 0.98*	7.91 ± 0.67*	9.35 ± 0.83▲	9.32 ± 0.68	10.05 ± 0.93	10.05 ± 0.92	10.94 ± 1.11*
TG (mmol/L)	0.57 ± 0.080	0.54 ± 0.070	0.58 ± 0.12	0.68 ± 0.11▲	0.52 ± 0.095	0.54 ± 0.083	0.57 ± 0.078	0.65 ± 0.11*
CHO (mmol/L)	1.47 ± 0.094	1.27 ± 0.12*	1.45 ± 0.11▲	1.62 ± 0.15▲	1.45 ± 0.11	1.61 ± 0.11*	1.66 ± 0.098*	1.68 ± 0.098*
Heart coefficient	0.32 ± 0.012	0.31 ± 0.018	0.31 ± 0.011	0.32 ± 0.0079	0.32 ± 0.016	0.33 ± 0.028	0.34 ± 0.030	0.32 ± 0.0094
Liver coefficient	3.25 ± 0.20	3.30 ± 0.13	3.31 ± 0.14	3.90 ± 0.27*▲	3.40 ± 0.19	3.38 ± 0.29	3.57 ± 0.34	4.00 ± 0.15*▲
Kidney coefficient	0.40 ± 0.019	0.39 ± 0.027	0.39 ± 0.013	0.39 ± 0.034	0.40 ± 0.019	0.40 ± 0.018	0.41 ± 0.028	0.40 ± 0.025
Lung coefficient	0.47 ± 0.021	0.45 ± 0.034	0.49 ± 0.015	0.47 ± 0.035	0.44 ± 0.026	0.49 ± 0.046	0.50 ± 0.049*	0.50 ± 0.032*
Spleen coefficient	0.31 ± 0.042	0.25 ± 0.056*	0.25 ± 0.022*	0.25 ± 0.037*	0.31 ± 0.030	0.28 ± 0.029	0.26 ± 0.059	0.25 ± 0.039

Note: Compared with the corresponding control group, *$p < 0.05$; compared with the corresponding model group, ▲$p < 0.05$.

TABLE 7 Results of T3, TSH, cAMP, cGMP, and routine blood test of rats (n = 8, $\bar{x} \pm s$).

Indexes	YANG-K	YANG-X	YANG-D	YANG-G	YIN-K	YIN-X	YIN-D	YIN-G
T3 (pg/ml)	2.72 ± 0.15	2.69 ± 0.17	2.78 ± 0.093	2.76 ± 0.11	2.75 ± 0.15	2.88 ± 0.15	2.95 ± 0.20*	3.10 ± 0.10*▲
TSH (pg/ml)	1.74 ± 0.16	1.86 ± 0.15	1.87 ± 0.15	1.98 ± 0.17*	1.71 ± 0.14	1.64 ± 0.14	0.62 ± 0.12	1.55 ± 0.097
cAMP/ cGMP	1.10 ± 0.027	1.06 ± 0.022*	1.06 ± 0.020*	1.08 ± 0.031	1.06 ± 0.015	1.11 ± 0.04*	1.13 ± 0.045*	1.15 ± 0.038*
WBC	6.95 ± 1.61	8.66 ± 1.55	8.61 ± 1.58	11.23 ± 1.77*▲	6.53 ± 1.88	10.26 ± 1.08*	10.90 ± 1.89*	12.18 ± 1.31*▲
RBC	6.66 ± 0.35	7.34 ± 0.30*	7.26 ± 0.36*	7.41 ± 0.34*	6.93 ± 0.41	7.21 ± 0.71	8.60 ± 0.72*▲	9.06 ± 0.59*▲
HGB	136.75 ± 6.65	144.63 ± 6.36	142.88 ± 4.46	149.50 ± 7.09*▲	138.63 ± 5.31	138.00 ± 5.89	178.50 ± 6.40*▲	182.6 ± 8.23*▲
PLT	1,172.25 ± 77.72	1,052.5 ± 57.50*	1,151.63 ± 87.76	1,124.25 ± 85.98	1,196.38 ± 46.63	1,168.50 ± 73.97	1,036.50 ± 62.31*▲	1,019.75 ± 60.79*▲
NEUT%	10.53 ± 1.22	15.97 ± 1.54*	12.89 ± 2.30	25.28 ± 2.73*▲	11.05 ± 1.21	11.68 ± 1.38	30.13 ± 3.54*▲	34.75 ± 3.13*▲
LYMPH %	71.45 ± 4.56	63.03 ± 5.21*	63.08 ± 5.13*	63.43 ± 7.50*	66.08 ± 6.63	61.45 ± 8.26	56.64 ± 8.13	52.83 ± 7.65*
MONO%	5.33 ± 1.32	7.18 ± 1.27*	7.15 ± 1.38*	8.00 ± 1.21*	5.40 ± 0.84	5.33 ± 1.01	5.60 ± 0.70	8.34 ± 0.98*▲

Note: Compared with the corresponding control group, *$p < 0.05$; compared with the corresponding model group, ▲$p < 0.05$.

serum samples of the YIN-K and YIN-G groups were determined using UHPLC-Q-Exactive Orbitrap/MS. According to the results of the multivariate data analysis in Figure 7, there was clear separation between the YIN-K and YIN-G groups, suggesting the metabolic profile might be different after continuous gavage of Euodiae Fructus for 15 days, and the details of PCA, OPLS-DA, and permutations are shown in the Supplementary Material.

3.5.2 Metabolites analysis

Based on the limitation of the variables with VIP>1 and simultaneous significant difference, ultimately there were 3212 endogenous metabolites in total, with 2060 (64.13%) in the positive ion mode, and the remaining in the negative mode (35.87%). After the identification, 34 corresponding metabolites were highlighted as the most discriminant in the rats of the YIN-

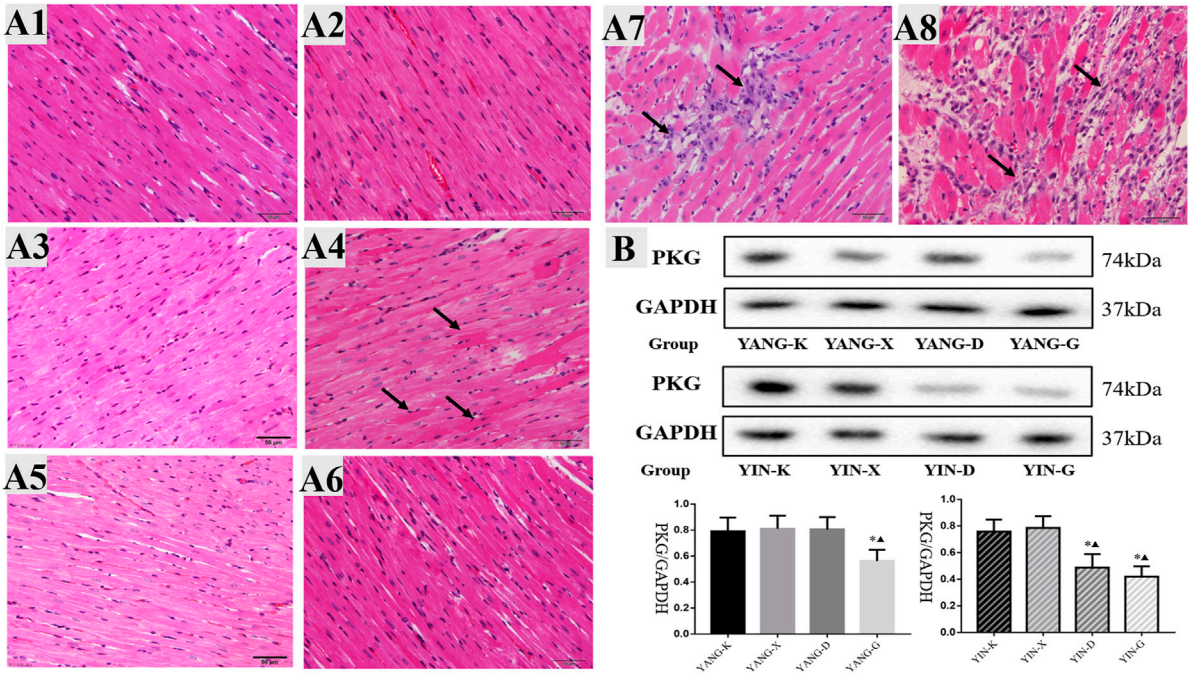

FIGURE 6
Cardiac histology and protein expression of PKG for rats in different groups. Note: **(A)** cardiac histology and **(B)** protein expression of PKG in heart issue: 1) YANG-K group, 2) YANG-X group, 3) YANG-D group, 4) YANG-G group, 5) YIN-K group, 6) YIN-X group, 7) YIN-D group, and 8) YIN-G group.

K and YIN-G groups, including D-proline, deoxycytidine, 5-hydroxyisourate, cytosine, uric acid, D-lysine, and so on (Supplementary Material).

The cluster analysis depicted in Figure 8A reveals that these discriminant metabolites were divided into two categories in a dendrogram, and there was close correlations or similar pathways for the metabolites in the same category. Furthermore, the results of the pathway analysis also pointed out that 10 metabolic pathways, including the purine metabolism, glycerophospholipid metabolism, glycerolipid metabolism, sphingolipid metabolism, and the phosphatidylinositol signaling system, as well as the arginine and proline metabolisms, were all strongly involved in the metabonomic signatures of rats exposed to Euodiae Fructus. This could induce cardiotoxicity in rats with Yin deficiency, and the most likely metabolic pathways and related discriminant metabolites are exhibited in Figures 8B,C and in the Supplementary Material.

4 Discussion

In recent decades, the therapeutic and beneficial effects of Chinese *materia medica* in preventing or ameliorating multiple cardiovascular and chronic diseases have become increasingly well known. Correspondingly, public awareness of medicinal herb safety has also heightened (Amadi and Orisakwe, 2018; Shaito et al., 2020). The present findings from *in vivo* and *in vitro* experiments and untargeted metabolomics research reveal that the mechanisms of potential cardiotoxicity induced by overdosage and irrational usage of Euodiae Fructus involve the cGMP-PKG pathway and the metabolic pathways concerned with energy metabolism, lipid metabolism, oxidative stress, and so on.

4.1 Cardiac cytotoxicity of evodiamine and rutaecarpine in *in vivo* experiments

The cGMP-PKG pathway has been closely linked with the cardiac cytotoxicity of evodiamine and rutaecarpine. Our data suggest the levels of LDH and CK, and the mitochondrial membrane potential and intensity of calcium fluorescence, changed remarkably in H9c2 cells undergoing the administration of evodiamine and rutaecarpine, which was similar to ways in which NRCMs shared frequency of spontaneous beat.

It is accepted that determination of LDH and CK activity provides one of the biochemical indexes for the evaluation and diagnosis of heart disease, due to the level of LDH in serum reflecting

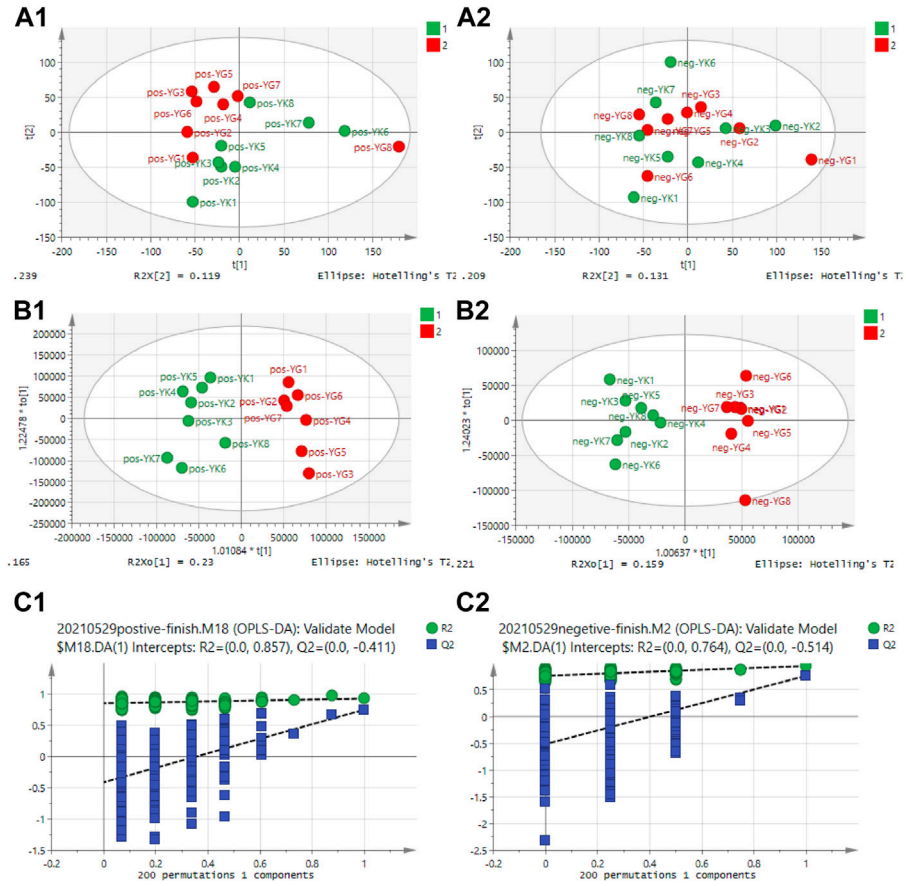

FIGURE 7
Results of multivariate data analysis for rats in YIN-K and YIN-G groups. Note: **(A1)** PCA scores plot-ESI$^+$; **(A2)** PCA scores plot-ESI$^-$; **(B1)** OPLS-DA scores plot-ESI$^+$; **(B2)** OPLS-DAscores plot-ESI$^-$; **(C1)** Permutation plot-ESI$^+$; **(C2)** Permutation-scores plot-ESI$^-$.

injury in the permeability of the cardiomyocytes, and the activity level of CK being directly related to the consumption and supply of myocardial oxygen and energy, muscle contraction, and mitochondrial function (Agress, 1965; Matschke et al., 2005; Ingwall, 2009; Zervou et al., 2016; Bak and Schousboe, 2017; Klein et al., 2020). Furthermore, the mitochondrial membrane potential and the intensity of calcium fluorescence, which were testing indexes in the present study, play an essential role in the mitochondrial function of myocardial tissue homeostasis (Skarka and Ostadal, 2002; Dibb et al., 2007; Kadenbach et al., 2011; Davlouros et al., 2016; Zorova et al., 2018; Schartner et al., 2019; Lai and Qiu, 2020). Understanding of the electrophysiological effects in cardiomyocyte contractile and mechanical function in response to cardiotoxic drugs has previously relied on primary cardiomyocytes from animal models (Liu et al., 2012; Tang et al., 2016; Blair and Pruitt, 2020). Therefore, this research selected the abovementioned indexes to quantify the myocardial cytotoxicity of evodiamine and rutaecarpine in an effort to understand how these bioactive compounds of Euodiae Fructus directly impact the cGMP-PKG pathway at the cellular and cardiomyocytes levels. Although there are empirical studies emphasizing the cardiovascular protective effects of evodiamine and rutaecarpine (Jiang et al., 2017; Zeng et al., 2019), some researchers have verified the risk of arrhythmia and cardiotoxicity *in vivo* and *in vitro*, findings consistent with the results of our study. For example, depending on dosage, dehydroevodiamine and hortiamine could prolong the action potential duration, eventually resulting in proarrhythmic effects (Baburin et al., 2018).

The cGMP-PKG signaling pathway plays a crucial role in various myocardial pathophysiological process, including cell growth and survival, interstitial fibrosis, endothelial permeability, myocardial contraction, and cardiovascular remodeling (Inserte and Garcia-Dorado, 2015; Nakamura and Tsujita, 2021). In particular, the cGMP-PKG pathway is a principal factor implicated in cardiovascular complications of diverse etiological processes because it stimulates downstream targets, including the Ca^{2+} channel, and a β3-adrenceptor in an inhibitory G protein-dependent manner (Takimoto, 2012; Zhang et al., 2014; Arioglu-Inan et al., 2019; Wan et al., 2020). With growing recognition of the

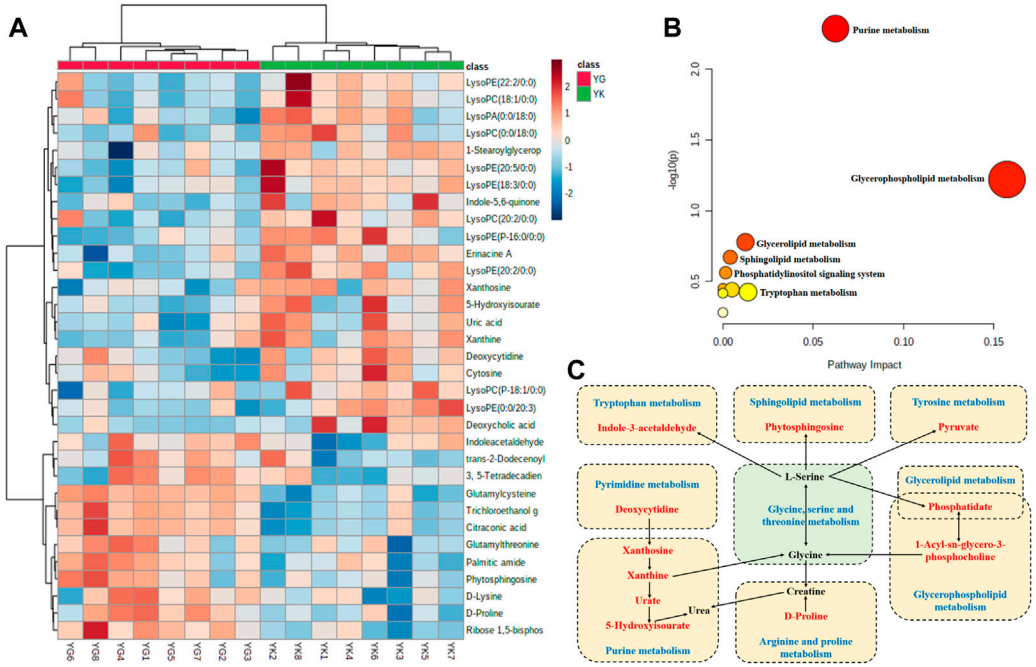

FIGURE 8
Metabolites analysis of rat serum samples in YIN-K and YIN-G groups. Note: **(A)** cluster analysis; **(B)** pathway analysis; **(C)** summary of metabolites and pathways (blue words indicate metabolic pathways, red words indicate identified discriminant metabolites in present research, and black words indicate the related endogenous metabolites).

cGMP-PKG pathway, there is increasing interest in envisaging it as a therapeutic target against the cardiotoxic effects of some drugs. Cumulatively and progressively developing the cardiomyopathy caused by adriamycin, levosimendan and tadalafil could produce greater benefits of anti-cardiotoxicity and prevent cardiomyocyte apoptosis by activating the cGMP-PKG pathway (Koka et al., 2010; Efentakis et al., 2020). Interference with hypotension and bradycardia among the molecular and cellular determinants of the cardiotoxicity induced by Crotalusdurissus cascavella venom, contributing to negative inotropic effects of the heart, have been associated with the NO/cGMP/PKG pathway (Simoes et al., 2021). Understanding the key role of the cGMP-PKG pathway in the cardiac cytotoxicity of evodiamine and rutaecarpine is essential for reducing risk in the clinical usage of Euodiae Fructus, and present research confirms the related mechanism through the agonist of the PKG protein, the PKG drug G1, as well as the following *in vitro* experiments and untargeted metabolomics research.

4.2 Cardiotoxicity induced by Euodiae Fructus in *in vitro* experiments

In general, the quintessence of TCM is syndrome differentiation and treatment, and the guarantee of clinical efficacy is the safe use of medications (Shaw et al., 2012; Xiang et al., 2019). Euodiae Fructus is considered slightly poisonous with hot or warm properties, and is used for treating gastro-intestinal disorders belonging to Yang deficiency in the theory of traditional Chinese medicine (TCM) (Chinese Pharmacopoeia Commission, 2020; Li et al., 2020). Clinical medication factors are complex in practice; the overdosage and irrational usage of Euodiae Fructus are cause for concern because some cases are associated with serious heart disorders and deaths. Accordingly, the current research illustrates the potential cardiotoxicity induced by Euodiae Fructus, and the results in rats with Yin deficiency suggest obvious cardiac physiological function, abnormal ECG, and pathological injury in the high-dosage group of Euodiae Fructus.

First, in order to further explore the clinical problems and simulate clinical symptoms, our study effected a hydrocortisone-induced Yang deficiency and a thyroxine-induced Yin deficiency model in rats, with the relevant modeling methods having certain recognition in syndrome animal modeling under TCM theoretical guidelines (Yao et al., 2007; Han et al., 2013; Ling and Xu, 2013; Reheman et al., 2019; Hu et al., 2021). Notably, the overall characterization, involving the general state, body weight, body temperature, and organ coefficients, in combination with the levels of T3 and TSH in serum, cAMP/cGMP in plasma, and glucose and lipid metabolism were comprehensively evaluated in our experiments.

Second, the transformation of "health benefit" into "cardiac toxicity" for Euodiae Fructus in terms of different syndromes and dosages was investigated based on ECG readings, serum myocardial zymogram results, and cardiac histology. The ECG was foundational in assessing cardiac function in terms of rate and rhythm, and is universally available for the diagnosis of heart diseases (Klabunde, 2017; Teplitzky et al., 2020). Moreover, the determination of cardiac enzyme profiles, including CK, CK-MB, HBDH, LDH, and AST, as evidence of myocardial injury, has been confirmed by substantial research, such as those studies exploring myocardial ischemic necrosis or changes to membrane permeability in myocardial cells (Pappas, 1989; Lee et al., 2009). Despite some promising biomedical approaches in the cardiac research field, cardiac histology is still irreplaceable in the diagnosis of cardiac injuries, owning to the ability of cardiac tissue slices to provide details of the native multicellularity, architecture, and physiology of the heart (Watson et al., 2019; George et al., 2020; Perbellini and Thum, 2020). In our study, an overdose of Euodiae Fructus could induce cardiotoxicity for rats in a state of Yin deficiency, including abnormal ECG and myocardial enzyme results, and cardiac pathological injuries, suggesting that irrational usage and overdosage of Euodiae Fructus is associated with increased risk of potential cardiotoxicity. Our study thus adequately identifies the urgent need to develop pharmacovigilance practices for herbal medicines, to monitor the cardiac function of patients, and to standardize clinical medication to avoid related adverse reactions (Barnes, 2003; Wang et al., 2009).

4.3 The interpretation of untargeted metabolomics research

As the terminal of an organism's biological process, an altered metabolism is one of the hallmarks of noxious effects in the heart, where changes in protein expression and injures in cardiac function ultimately lead to aberrant cellular metabolism (Kroemer et al., 2018; Luz and Tokar, 2018). Fortunately, the emergence of metabolomics research has provided a new approach to statistically and quantitatively visualizing evidence according to the dynamic information in overall profiles of endogenous metabolites after the biological system has suffered from exogenous disturbance and stimulation (Al-Ansari et al., 2021; Shibutami and Takebayashi, 2021; Spyroglou et al., 2021). Indeed, a burst of research utilizing untargeted metabolomics technology has been published in the field of cardiac toxicology over the past decades, based on the dual advantages of global material scanning and the accuracy of material annotation, and contributing to numerous methodological advances in interpreting the enrolled metabolic pathway and toxic mechanism (Parry et al., 2018; Palmer et al., 2020). Here, the methods of untargeted metabolomics research and multivariate statistics were used to detect changes in endogenous metabolisms induced by overdosage of Euodiae Fructus in rats with Yin deficiency. Our result highlights 34 kinds of metabolites, including D-proline, deoxycytidine, 5-hydroxyisourate, cytosine, uric acid, and D-lysine, and a total of 10 metabolic pathways involving the purine metabolism, glycerophospholipid metabolism, glycerolipid metabolism, sphingolipid metabolism, and the phosphatidylinositol signaling system, as well as the arginine and proline metabolisms.

On the one hand, through investigation of potential molecular mechanisms underlying different conditions in biological systems, the expression patterns of some differential metabolites were similar, due to involving the associated metabolic pathways, resulting in the presentation of a close concentration-dependent correlation (You et al., 2019; Jahagirdar and Saccenti, 2020). In this study, the levels of lysophospholipids (lysophosphatidic acid, lysophosphatidylcholine) and lysosphingolipids, namely LysoPC (18:1/0:0), LysoPC (0:0/18:0), LysoPE (22:2/0:0), and LysoPA (0:0/18:0), decreased in the YIN-G group, suggesting that in a Yin deficiency state, high-dose Euodiae Fructus can reduce the lysophosphatidic content and cause possible heart risk. According to published research, LPC (14:0) and LPC (20:2) were verified as highly specific biomarkers of cardiotoxicity from rat plasma samples *via* ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry, and subsequently used a support vector machine to develop a predictive model (Li et al., 2015). As a critical biomarker positively associated with cardiovascular issues, there is increasing evidence showing that lysophospholipids and lysosphingolipids can specifically bind to G-protein coupled receptors, thus directly control secondary messengers involving the Ca^{2+} signaling pathway, Rho Kinase (ROCK), diacylglycerol (DAG), IP3 receptor (IP3R), mitogen-activated protein kinase (MAPK), adenylate cyclase (AC), and phosphatidylinositol 3-kinase (PI3K), etc. (Schilling et al., 2002; Torkhovskaya et al., 2007; Li et al., 2016; Law et al., 2019). Hence, the regulation of lysophospholipids on the G-coupled protein and calcium pathway is similar to the expression level and regulatory function of cGMP-PKG pathway involved in this study.

On the other hand, the cardiotoxicity induced by overdosage and irrational usage of Euodiae Fructus is associated with the purine metabolism, glycerophospholipid metabolism, glycerolipid metabolism, and the sphingolipid metabolism, as well as the phosphatidylinositol signaling system, suggesting that the related cardiotoxic metabolic pathways could mediate oxidative stress, energy metabolism, lipid metabolism, amino acid metabolism and other biological processes. With regard to the purine metabolism in cardiac pathological process, findings demonstrate overwhelmingly that purine release is directly related to the rate of energy

consumption in the heart, and is significantly connected to a wide range of cardiovascular activity, including dilating the coronary artery, reducing reperfusion injury, inhibiting cardiomyocyte apoptosis, and so on. Furthermore, this metabolic pathway is involved in the oxidative stress injury of cardiomyocytes caused by the release of reactive oxygen species (Hisatome et al., 1990; Zucchi et al., 1990; Safranow et al., 2005; Sansbury et al., 2014). The sphingolipids are also known to play a pivotal role in signal transduction; growth and differentiation; immune response, proliferation, and apoptosis; inflammatory response; and other important signal molecules. Sphingolipid metabolism disorder has been widely identified as a prognostic and diagnostic marker for cardiovascular diseases, such as ischemia-reperfusion injury, lipotoxic cardiomyopathy, and cardiac insufficiency in recent lipomics studies, while some specific sphingolipids are new biomarkers for cardiovascular diseases (Baranowski and Gorski, 2011; Iqbal et al., 2017; Hannun and Obeid, 2018; Matanes et al., 2019; Iessi et al., 2020).

5 Conclusion

To the best of our knowledge, no previous study has specifically focused on the mechanisms of potential cardiotoxicity induced by Euodiae Fructus. The present research can thus provide a useful overview of how overdosage and irrational usage of Euodiae Fructus can induce cardiac side effects at macroscopic and microscopic levels, including the organism, tissue, cell, protein, and molecular levels, and hence what needs to be done to improve the safety of herbal medicines, especially herbs with poisonous components. Inevitably, this study is only a preliminary investigation into the cardiac cytotoxicity of evodiamine and rutaecarpine through *in vivo* experiments, and into the expression of the cGMP-PKG pathway in discussions of the differential metabolites in rat serum. Based on our data, it is clear that further research needs to be performed using mass spectrometry and gas chromatography to detect and analyze tissue samples, such as those of myocardium, liver, and kidney, so as to fully tap the overall metabolomic information. Further investigations are warranted to explore the cardiotoxicity profiles and other toxicity correlations of Euodiae Fructus, as well as its toxic ingredients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, and further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the animal ethics committee of Beijing University of Traditional Chinese Medicine.

Author contributions

DZ and BZ contributed to the conception and design of the study; DZ and JL drafted the manuscript; DZ, JL, ZR, HW, RS, and XW conducted the experiments; XZ, YW, ZL, and BZ revised the work critically for important intellectual content; DZ, JL, ZR, and XZ performed the statistical analysis and visualization. All authors have read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1028046/full#supplementary-material

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Clinical features, diagnosis and management of amoxicillin-induced Kounis syndrome

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Background: The available evidence suggests that amoxicillin is often associated with the occurrence of Kounis syndrome (KS). The purpose of this study is to explore the clinical characteristics of KS induced by amoxicillin.

Methods: We searched for case reports of amoxicillin-induced KS through Chinese and English databases from 1972 to May 2022.

Results: A total of 33 patients with KS were included, including 16 patients (48.5%) receiving amoxicillin treatment and 17 patients (51.5%) receiving amoxicillin-clavulanate. The median age was 58 years (range 13–82), 75.8% were from Europe and 81.8% were male. Nearly 70% of KS patients develop symptoms within 30 min after administration. Chest pain (63.6%) and allergic reaction (75.8%) were the most common clinical manifestations. Diagnostic evaluation revealed elevated troponin (72.7%), ST-segment elevation (81.2%) and coronary artery stenosis with thrombosis (53.6%). Thirty-two (97.0%) patients recovered completely after discontinuation of amoxicillin and treatments such as steroids and antihistamines.

Conclusion: KS is a rare adverse reaction of amoxicillin. Amoxicillin-induced KS should be considered when chest pain accompanied by allergic symptoms, electrocardiogram changes and or elevated levels of myocardial injury markers. Therapeutic management of KS requires simultaneous treatment of cardiac and allergic symptoms. Epinephrine should be used with caution in patients with suspected KS.

KEYWORDS

Kounis syndrome, amoxicilline, coronary spasm, acute coronary syndrome, hypersensitivity

Introduction

Kounis syndrome (KS) is an acute coronary syndrome caused by an allergic reaction, and first reported by Kounis and Zavras in 1991 (ounis and Zavras, 1991). Patients with a history of allergies, hypertension, smoking, diabetes, and hyperlipidemia are more likely to be affected. KS can occur at any age, but the most commonly affected age group is 40–70 years (68%) of male patients (74.3%) (Abdelghany et al., 2017). KS seems to have a geographical distribution and is mostly reported in southern Europe, especially in Greece and Turkey (Kounis, 2016). Three variants of KS have been defined. The Type I variant (coronary spasm) includes patients with normal or near-normal coronary arteries but without predisposing factors for coronary artery disease. Allergic reactions result in coronary spasm, with or without elevation of markers of myocardial injury. The Type II variant include patients with pre-existing atherosclerotic disease, acute allergy causing plaque erosion or rupture, presenting as acute myocardial infarction. The type III variant variant refers to allergic manifestations and stent thrombosis after coronary drug stent implantation (Kounis, 2016).

A variety of reasons have been found to induce KS, including many drugs, diseases, food, environmental exposure or certain other conditions (Kounis, 2016). Among them, antibiotics are the most common cause of KS, accounting for about 27%, mosquito bites account for about 23% (Abdelghany et al., 2017). Amoxicillin is a commonly used beta-lactam antibiotic and is usually associated with possible adverse events such as gastrointestinal, allergic reactions and haematological reactions (Salvo et al., 2007). KS is a rare and serious complication after the administration of amoxicillin. Knowledge of amoxicillin-induced KS is largely based on case reports. The clinical features of KS induced by amoxicillin are still unclear. The purpose of this study is to explore the clinical characteristics of KS induced by amoxicillin, and to provide a basis for the rational use of amoxicillin.

Methods

Search strategy

We searched the literature related to amoxicillin-induced KS by searching Chinese databases (Wanfang Data, China National Knowledge Infrastructure (CNKI), Chinese VIP) and English databases (PubMed/Medline, Embase, Web of Knowledge, OVID, Elsevier, Springer Link and Cochrane Library databases) from 1972 to May 2022. The search combined subject and free words such as amoxicillin, amoxicillin-clavulanate, Kounis syndrome, antibiotics, allergic reactions, β-lactams, thrombosis, myocardial infarction, acute coronary syndrome, coronary spasm, hypersensitivity.

Inclusion and exclusion criteria

Inclusion criteria: case report and case analysis of KS induced by amoxicillin. The clinical data is relatively complete, including the amoxicillin application, clinical manifestations, laboratory examinations, treatment and prognosis etc. Exclusion criteria: duplicate literature, reviews, mechanistic studies, animal studies and articles for which the full text was not available.

Data extraction

Two researchers independently conducted a preliminary screening of the literature according to the inclusion and exclusion criteria, and then the group discussed the included literature. We extract the following information of patients: region, gender, age, medical history, drug combination, amoxicillin application, indication, symptom onset time, clinical manifestations, laboratory examinations, imaging examinations, treatment and prognosis by using self-designed data extraction table.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL). Continuous data is represented by median value with ranges, counting data is represented by number of cases and percentage (%).

Results

Basic information

As shown in Figure 1, a total of 456 relevant studies were initially identified. One hundred and fifty-six duplicate studies were excluded. After an initial screening of titles and abstracts, 218 articles were removed. Of the remaining 79 studies, 48 were excluded from the full-text review. A total of 31 studies were included (Alemparte Pardavila et al., 1999; López-Abad et al., 2004; Moreno-Ancillo et al., 2004; Gikas et al., 2005; Del Furia et al., 2007; Tigen et al., 2007; Tavil et al., 2008; Vivas et al., 2008; Biteker et al., 2009; Caglar et al., 2011; Venturini et al., 2011; Viana-Tejedor et al., 2011; Mazarakis et al., 2012; Bezgin et al., 2013; Calf et al., 2013; Ilhan et al., 2013; Kilickesmez et al., 2013; Lombardi et al., 2013; González-de-Olano et al., 2014; Ralapanawa and Kularatne, 2015; Molina Anguita et al., 2016; Salouage et al., 2016; Shimi et al., 2016; Antonelli et al., 2017; Canpolat et al., 2017; Omri et al., 2017; Pradhan et al., 2018; Lopes and Agarwal, 2019; Moloney et al., 2019; Caragnano et al., 2020; Duarte et al., 2020). The basic information of these 33 patients is summarized in Table 1. These patients included

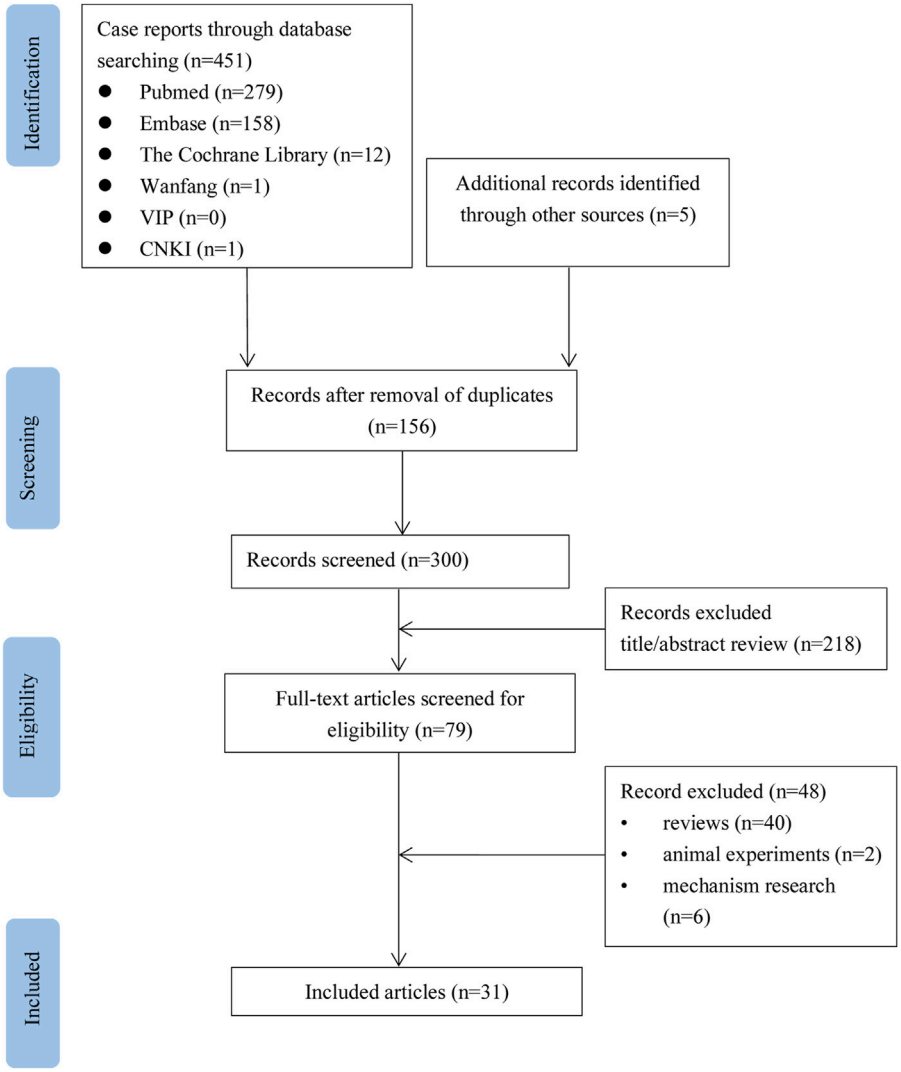

FIGURE 1
Flow chart of the study selection process for reported cases of amoxicillin-induced Kounis syndrome.

27 males (81.8%) and 6 females with a median age of 58years (range 13–82). These patients include 25 cases (75.8%) in Europe, 3 patients (9.1%) in Africa, 2 patients (6.1%) in Asia, and 2 patients (6.1%)) in the United States, 1 patient (3.0%) in Oceania. Amoxicillin and amoxicillin-clavulanate are primarily used for infection and dental care prophylaxis. There were 16 patients with other diseases, including hypertension in 8 patients (24.2%), dyslipidemia in 6 patients (18.2%), diabetes in 4 patients (12.1%), ischemic heart disease in 3 patients (9.1%). Eight patients (24.2%) had a history of smoking habits (Vivas et al., 2008; Venturini et al., 2011; Mazarakis et al., 2012; Lombardi et al., 2013; Salouage et al., 2016; Pradhan et al., 2018; Caragnano et al., 2020). Four patients (12.1%) had previous history of penicillin-allergy (Antonelli et al., 2017; Omri et al., 2017; Caragnano et al., 2020; Duarte et al., 2020). Five patients (15.2%) were treated with other drugs concurrently.

Administration of amoxicillin

In these patients, 16 patients (48.5%) received amoxicillin and 17 patients (51.5%) received amoxicillin-clavulanate (Table 2). The daily dose of amoxicillin ranged from 0.25 g to 2 g, and the dose range of amoxicillin-clavulanate ranged from 0.5 g to 2 g. The route of administration was oral in 29 patients

TABLE 1 Summary of clinical information of 33 patients with amoxicillin-induced Kounis syndrome.

Reference	Age/ Gender	Drug	Daily dosage (g)	Route of administration	Time of symptom onset	Symptoms	Troponin I (ng/ml)	ECG	Echocardiography	Coronarography	KS type	Treatment
5	M/62	AC	0.5	oral	15 min	Unconsciousness, skin vasodilation	NA	ST elevation	NA	Stenosis	II	dopamine
6	M/56	AC	0.5	oral	30 min	CP, D, R	1.37	normal	normal	NA	NA	steroids, antihistamines, NG
7	M/32	AC	0.5	oral	2 h 15 min	dizziness, blurred vision, D, abdominal pain, anaphylaxis	45.5	ST elevation	EF 60%	normal	I	steroids, E, aspirin, UF, pethidine, NG, reteplase
8	F/70	AC	0.5	oral	Few min	P, warmth, flushing, lips and hands swelling, D, unconscious	0.31	ST elevation	hypokinesis	stenosis	II	steroids, antihistamines, aspirin, nitrates
9	M/69	AC	first dose	IV	Few min	CP, epigastric discomfort	normal	ST elevation	normal	normal	I	steroids, NG, CCB
10	F/40	AC	first dose	oral	20 min	CP, swollen extremities, R, tongue swelling, dysphagia	11.65	ST elevation	EF 50%, hypokinesis	Stenosis	II	steroids, antihistamines, tirofiban, clopidogrel, metoprolol, ramipril, atorvastatin.
11	M/64	AC	NA	oral	immediately	CP, erythema, N, V	4.48[a]	ST elevation	normal	thrombosis	II	NG, antihistamines
12	M/48	AC	2	oral	3d	CP, R, S	3.7	ST elevation	EF 40%, hypokinesis	stenosis	III	steroids, antihistamines, simvastatin, aspirin, atenolol, nitrates, clopidogrel
13	M/64	AC	1	oral	5 min	chest discomfort, unconsciousness, R, S	2.1	ST elevation	NA	stenosis	II	steroids, antihistamines, aspirin, clopidogrel, UF
14	M/71	AC	a tablet	oral	within min	V, U, P, dizziness, hypotension	0.266	ST depression	NA	NA	NA	steroids, antihistamines, E
15	F/61	AC	0.25	oral	10min	V, despair, diarrhea, dizziness, fainting	0.245	negative T-wave	normal	NA	I	steroids, antihistamines

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10.3389/fphar.2022.998239

TABLE 1 (*Continued*) Summary of clinical information of 33 patients with amoxicillin-induced Kounis syndrome.

Reference	Age/ Gender	Drug	Daily dosage (g)	Route of administration	Time of symptom onset	Symptoms	Troponin I (ng/ml)	ECG	Echocardiography	Coronarography	KS type	Treatment
16	M/53	AC	NA	oral	Few min	CP, U, P, ventricular, fibrillation	1,272	ST elevation	NA	NA	NA	steroids, antihistamines
17	M/60	AC	1	oral	immediately	CP, altered mental status, dizziness, P, warmth, flushing, D, U	0.0047	ST elevation	EF 30%, hypokinesis	Stenosis, occlusion	II	steroids, antihistamines, UF, clopidogrel, streptokinase
18	M/62	AC	0.5	oral	immediately	general asthenia, face erythema	0.064	ST elevation	EF 55%	stenosis	II	adrenaline, mechanical ventilation
19	M/22	AC	single	oral	1 h	CP, chest tightness	0.550	ST elevation	normal	normal	I	aspirin, UF
20	M/64	AC	1	oral	After 5 tablets	CP, weakness, S	0.14	repolarization abnormalities	NA	subocclusion	I	isosorbide dinitrate, aspirin, LMWH
20	M/57	ACA	0.875	oral	15 min	CP, N, R, P	3.78	ST elevation	EF 55%, hypokinesis	normal	I	steroids, antihistamines
20	M/58	ACA	0.875	oral	Few min	CP, R, D	normal	ST elevation	NA	normal	I	steroids, antihistamines
21	F/58	ACA	NA	oral	1 h	CP, flushing, P, warmth, facial oedema, U, dizziness	0.51	Pardee waves	NA	thrombosis	III	steroids, morphine, E, UF
22	F/56	ACA	2	oral	Immediately	chest discomfort, N, V, S, erythema, U	7.9	ST depression	EF 60%	normal	I	steroids, antihistamines, ephedrine, aspirin, clopidogrel
23	M/54	ACA	1	oral	30 min	CP, P, N	NA	ST elevation	EF 30%, hypokinesis	vasospasm	I	aspirin, clopidogrel, UF, nitrate
24	M/53	ACA	NA	oral	NA	CP, D, S, N, dizziness, cyanosed lips, U	NA	ST-elevation	NA	stenosis	II	steroids, adrenaline, aspirin, fentanyl, UF, ticagrelor
25	F/73	ACA	NA	IV	1 min	R, altered state of consciousness	2046	ST elevation	NA	stenosis	II	steroids, antihistamines, advanced life support,

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10.3389/fphar.2022.998239

TABLE 1 (*Continued*) Summary of clinical information of 33 patients with amoxicillin-induced Kounis syndrome.

Reference	Age/ Gender	Drug	Daily dosage (g)	Route of administration	Time of symptom onset	Symptoms	Troponin I (ng/ml)	ECG	Echocardiography	Coronarography	KS type	Treatment
												mechanical ventilation
26	M/74	ACA	1.2	IV	20 min	anaphylactic shock, R, P, palpitations, chest tightness, S	2.2	ST elevation	hypokinesis	NA	NA	steroids, antihistamines, adrenalin, aspirin, clopidogrel, atovastatin
27	M/43	ACA	NA	oral	NA	CP, P, erythema	NA	ST elevation	NA	normal	I	steroids, antihistamines, oxygen
28	M/61	ACA	1	oral	10 min	CP, R	0.288[a]	ST elevation	NA	normal	II	antihistamine
29	M/13	ACA	0.5	oral	30 min	CP, R	13	ST elevation	hypokinesis	normal	I	steroids, antihistamines
30	M/31	ACA	NA	oral	1 h	angina pectoris	↑	ST elevation	normal	stenosis	NA	thrombolytic therapy (tPA)
31	M/29	ACA	1	oral	NA	CP, D	29	ST elevation	normal	normal	I	steroids, antihistamines, morphine
32	M/16	ACA	1	oral	NA	ischemic pain	NA	ST elevation	normal	normal	I	NA
33	M/82	ACA	NA	IV	Immediately	abdominal pain, D, erythematous, R	NA	ST elevation	NA	normal	I	steroids, antihistamines, aspirin, UF
34	M/58	ACA	0.875	oral	30 min	CP, D, weakness, S, hypotension	normal	ST elevation	NA	normal	I	E
35	M/25	ACA	1	oral	20 min	CP, R	2.40[a]	ST elevation	hypokinesis	normal	I	NG, anti-ischemic and anti-platelet drugs

Abbreviation: AC, amoxicillin; ACA, amoxicillin and clavulanic acid; IV, intravenous; CP, chest pain; V, vomiting, N, nausea; P, pruritus; S, sweating; D, dyspnea; R, rash, U, urticaria; Electrocardiogram; LMWH, low molecular weight heparin; UF, unfractionated heparin; tPA, tissue plasminogen activator; CCB, calcium channel blockers; NG, nitroglycerine; ECG, E, epinephrine. Na, Not applicable.

[a]Represents the value of Troponin T.

10.3389/fphar.2022.998239

TABLE 2 Basic information of 33 patients with amoxicillin-induced Kounis syndrome.

Parameter	Clinical features	Value
Sex	males	27 (81.8%)
	females	6 (18.2%)
Age	years	58 (13-82)[b]
Race	Europe	25 (75.8%)
	Africa	3 (9.1%)
	Asia	2 (6.1%)
	USA	2 (6.1%)
	Oceania	1 (3.0%)
Drug	amoxicillin	16 (48.5%)
	amoxicillin-clavulanate	17 (51.5%)
Route of administration	oral	29 (87.9%)
	intravenous injection	4 (12.9%)
Symptom onset time (29)[a]	immediately	5 (15.2%)
	within 30 min	18 (54.5%)
	1 h	3 (9.1%)
	2 h 15 min	1 (3.0%)
	3 days	1 (3.0%)
	5th tablet	1 (3.0%)
Indication	upper respiratory tract infection	9 (27.3%)
	dental infection	5 (15.2%)
	dental care prophylaxis	3 (9.1%)
	pulmonary infection	2 (6.1%)
	flu	2 (6.1%)
	urinary tract infection	2 (6.1%)
	trauma	1 (3.0%)
	systemic inflammatory response syndrome	1 (3.0%)
	na	8 (24.2%)
Medical history (16)[a]	hypertension	8 (24.2%)
	dyslipidemia	6 (18.2%)
	diabetes	4 (12.1%)
	ischemic heart disease	3 (9.1%)
	thyroid disease	2 (6.1%)
	cerebrovascular disease	2 (6.1%
	asthma	2 (6.1%)
	vesical neoplasy	1 (3.0%)
	volvulus	1 (3.0%)
	epilepsy	1 (3.0%)
Risk factors	penicillin-allergy	4 (12.1%)
	non-steroidal anti-inflammatory drugs food allergy	1 (3.0%)
	smoking	1 (3.0%)
		8 (24.2%)
combination therapy	nebivolol/hydrochlorothiazide, doxazosin, insulin, clopidogrel, captopril, furosemide, metformin, aspirin, statins, tapazole	5 (15.2%)

[a]Represents the number of patients out of 33 on which information regarding this particular parameter was provided.
[b]Median (minimum-maximum).
Abbreviations: na, not applicable.

(87.9%) and intravenous in 4 patients (12.9%). KS had a wide range of onset times, from immediately after taking the medicines to 3 days. Symptoms occurred immediately after taking the medicines in 5 patients (15.2%), within half an hour in 18 patients (54.5%), 1 h in 3 patients (3.0%), 2 h 15 min in 1 patient (3.0%), after taking the 5th tablet in

TABLE 3 Clinical symptoms, imaging and laboratory tests of 33 patients with amoxicillin-induced Kounis syndrome.

Parameter	Clinical features	Value
Symtoms	chest pain	21 (63.6%)
	allergic reactions (rash, pruritus, erythema)	25 (75.8%)
	neurological adverse reactions (alteration of consciousness, dizziness)	10 (30.3%)
	gastrointestinal adverse reactions (nausea, vomiting, abdominal pain)	10 (30.3%)
	hypotension	13 (36.3%)
	dyspnea	6 (18.2%)
	cardiac arrest	4 (12.1%)
	chest discomfort	2 (6.1%)
	swelling (lips, hands, tongue, face)	2 (6.1%)
	chest tightness	1 (3.0%)
Electrocardiogram	ST elevation	27 (81.2%)
	depression	2 (6.1%)
	pardee waves	1 (3.0%)
	left ventricular repolarization abnormalities	1 (3.0%)
	negative T-wave	1 (3.0%)
	normal	1 (3.0%)
Echocardiography (20) [a]	normal	13 (65.0%)
	hypokinesis	9 (45.0%)
	reduced ejection fraction	3 (15.0%)
Coronary angiography (28) [a]	normal	14 (50.0%)
	stenosis	12 (42.9%)
	thrombosis	2 (7.1%)
	left coronary artery	8 (28.6%)
	right coronary artery	4 (14.3%)
Laboratory examination (27)[a]	troponin T	3 (11.1%)
	elevated	3 (11.1%)
	troponin I	24 (88.9%)
	normal	3 (11.1%)
	elevated	21 (77.8%)
		2.2 (0.064, 2046) [b]
	creatine kinase	15 (45.5%)
	normal	5 (15.2%)
	elevated	10 (30.3%)
	creatine kinase-myocardial band	9 (27.3%)
	elevated	9 (27.3%)
	serum tryptase	5 (15.2%)
	elevated	5 (15.2%)
	skin prick tests	10 (30.3%)
	positive	10 (30.3%)

[a]Represents the number of patients out of 33 on which information regarding this particular parameter was provided.
[b]Median (minimum-maximum).

1 patient (3.0%), and 3 days in 1 patient (3.0%). Two patients (6.1%) developed similar symptoms after taking amoxicillin in their previous medical history (Del Furia et al., 2007; Tavil et al., 2008). One patient (3.0%) took amoxycillin in the past without any related symptoms (Gikas et al., 2005). Symptoms reappeared in 1 patient (3.0%) who received amoxicillin again (Moreno-Ancillo et al., 2004).

Clinical manifestations

The clinical symptoms of 33 KS patients are summarized in Table 3. The main clinical manifestations of these patients included chest pain in 21 patients (63.6%), allergic reactions (rash, pruritus, erythema) in 25 patients (75.8%), neurological adverse reactions (alteration of consciousness, dizziness) in 10 patients (30.3%), and

TABLE 4 Treatment and prognosis of 33 patients with amoxicillin-induced Kounis syndrome.

Parameter		Value
Treatment	discounted	33 (100%)
	steroids	22 (66.7%)
	antihistamines	20 (66.0%)
	epinephrine	6 (18.2%)
	nitrate	8 (24.2%)
	anti-platelet drugs	14 (42.4%)
	anticoagulant drugs	10 (30.3%)
	thrombolytic therapy	1 (3.0%)
	cardiac resuscitation	1 (3.0%)
	percutaneous coronary intervention	8 (24.2%)
	coronary artery bypass surgery	1 (3.0%)
Outcome	recovered	32 (97.0%)
	died	1 (3.0%)
Kounis syndrome variants	I	16 (48.5%)
	II	10 (30.3%)
	III	2 (6.1%)
	na	5 (15.2%)

Abbreviations: na, not applicable.

gastrointestinal adverse reactions (nausea, vomiting, abdominal pain) in 10 patients (30.3%), dyspnea in 6 patients (18.2%), swelling (lips, hands, tongue, face) in 2 patients (6.1%). Thirteen patients had hypotension at the onset of symptoms (Alemparte Pardavila et al., 1999; López-Abad et al., 2004; Gikas et al., 2005; Vivas et al., 2008; Mazarakis et al., 2012; Kilickesmez et al., 2013; Lombardi et al., 2013; González-de-Olano et al., 2014; Ralapanawa and Kularatne, 2015; Salouage et al., 2016; Shimi et al., 2016; Duarte et al., 2020). Cardiac arrest occurred in 4 patients (12.1%) (Calf et al., 2013; Canpolat et al., 2017; Caragnano et al., 2020; Duarte et al., 2020).

Laboratory examination

The laboratory tests of 33 KS patients are summarized in Table 2. Laboratory exams of troponin and tryptase performed after the beginning of the episode in some patients. Of the 27 recorded cases, 3 patients (11.1%) had elevated troponin T, 3 patients (11.1%) had normal troponin I, and 21 patients (77.8%) had elevated troponin I, with a median of 2.2 ng/ml (range 0.064–2046). Creatine kinase (CK) was reported in 15 patients(45.5%), with elevations in 10 patients (30.3%) (Alemparte Pardavila et al., 1999; Moreno-Ancillo et al., 2004; Gikas et al., 2005; Tigen et al., 2007; Caglar et al., 2011; Viana-Tejedor et al., 2011; Bezgin et al., 2013; Antonelli et al., 2017; Pradhan et al., 2018; Duarte et al., 2020). Nine patients (27.3%) reported elevated creatine kinase-myocardial band (CK-MB) (Moreno-Ancillo et al., 2004; Gikas et al., 2005; Tigen et al., 2007; Tavil et al., 2008; Biteker et al., 2009; Venturini et al., 2011; Bezgin et al., 2013; Kilickesmez et al., 2013; Omri et al., 2017). The serum tryptase levels were significantly elevated in 5 patients (15.2%) undergoing examination (Biteker et al., 2009; González-de-Olano et al., 2014; Molina Anguita et al., 2016; Lopes and Agarwal, 2019; Duarte et al., 2020). The results were positive in 10 patients (30.3%) undergoing skin prick tests (Alemparte Pardavila et al., 1999; López-Abad et al., 2004; Moreno-Ancillo et al., 2004; Gikas et al., 2005; Del Furia et al., 2007; Vivas et al., 2008; Bezgin et al., 2013; Kilickesmez et al., 2013; González-de-Olano et al., 2014; Molina Anguita et al., 2016).

Imaging examination

The imaging examinations of 33 KS patients are summarized in Table 2. Electrocardiogram (ECG) examination mainly showed ST segment elevation in 27 patients (81.2%). Very few patients showed pardee waves (3.0%), depression (6.1%), left ventricular repolarization abnormalities (3.0%) and negative T-wave (3.0%) on ECG. Only 1 patient had normal ECG (3.0%). Echocardiography in 20 patients (60.6%) at the onset of KS showed normal in 13 patients (65.0%), hypokinesis in 9 patients (45.0%), and reduced ejection fraction in 3 patients (15.0%) (Venturini et al., 2011; Canpolat et al., 2017; Caragnano et al., 2020). Coronary angiography in 28 patients showed normal in 14 patients (50.0%), stenosis in 12 patients (42.9%), and thrombosis in 2 patients (7.1%) (Viana-Tejedor et al., 2011; Salouage et al., 2016). The left coronary artery (LCA) was affected in 8 patients (28.6%), (Del Furia et al., 2007; Caglar et al., 2011; Viana-Tejedor et al., 2011; Mazarakis et al., 2012; Lombardi et al., 2013; Salouage et al., 2016; Omri et al., 2017; Moloney et al., 2019), and the right coronary artery (RCA) was affected in 4 patients (14.3%) (Tigen et al., 2007; Viana-Tejedor et al., 2011; Mazarakis et al., 2012; Canpolat et al., 2017).

Treatment and prognosis

The treatment and prognosis of the 33 KS patients are summarized in Table 4. All patients immediately withdrew amoxicillin and amoxicillin-clavulanate after the onset of symptoms (Table 2). The remaining treatment options included steroids in 22 patients (66.7%), antihistamines in 20 patients (66.0%), epinephrine in 6 patients (18.2%), nitrate in 8 patients (24.2%), anti-platelet drugs in 14 patients (42.4%), anticoagulant drugs in 10 patients (30.3%), thrombolytic therapy in 1 patient (3.0%). Revascularization was performed in 9 patients (27.3%), including percutaneous coronary intervention (PCI) in 8 patients (24.2%), coronary artery bypass surgery (CABS) in 1 patient (3.0%) (Alemparte Pardavila et al., 1999; Del Furia et al., 2007; Caglar et al., 2011; Venturini et al., 2011; Mazarakis et al., 2012; Lombardi et al., 2013; Antonelli et al., 2017; Omri et al., 2017; Moloney et al., 2019). Thirty-two patients (97.0%) recovered completely, and only one patient (3.0%) died (Omri et al., 2017).

Types of Kounis syndrome

Sixteen patients (48.5%) belonged to type I KS variant, 10 patients (30.3%) belonged to type II KS variant, 2 patients (6.1%) belonged to type III KS variant. The KS variant could not be identified in the remaining 5 patients (15.2%).

Discussion

KS is an allergic acute coronary syndrome that can occur at any age, but the most commonly affected age group is 40–70 years (68%) of male patients (74.3%). Patients with a history of allergies, hypertension, smoking, diabetes, and hyperlipidemia are more likely to be affected (Abdelghany et al., 2017). Among the 33 reported cases of KS induced by amoxicillin, the majority were type I variant, the patients were mainly middle-aged men from Europe. Approximately 70% of cases occur within 30 min after administration. The diagnosis of KS mainly relies on clinical symptoms and signs as well as laboratory tests, electrocardiogram, echocardiography and coronary angiography. In addition to the typical symptoms of chest pain, allergic reactions will appear, including rash, hives. Cardiac troponin I or T and myocardial enzymes (CK, CK-MB) are important markers of myocardial injury. ECG usually showed ischemia-related ST-segment changes, of which ST-segment elevation was the most common manifestation. Coronary angiography may show spasm or stenosis of coronary vessels. The study showed that LCA was the culprit in approximately one-third of patients with coronary vasospasm or stenosis.

It is currently believed that the occurrence of KS is caused by allergic reactions in people with allergies after exposure to specific antigens. The main inflammatory cells that are involved in the development of KS are mast cells that interact with macrophages and T-lymphocytes (Fassio et al., 2016). Infiltration of activated mast cells into plaque erosion or rupture areas is a common pathway between allergic and non-allergic coronary events (Kovanen et al., 1995). These activated cells release inflammatory mediators, including histamine, neutral proteases, arachidonic acid products, platelet activating factor and heparin, etc., leading to peripheral vasodilatation, hypotension, coronary spasm, and coronary atherosclerosis erosion, rupture of plaque-like plaques or thrombosis in coronary stents (Abdelghany et al., 2017).

At present, the guideline for the treatment of KS have not been established, and the treatment recommendations are mainly derived from the experience summary of case reports. The treatment of KS should consider two aspects of acute coronary syndrome (ACS) and allergic reaction. Patients with ACS should be treated according to the ACS guidelines. Anti-allergic treatment often has a better effect in patients with type I KS variant, while patients with type II variant and III KS variant need to treat acute coronary syndromes while being anti-allergic (Abdelghany et al., 2017). Corticosteroids and H1 and H2 antihistamines can all reduce or eliminate allergy symptoms. The administration of vasodilators such as calcium channel blockers and nitrates can abolish hypersensitivity induced vasospasm. Epinephrine should be used with caution in KS, because it can aggravate myocardial ischemia, prolong the QTc interval and induce coronary vasospasm and arrhythmia (Fassio et al., 2016). Stabilizing mast cells and preventing the release of inflammatory mediators may be a new therapeutic strategy for KS. Drugs and natural molecules that stabilize mast cells include mediator antagonists, mediator biosynthesis inhibitors, leukotriene antagonists, mediator receptor blockers such as sodium nedocromil, sodium cromoglycate, ketotifen, lodoxamide, humanized IgG1 monoclonal antibodies and others which interfere with mast cell stabilization (Cevik et al., 2010).

KS has a good prognosis and can fully recover with appropriate treatment in most patients. Our research showed that amoxicillin-induced KS may have serious complications, such as cardiac arrest in 12.1% of patients and death in 3% of patients.

Conclusion

In conclusion, KS is a rare adverse reaction of amoxicillin. Amoxicillin-induced KS should be considered when chest pain accompanied by allergic symptoms, ECG changes and or elevated levels of myocardial injury markers.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

CW and SZ conceived of the presented idea. CW, YZ, WF, ZL and SZ wrote the manuscript. All authors discussed the results and contributed to the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Do antibody–drug conjugates increase the risk of sepsis in cancer patients? A pharmacovigilance study

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Introduction: Antibody–drug conjugates (ADCs) produce unparalleled efficacy in refractory neoplasms but can also lead to serious toxicities. Although ADC-related sepsis has been reported, the clinical features are not well characterized in real-world studies.

Objective: The aim of this study was to identify the association between ADCs and sepsis using FAERS data and uncover the clinical characteristics of ADC-related sepsis.

Methods: We performed disproportionality analysis using FAERS data and compared rates of sepsis in cancer patients receiving ADCs vs. other regimens. Associations between ADCs and sepsis were assessed using reporting odds ratios (RORs) and information component (IC). For each treatment group, we detected drug interaction signals, and conducted subgroup analyses (age, gender, and regimens) and sensitivity analyses.

Results: A total of 24,618 cases were reported with ADCs between Q1, 2004 and Q3, 2021. Sepsis, septic shock, multiple organ dysfunction syndrome, and other sepsis-related toxicities were significantly associated with ADCs than other drugs in this database. Sepsis and multiple organ dysfunction syndrome have the highest safety concerns with ADCs compared with other anticancer monotherapies. Gemtuzumab ozogamicin and inotuzumab ozogamicin showed increased safety risks than other ADCs. For the top nine ADC-related sepsis, males showed higher sepsis safety concern than females (p <0.001); however, age did not exert influence on the risk of sepsis. We identified that 973 of 2,441 (39.9%) cases had acute myeloid leukemia (AML), and 766 of 2613 (29.3%) cases on ADCs died during therapy. Time-to-onset analysis indicated ADC-related sepsis is prone to occur within a month after administration. Co-administration of ADCs with colony-stimulating factors, proton pump inhibitors, H2-receptor antagonists, or CYP3A4/

5 inhibitors showed to synergistically increase the risk of sepsis-related toxicities.

Conclusion: Antibody–drug conjugates may increase the risk of sepsis in cancer patients, leading to high mortality. Further studies are warranted to characterize the underlying mechanisms and design preventive measures for ADC-related sepsis.

KEYWORDS

antibody–drug conjugates, sepsis, pharmacovigilance, FAERS, data mining

Introduction

Antibody–drug conjugates (ADCs) are a relatively new class of anticancer agents designed to merge the selectivity of monoclonal antibodies with cell-killing properties of chemotherapy. They are commonly described as the "Trojan horses" of therapeutic armamentarium because of their capability of directly conveying cytotoxic drug (payloads) into the tumor space, thus transforming chemotherapy into a targeted agent (Criscitiello et al., 2021). The FDA has approved 12 ADCs, which could be categorized by different kinds of payload, tubulin polymerization inhibitors (trastuzumab emtansine, enfortumab vedotin, brentuximab vedotin, polatuzumab vedotin, belantamab mafodotin, and tisotumab vedotin), DNA-damaging agents (gemtuzumab ozogamicin, inotuzumab ozogamicin, trastuzumab deruxtecan, and sacituzumab govitecan), pyrrolobenzodiazepine (loncastuximab tesirine), and truncated exotoxin (moxetumomab pasudotox). ADCs have an excellent risk-to-benefit ratio (Chau et al., 2019) in many types of neoplasms and seem suited to provide benefit for patients with treatment-refractory cancers (Drago et al., 2021). A recent study indicated that grade 3/4 anemia, neutropenia, and peripheral neuropathy were consistently reported for ADCs whose payload is monomethyl auristatin E (MMAE), thrombocytopenia and hepatic toxicity for emtansine (DM1), and ocular toxicity for monomethyl auristatin F (MMAF) (Masters et al., 2018). Another study showed that despite the use of antibodies targeting antigens abundantly and exclusively expressed on cancer cells (i.e., target cells), dose-limiting toxicities (DLTs) in normal cells/tissues are frequently reported even at suboptimal therapeutic doses (Mahalingaiah et al., 2019).

Sepsis is a condition that is associated with extremely high mortality and, for many of those who survive, severe morbidity. Cancer patients with sepsis have higher mortality rates than non-cancer patients (Hensley et al., 2019; Manjappachar et al., 2022). A recent study further indicated that septic shock in patients with hematologic malignancies is associated with a high mortality rate and poor 90-day survival compared with the control group. The World Health Organization (WHO) designated sepsis a global health priority in 2017 and adopted a resolution to improve the prevention, diagnosis, and management of sepsis (Cecconi et al., 2018).

The first case of sepsis was reported with brentuximab vedotin in 2014 (Schaefer et al., 2014). Since then, several sepsis cases have been reported with ADCs, such as enfortumab vedotin, polatuzumab vedotin, and inotuzumab ozogamicin, in clinical trials (DeAngelo et al., 2020; Sehn et al., 2020; Powles et al., 2021). A pool analysis of clinical trials showed that 28% of cancer patients who received gemtuzumab ozogamicin developed grade 3 to 4 infection, of which 16% progressed to sepsis (Koo and Baden, 2008). However, there are no reviews, meta-analyses, or large cohort studies to identify the association between sepsis and ADCs. The clinical characteristics, broad spectrum, and outcome of sepsis-related toxicities correlated with ADCs remain unknown. Herein, our pharmacovigilance study analyzes the association between ADCs and sepsis-related toxicities using data from the FDA's Adverse Event Report System (FAERS).

Materials and methods

Study design and data sources

The study protocol for our observational, retrospective, cross-sectional pharmacovigilance study of the FAERS database (evaluation of reporting of antibody–drug conjugate-associated sepsis-related toxicities) was registered on ClinicalTrials.gov, NCT05349383. AERS*Mine* (Sarangdhar et al., 2016), a validated web-based platform that analyzes FAERS reports for AE (adverse event) association with drugs, indications, and other features including demographics, reporting period, and report source, was used to conduct this pharmacovigilance analysis. Several high-impact studies (Sarangdhar et al., 2016; Fadini et al., 2018; Fadini et al., 2019; Suarez-Almazor et al., 2019; Sarangdhar et al., 2021) have used AERS*Mine* to analyze FAERS data, including a recent study (van Hasselt et al., 2020) which combined post-marketing data with cell line-derived transcriptomic datasets to identify a gene signature to predict the risk of cardiotoxicity with protein kinase inhibitors. Ethical approval was not required because this study was conducted by using deidentified data.

Procedures

This study included all sepsis-related toxicities in cancer patients reported between 2004 and 2021 (Q3) and classified by preferred term (PT) under sepsis (SMQ, Standardised MedDRA Query), according to the Medical Dictionary for Regulatory Activities (MedDRA 25.0; Supplementary Material S1 sepsis reports with counts >0 were included). We used case/non-case analysis to study if sepsis was differentially reported with ADCs as compared to other drugs in the complete database. To highlight the underlying association between ADCs and sepsis, we compared the safety signals of sepsis among ADCs and other common cancer regimens, such as chemotherapy, targeted therapy, and their combinations. First, we identified relevant National Comprehensive Cancer Network (NCCN) guidelines (Supplementary Material S1), according to FDA-approved indications of ADCs. Then we extracted different cancer regimens (Supplementary Material S1) from those selected NCCN guidelines. AERS*Mine* was used to analyze sepsis safety signals among different regimens. We used a heatmap to display the landscape of sepsis-related toxicities among anticancer therapies.

For detailed clinical features, we analyzed the sepsis frequencies by age, gender, and different ADCs regimens, and used the forest plot to visualize the difference. The outcome of ADC-related sepsis was also detected. Furthermore, a previous study showed the time-to-onset analysis method does not share the major drawback of disproportionality analysis (DPA) known as the masking effect and could be a complementary tool to detect safety signals apart from traditional DPA (Van Holle et al., 2012). Another study displayed the process of the time-to-onset analysis in detail by using the Weibull distribution (Ando et al., 2019). We detected time to onset of ADC-related sepsis leveraging FAERS raw data in this study.

Drug–drug interaction (DDI) may affect the occurrence and severity of adverse drug reactions. For instance, a higher proportion of patients reported interstitial pneumonitis for nivolumab in combination with epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) vs. treatment with either drug alone (Oshima et al., 2018). Granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) is usually used to augment myeloid cell functions in cancer patients receiving chemotherapy. Previous research studies showed that granulocyte colony-stimulating factor could enhance the effect of gemtuzumab ozogamicin in acute myeloid leukemia (Leone et al., 2004) and primary prophylaxis with G-CSF may improve outcomes in patients with newly diagnosed stage III/IV Hodgkin lymphoma treated with brentuximab vedotin in addition to chemotherapy (Straus et al., 2020). The expert consensus on the clinical application of antibody–drug conjugates in the treatment of malignant tumors (2020 edition) of China (Professional Committee on Clinical Research of Oncology Drugs CA-CAExpert Committee for Monitoring the Clinical Application of Antitumor DrugsBreast Cancer Expert Committee of National Cancer Quality Control CenterCancer Chemotherapy Quality Control Expert Committee of Beijing Cancer Treatment Quality Control and Improvement Center, 2021) also recommended that colony-stimulating factors could be used to prevent the neutropenia associated with ADCs. So we detected the safety signal of sepsis when colony-stimulating factors were combined with ADCs in the DDI analysis. A previous research study (Zhang et al., 2022) showed that proton pump inhibitors (PPIs) interfere with the antitumor potency of HER2-targeting ADCs due to the inhibition of vacuolar H+-ATPase activity. We inferred that drugs that inhibit gastric acid secretion, such as proton pump inhibitors and H2-receptor antagonists, may alter the risk of sepsis when co-administered with ADCs. Moreover, we searched the DrugBank (Wishart et al., 2018) and found that enfortumab vedotin, brentuximab vedotin, polatuzumab vedotin, tisotumab vedotin, trastuzumab deruxtecan, and loncastuximab tesirine are mainly metabolized by the CYP3A4/5 enzyme. When the activity of the CYP3A4/5 enzyme was affected by other drugs, the metabolism process of ADCs would also be affected. So we also detected the safety signal of sepsis when ADCs were combined with proton pump inhibitors, H2-receptor antagonists, and CYP3A4/5 strong inhibitors.

Since pharmacovigilance studies based on spontaneous reporting systems can be impacted by reporting bias (Noguchi et al., 2021), we further conducted sensitivity analysis by excluding known drugs and indications which may increase susceptibility to sepsis.

Statistical analysis

In this study, two calculation indicators of disproportionality were used, the reporting odds ratio (ROR) (Rothman et al., 2004) based on the frequentist statistical method and the information component (IC) (Bate et al., 1998) based on the Bayesian statistical method used at the Uppsala Monitoring Centre (UMC). When the lower limit of the 95% credibility interval of ROR (ROR_{025}) >1 (Rothman et al., 2004) or the lower limit of the 95% credibility interval of IC (IC_{025}) >0 (Bate et al., 1998), significant adverse events were detected. Noren et al. (2013) put forward shrinkage observed-to-expected ratios to provide effective protection against spurious associations in signal detection. This adjustment calculation method was used in our analysis. These IC and ROR are standard pharmacovigilance metrics and have recently been shown to quantitate the spectrum and characteristics of immune

TABLE 1 Sepsis-related toxicities reported with ADC therapy *vs.* the full FAERS database.

	Overall ADCs	Full database	ROR_{025}	IC_{025}
Total number of ICSRs available	24618	16849672		
Number of ICSRs by sepsis subgroups				
Sepsis	1054	108277	6.55	**2.63**
Escherichia bacteremia	25	1848	6.32	2.33
Septic shock	419	38950	6.85	**2.71**
Bacteremia	114	10139	6.49	**2.59**
Systemic inflammatory response syndrome	31	4066	3.69	1.69
Neutropenic sepsis	193	7238	16.34	**3.89**
Escherichia sepsis	43	2915	7.58	2.68
Klebsiella sepsis	21	1040	9.15	2.68
Staphylococcal sepsis	61	5561	5.9	2.41
Staphylococcal bacteremia	59	3880	8.18	2.84
Enterococcal bacteremia	22	853	11.86	2.97
Candida sepsis	14	923	6.21	2.07
Streptococcal bacteremia	20	895	10.03	2.75
Blood culture positive	71	3668	10.69	3.22
Fungal sepsis	13	854	6.11	2.01
Urosepsis	42	8939	2.38	1.13
Multiple organ dysfunction syndrome	408	17778	14.77	**3.78**
Pseudomonal sepsis	62	1708	20.02	3.96
Systemic *candida*	29	1883	7.41	2.56
Fungemia	23	1313	8.07	2.58
Streptococcal sepsis	36	1238	14.71	3.43
Device-related sepsis	19	1803	4.63	1.87
Bacterial sepsis	34	3217	5.21	2.16
Enterococcal sepsis	15	922	6.79	2.2
Biliary sepsis	21	456	21.31	3.47

ADCs, antibody–drug conjugates; FAERS, FDA's Adverse Event Report System; IC_{025}, the lower limit of the 95% credibility interval of information component; ICSR, individual case safety report; ROR_{025}, the lower limit of the 95% credibility interval of reporting odds ratio. When $IC_{025} > 0$ or $ROR_{025} > 1$, a significant safety signal was detected.

checkpoint inhibitor-related cardiovascular toxicity (Salem et al., 2018).

Several methods for detecting DDI have been reported (Noguchi et al., 2019); however, the omega (Ω) shrinkage observed-to-expected ratio measure (Noren et al., 2008; Noguchi et al., 2019) used by the UMC (UMC, 2016) has shown to be the most conservative in DDI signal detection (Noguchi et al., 2020). The detection criterion is the lower limit of the 95% credibility interval of Ω ($Ω_{025}$) >0 (calculation of IC, ROR, and Ω are included in Supplementary Material S1). Safety signals of sepsis-related toxicities among diverse treatment regimens were conducted using the χ2 test (Bonferroni adjustment). All data analyses were performed independently by two or more authors, and all statistical analyses were performed with JMP Pro 16 (SAS Institute Inc., Cary, NC, United States) and Microsoft Excel (2021).

Results

ADCs-sepsis disproportionate analysis

Our post-marketing safety signal analysis showed that sepsis and other related toxicities were significantly associated with ADCs. Sepsis (ROR_{025} 6.55 and IC_{025} 2.63), septic shock (ROR_{025} 6.85 and IC_{025} 2.71), multiple organ dysfunction syndrome (ROR_{025} 14.77 and IC_{025} 3.78), neutropenic sepsis (ROR_{025} 16.34 and IC_{025} 3.89), and bacteremia (ROR_{025} 6.49 and IC_{025} 2.59) were the five most common sepsis-related toxicities correlated with ADCs (Table.1). The IC values and their 95% credibility intervals over time for sepsis, septic shock, neutropenic sepsis, and multiple organ dysfunction syndrome, which are top four of the most reported sepsis-related toxicities, are shown in Figure 1.

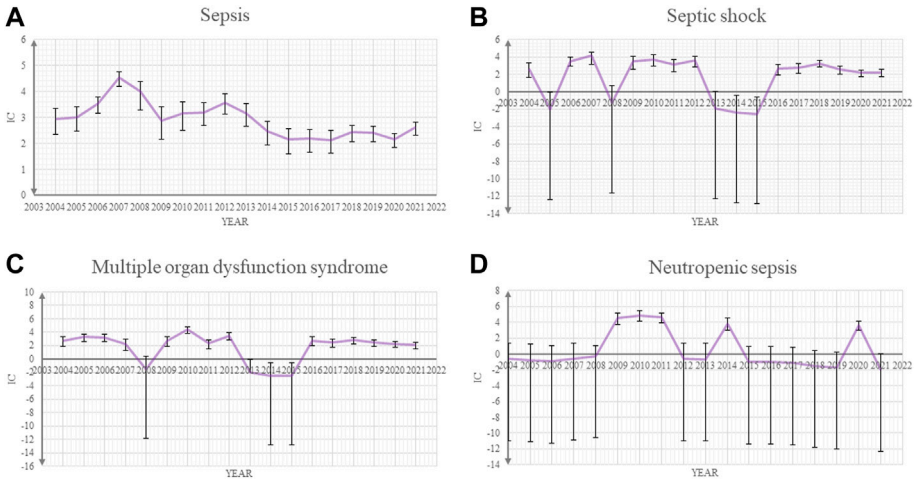

FIGURE 1
Information component (IC) and its 95% credibility interval over time for **(A)** sepsis, **(B)** septic shock, **(C)** multiple organ dysfunction syndrome, and **(D)** neutropenic sepsis.

Clinical features of sepsis-related toxicities during ADC therapies

We further analyzed the clinical characteristics of the five most common sepsis-related toxicities correlated with ADCs (Table 2). About 71.1% (1,556/2,188) of cases were reported by medical professionals, and 48.7% (1,065/2,188) of cases were reported in 2019–2021. ADC-related sepsis cases were predominantly reported largely in patients with acute myeloid leukemia (39.9% of all cases; n = 973/2,441). The co-reported toxicities' landscape among sepsis-related toxicities shows that sepsis, septic shock, and multiple organ dysfunction syndrome not only overlap with each other but also with other serious toxicities such as veno-occlusive liver disease (Figure 2). To evaluate the onset of ADC-induced sepsis, we conducted time-to-onset analysis using a curated FAERS dataset (Khaleel et al., 2022). The β-coefficient and its 95% CI for sepsis, septic shock, and bacteremia were less than one, suggesting that the onset time of ADC-induced sepsis is the early failure type, and approximately 60% of sepsis, septic shock, and bacteremia due to ADC therapies developed within 26.6–32.3 days. However, the β and 95% CI of neutropenic sepsis include 1, and nearly 60% of patients who received ADCs would develop neutropenic sepsis within 3 weeks (Table 3; Figure 3).

We further identified that death, as an outcome, was common in patients with ADC-related sepsis. We identified that 340 of 1,177 (28.9%), 165 of 562 (29.4%), 129 of 322 (40.1%), 57 of 189 (30.1%), and 21 of 119 (17.7%) death cases were reported in patients who developed sepsis, septic shock, multiple organ dysfunction syndrome, neutropenic sepsis, and bacteremia, respectively (Figure 4). We conducted the subgroup analysis of sepsis-related toxicities according to ADC categories, gender, and age. Subgroup analysis of sepsis-related toxicities stratified by ADC categories, gender, and age revealed that both gemtuzumab ozogamicin and inotuzumab ozogamicin, with calicheamicin payload, showed higher safety concerns for sepsis than any other ADCs (Figure 5; Table 4). Males showed significantly higher safety concern for sepsis-related events than females (Figure 6, p <0.0001). There was no significant difference for sepsis-related toxicities among different age-groups (0–14 years, 15–24 years, 25–65 years, or >65 years, p >0.05).

Sepsis signals in ADCs and other anticancer regimens

We further compared the incidence of sepsis-related toxicities across different cancer regimens (Figure 7, Figure 8). When compared with the global controls (all cancer patients) or within class (e.g., ADCs, targeted therapy, and immunotherapy), we found that ADCs presented the highest safety concern for sepsis, multiple organ dysfunction syndrome, pseudomonal sepsis, fungemia, and blood culture positive compared with any other cancer drug regimens (p <0.05). We also noted that the combination of ADCs and chemotherapy significantly increased the safety concern of septic shock, neutropenic sepsis, and bacteremia (Supplementary Material S3).

Co-administration and drug–drug interaction signal analysis

First, we analyzed the safety signal for sepsis when ADCs were co-administrated with the granulocyte colony-stimulating factor

TABLE 2 Clinical characteristics of sepsis-related toxicities correlated with ADCs.

Characteristics	Sepsis	Septic shock	Multiple organ dysfunction syndrome	Neutropenic sepsis	Bacteremia
	N (%)	N (%)	N (%)	N (%)	N (%)
Total number of reporting source	1,054	419	408	193	114
Medical staff	742 (70.4)	306 (73.0)	281 (68.9)	151 (78.2)	76 (66.7)
Non-medical staff	312 (29.6)	113 (27.0)	127 (31.1)	42 (21.8)	38 (33.3)
Reporting year					
2016–2021 (Q3)	511 (48.5)	230 (54.9)	189 (46.3)	80 (41.5)	55 (48.2)
2010–2015	236 (22.4)	86 (20.5)	112 (27.5)	79 (40.9)	29 (25.4)
2004–2009	307 (29.1)	103 (24.6)	107 (26.2)	34 (17.6)	30 (26.3)
Gender					
Male	496 (54.3)	215 (58.6)	211 (59.4)	94 (54.0)	52 (52.0)
Female	418 (45.7)	152 (41.4)	144 (40.6)	80 (46.0)	48 (48.0)
Data available	914	367	355	174	100
Age-group, years					
0–14	26 (3.6)	1 (0.3)	22 (6.8)	3 (2.0)	1 (1.1)
15–65	409 (56.5)	232 (71.8)	193 (59.6)	106 (70.2)	60 (66.7)
>=66	289 (39.9)	90 (27.9)	109 (33.6)	42 (27.8)	29 (32.2)
Data available	724	323	324	151	90
Drugs (different payloads)					
Tubulin polymerization inhibitors	442 (42.0)	203 (48.5)	145 (35.5)	78 (40.4)	47 (41.2)
Trastuzumab emtansine	89 (20.1)	19 (9.4)	15 (10.3)	32 (41.0)	8 (17.0)
Enfortumab vedotin	5 (1.1)	2 (1.0)	8 (5.5)	0	2 (4.3)
Brentuximab vedotin	297 (67.3)	157 (77.3)	113 (78.0)	36 (46.2)	17 (36.2)
Polatuzumab vedotin	35 (7.9)	21 (10.3)	7 (4.8)	8 (10.3)	15 (31.9)
Belantamab mafodotin	16 (3.6)	4 (2.0)	2 (1.4)	2 (2.5)	5 (10.6)
DNA-damaging agents	612 (58.0)	216 (51.5)	263 (64.5)	115 (59.6)	67 (58.8)
Gemtuzumab ozogamicin	501 (81.9)	186 (86.1)	214 (81.4)	85 (73.9)	52 (77.6)
Inotuzumab ozogamicin	91 (14.9)	27 (12.5)	49 (18.6)	30 (26.1)	15 (22.4)
Trastuzumab deruxtecan	6 (1.0)	0	0	0	0
Sacituzumab govitecan	14 (2.2)	3 (1.4)	0	0	0
Indications					
Breast cancer	96 (9.1)	10 (2.4)	10 (2.5)	21 (10.9)	0
Non-Hodgkin's lymphoma	0	0	0	12 (6.2)	0
Hodgkin's disease	139 (13.2)	71 (16.9)	64 (15.7)	21 (10.9)	0
Diffuse large b-cell lymphoma	46 (4.4)	15 (3.6)	0	0	0
T-cell lymphoma	41 (3.9)	11 (2.6)	10 (2.5)	0	0
Anaplastic large-cell lymphoma	15 (1.4)	0	0	0	0
Acute myeloid leukemia	426 (40.4)	148 (35.3)	169 (41.4)	65 (33.7)	38 (33.3))
Acute lymphocytic leukemia	62 (5.9)	21 (5.0)	34 (8.3)	11 (5.7)	0
Plasma cell myeloma	12 (1.1)	0	0	0	0
Concurrent symptoms/syndromes					
Sepsis	N/A	51 (12.2)	130 (31.9)	0	0
Septic shock	51 (4.8)	N/A	102 (25.0)	0	0
Multiple organ dysfunction syndrome	130 (12.3)	102 (24.3)	N/A	36 (18.7)	0
Neutropenic sepsis	0	0	22 (5.4)	N/A	0
Bacteremia	0	0	0	0	N/A

(Continued on following page)

TABLE 2 (*Continued*) Clinical characteristics of sepsis-related toxicities correlated with ADCs.

Characteristics	Sepsis	Septic shock	Multiple organ dysfunction syndrome	Neutropenic sepsis	Bacteremia
	N (%)	N (%)	N (%)	N (%)	N (%)
Other co-reported AEs					
Febrile neutropenia	156 (14.8)	50 (11.9)	40 (4.8)	0	26 (22.8)
Veno-occlusive liver disease	69 (6.6)	0	59 (14.5)	15 (7.8)	13 (11.4)
Pneumonia	168 (15.9)	92 (22.0)	0	17 (8.8)	0
Acute respiratory distress syndrome	35 (3.3)	33 (7.9)	0	0	0
Disseminated intravascular coagulation	34 (3.3)	0	0	0	0

ADCs, antibody–drug conjugates; N/A, not applicable; AEs, adverse events.

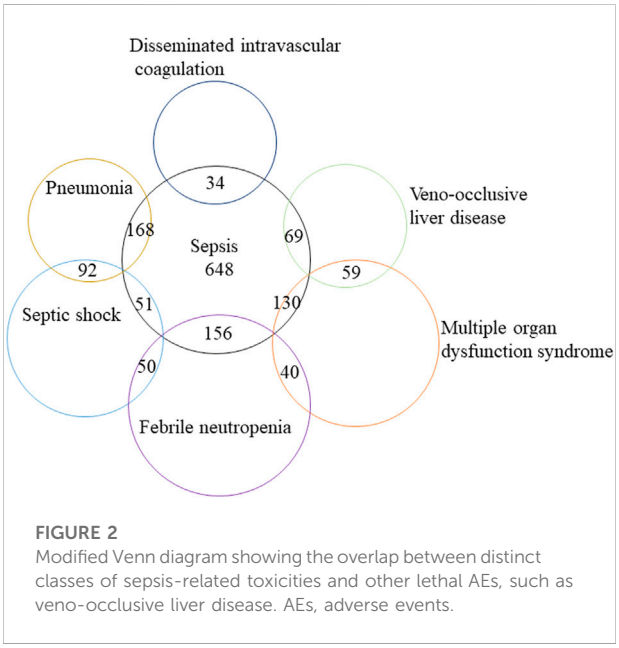

FIGURE 2
Modified Venn diagram showing the overlap between distinct classes of sepsis-related toxicities and other lethal AEs, such as veno-occlusive liver disease. AEs, adverse events.

(G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), proton pump inhibitors, H2-receptor antagonists, and CYP3A4/5 strong inhibitors. ADCs, when combined with colony-stimulating factors (G-CSF/GM-CSF), have a comparable safety signal (IC_{025}, 2.58 vs. 2.63) for sepsis than ADCs' monotherapy (Table 5). We analyzed the safety profiles for the combination therapy of ADCs with PPIs or H2-receptor antagonists. Sepsis (IC_{025} 2.75), septic shock (IC_{025} 2.48), neutropenic sepsis (IC_{025} 3.44), multiple organ dysfunction syndrome (IC_{025} 3.26), pseudomonal sepsis (IC_{025} 4.66), biliary sepsis (IC_{025} 4.25), and streptococcal sepsis (IC_{025} 3.32) were significantly associated with ADC and PPI combination therapy (Table 6). Safety concerns for sepsis (IC_{025} 3.66) and septic shock (IC_{025} 2.38) were detected for the combination of ADCs and H2-receptor antagonists (Table 7). We then included the most common CYP3A4/5 strong inhibitors which contain protease inhibitors, imidazole and triazole derivatives, and macrolides, and analyzed sepsis-related toxicities when ADCs were combined with the previously listed CYP3A4/5 inhibitors. We identified that risk for sepsis (ROR_{025} 11.57 and IC_{025} 3.06) and septic shock (ROR_{025} 13.91 and IC_{025} 3.95) was significantly higher for the combination than for ADC monotherapy (Table 8).

Second, we analyzed the drug–drug interaction signals for sepsis between ADCs and granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), proton pump inhibitors, H2-receptor antagonists, and CYP3A4/5 strong inhibitors. We did not detect other drug interaction signals for sepsis-related toxicities, except for *Escherichia* bacteremia (Ω_{025} 0.37), for the ADCs-G-CSF/GM-CSF combination. We further

TABLE 3 Time-to-onset analysis of ADC-induced sepsis and related toxicities in the FAERS database.

Adverse event	Case (N)	α (95% CI)	β (95% CI)
Sepsis	405	31.2 (26.8–36.2)	0.69 (0.42–0.74)
Septic shock	159	30.1 (24.0–37.6)	0.74 (0.65–0.82)
Neutropenic sepsis	81	21.0 (16.4–26.7)	0.96 (0.81–1.11)
Multiple organ dysfunction syndrome	77	32.3 (22.7–45.3)	0.69 (0.58–0.81)
Bacteremia	34	26.6 (14.3–47.8)	0.61 (0.46–0.78)

The Weibull distribution is a continuous probability distribution used to analyze life data, model failure times, and access product reliability, which also could be used to conduct time-to-onset in pharmacovigilance. N, we included available data which contain the event date and ADCs therapy start date. α, scale parameter, could be used to express time-to-onset duration. β, shape parameter, could be used to confirm the distribution type: early failure type (β<1), random failure type (95% CI of β include 1), and wear-out type (β>1). 95% CI, 95% credibility interval.

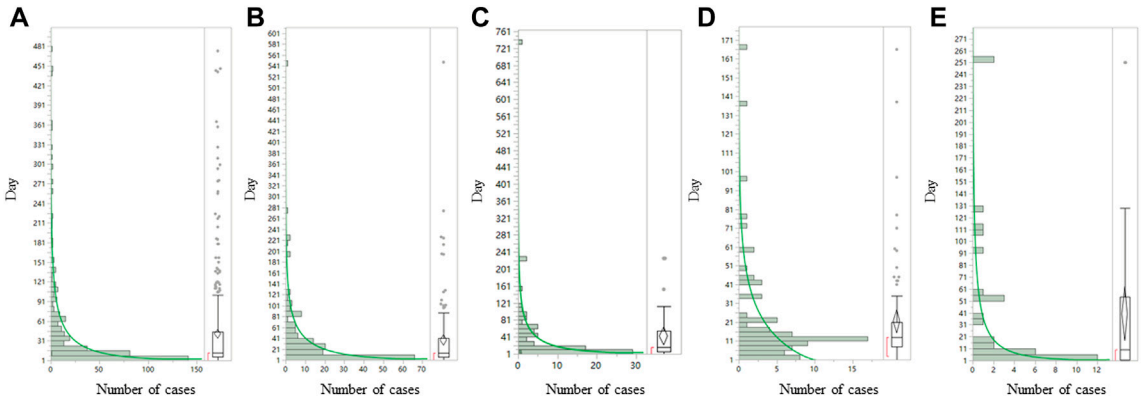

FIGURE 3
Time-to-onset analysis for **(A)** sepsis, **(B)** septic shock, **(C)** multiple organ dysfunction syndrome, **(D)** neutropenic sepsis, and **(E)** bacteremia associated with ADCs. ADCs, antibody–drug conjugates. The *Y* axis is days to onset and the *X* axis is the number of case *(N)*. The left side of each graph is a histogram and the right side is a box-and-whisker diagram with outlies. The green line on the histogram was drawn by fitting the Weibull distribution.

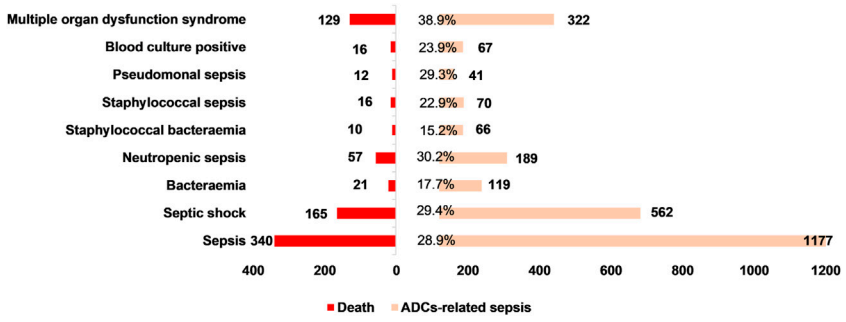

FIGURE 4
Death cases and their proportion in ADC-related sepsis.

identified drug interaction signals for ADCs–PPIs combination therapy and found increased safety concerns for enterococcal bacteremia (Ω_{025} 0.34), systemic inflammatory response syndrome (Ω_{025} 0.09), pseudomonal sepsis (Ω_{025} 1.59), streptococcal sepsis (Ω_{025} 0.95), and biliary sepsis (Ω_{025} 3.24). In contrast, DDI signals were detected for sepsis (Ω_{025} 0.79) and bacteremia (Ω_{025} 0.43) when ADCs were combined with H2-receptor antagonists. DDI signals were detected for sepsis (Ω_{025} 0.28) and fungemia (Ω_{025} 0.02) for the combination of ADCs and CYP3A4/5 inhibitors (Table 9) (Supplementary Material S2).

Sensitivity analysis

Some confounding factors such as indications and other known drug reactions may affect the safety signals of ADC-related toxicities. We excluded diseases (autologous hematopoietic stem cell transplant, allogenic stem cell transplantation, diabetes, organ transplant, chronic obstructive pulmonary disease, alcohol abuse, indwelling catheter management, surgery, and HIV infection) as sepsis may occur preferentially in patients with these conditions. We also excluded known drug reactions of sepsis (extracted from FDA's labels) and chose the role code as "primary suspect drug." After adjusting for confounders, no significant change was observed in the safety signals (Table 10).

Discussion

To the best of our knowledge, this is the first large-scale study to identify the association between ADCs and sepsis.

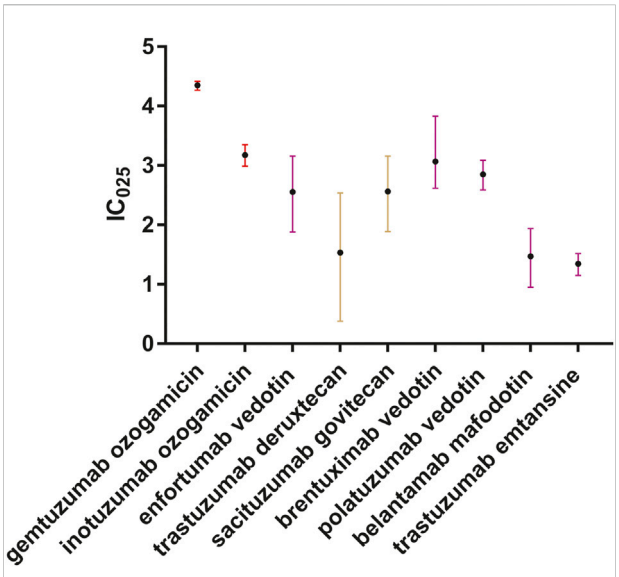

FIGURE 5
Forest plot of IC_{025} values of ADC-related sepsis. We included all sepsis-related toxicities correlated with ADCs. Different colors stand for different payloads. Red indicates calicheamicin, brown indicates DNA-damaging agents, and purple indicates tubulin polymerization inhibitors.

Meanwhile, the first case of sepsis (Schaefer et al., 2014) correlated with brentuximab vedotin was reported in 2014, and there have been no reviews, meta-analyses, or retrospective studies focusing on the underlying association between ADCs and sepsis. This study is the first effort to systematically associate sepsis-related toxicities occurrence with ADCs and characterize a large population of affected patients. The novelty and significance of our study is summarized in three aspects:

First, we analyzed the significant association between ADCs and sepsis, and further uncovered the clinical features of ADC-related sepsis. Through disproportionality analysis of the FAERS database, we detected significantly high safety concern for sepsis and related toxicities (including septic shock, multiple organ dysfunction syndrome, neutropenic sepsis, and bacteremia) in ADCs compared to other drugs. Safety profiles for sepsis, septic shock, and multiple organ failure syndrome have consolidated in the recent years without significant fluctuations year on year. Although spontaneous reporting systems are subject to various reporting biases which may impact signal scores, in this study, signal scores remained stable across the study years. Certainly, we cannot validate this beyond the limitations of the spontaneous reporting system, but the stable safety signal may enhance the validity of the signal. The sepsis-related toxicities that correlated with ADCs not only overlapped with each other but also with other serious toxicities such as veno-occlusive liver disease. A previous study (Kim et al., 2019) showed that patients with chemotherapy-induced febrile neutropenia are vulnerable to extended-spectrum b-lactamase-producing Enterobacteriaceae infection, which is prone to cause septic shock. We also detected the overlap of febrile neutropenia with sepsis or septic shock. The time-to-onset information of ADC-related sepsis is scarce in the published literature or FDA's drug labels. We fitted a Weibull distribution to estimate the duration between ADC administration and sepsis occurrence. The results of Weibull parameter α and β values of ADC-related sepsis suggested that sepsis, septic shock, and bacteremia occurred within a month and classified into the early failure type, while neutropenic sepsis classified into the random failure type. Clinicians should be vigilant in the early recognition and prevention of this kind of toxicity. Different ADCs are constituted of different cytotoxic payloads and targeted monoclonal antibody. A recent review (Lievano et al., 2021) indicated that key toxicities for ADCs are primarily associated with off-target effects from the payload. We found that gemtuzumab ozogamicin and inotuzumab ozogamicin presented higher safety concerns for sepsis than any other kinds of ADCs, which may indicate the role of calicheamicin in the elevated risk of sepsis than other kinds of payloads. Males showed significantly higher safety concern for sepsis than females; however, age did not correlate with ADC-related

TABLE 4 Safety signals among different ADC drugs (vs. other drugs in the full database).

Drug name	All AEs	Targeted AEs	ROR (95% CI)	IC (95% CI)
Gemtuzumab ozogamicin	4,923	1,389	28.52 (26.80–30.35)	4.36 (4.27–4.42)
Inotuzumab ozogamicin	1,596	167	10.53 (9.22–12.01)	3.19 (2.99–3.35)
Enfortumab vedotin	191	14	7.80 (4.92–12.37)	2.63 (1.88–3.16)
Trastuzumab deruxtecan	129	8	3.98 (1.86–8.51)	1.69 (0.38–2.54)
Sacituzumab govitecan	178	19	7.84 (4.94–12.44)	2.64 (1.89–3.16)
Brentuximab vedotin	7,632	543	7.33 (6.80–7.90)	2.75 (2.62–2.83)
Polatuzumab vedotin	1,196	114	8.30 (6.92–9.95)	2.88 (2.59–3.09)
Belantamab mafodotin	457	24	3.05 (2.15–4.32)	1.53 (0.95–1.94)
Trastuzumab emtansine	4,841	106	2.65 (2.33–3.01)	1.37 (1.15–1.52)

ADCs, antibody–drug conjugates; AEs, adverse events; IC, information component; and ROR, reporting odds ratio. 95% CI, 95% credibility interval.

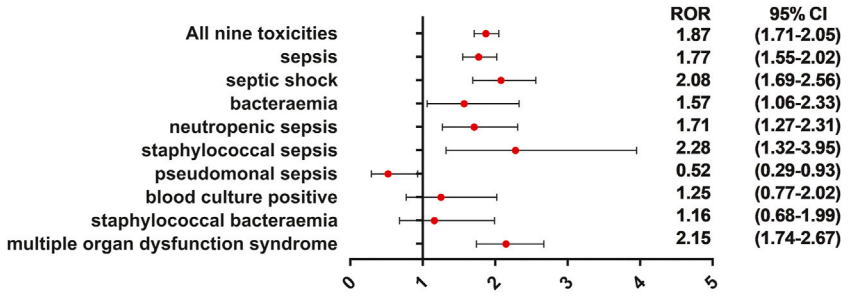

FIGURE 6
ROR of sepsis-related toxicities when males vs. females. This forest plot showed that sepsis, septic shock, bacteremia, neutropenic sepsis, staphylococcal sepsis, and multiple organ dysfunction syndrome were significantly more reported with males. This may provide alert of sepsis for clinicians when they are using ADC therapies to treat male cancer patients.

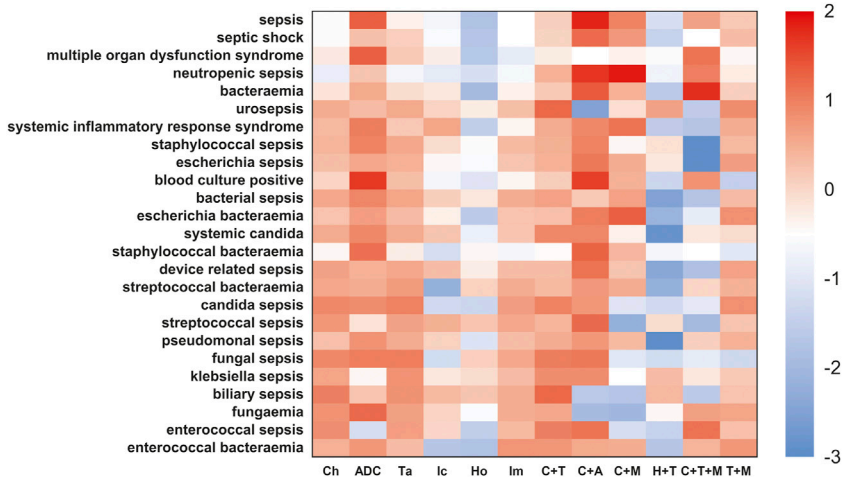

FIGURE 7
Sepsis-related toxicities landscape among ADCs and other anticancer therapies (vs. global control). The heatmap represents a comparative analysis of differential risk profiles of sepsis and related AEs across cancer drug regimens, such as Ch-chemotherapy, ADC-antibody–drug conjugates, Ta-targeted therapy, Ic-immunotherapy, HO-endocrine therapy, Im-immunomodulatory drugs, and C+T-chemotherapy combined with targeted therapy. These regimens were extracted from guidelines of FDA-approved indications for ADCs. For the more conservative global controls, we selected cancer patients not on any of the aforementioned cancer regimens, that is, patients not taking Ic, Ta, Ch, Ic+Ta, Ch+Ic, and Ch+Ta. The red color indicates a high risk of adverse effect in different cancer drug regimens. This analysis demonstrated ADCs had the highest safety concern for sepsis, multiple organ dysfunction syndrome, pseudomonal sepsis, fungemia, and blood culture positive than any other cancer drug regimens (p <0.05).

sepsis. We noticed that the first case of sepsis associated with ADCs is male (Schaefer et al., 2014), and another pharmacovigilance study related to cutaneous toxicity associated with enfortumab vedotin (Yang et al., 2021) indicated that most cases were male (76.42%). This is in line with our study. But another study (Li et al., 2022) focusing on arrhythmia association with antibody–drug conjugates showed that gender differences among affected patients are not significant (female vs. male = 43.57 vs. 42.86%). The aforementioned result showed that gender difference is not the same in different adverse events of ADCs. The International Conference on Harmonization considers older people a “special population” as they differ from younger adults in terms of comorbidity, polypharmacy, pharmacokinetics, and greater vulnerability to adverse drug reactions (Davies and O’Mahony, 2015). However, another research study (Begaud et al., 2002) analyzed 92,043 spontaneous domestic reports in the French pharmacovigilance database and argued the main factor for the risk of adverse drug reaction is the number of drug

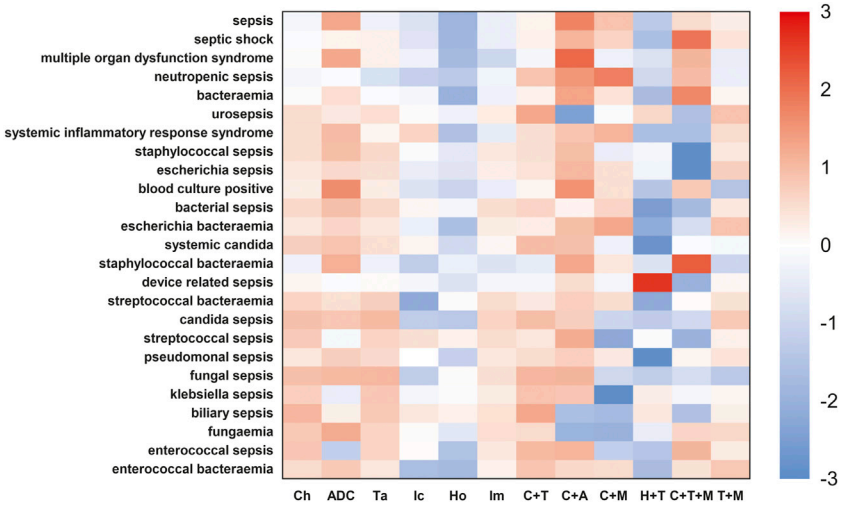

FIGURE 8
Sepsis-related toxicities landscape among ADCs and other anticancer therapies (vs. in-class control). In-class control represented other cancer patients not on a specific drug class, for instance, the controls for the Ic group were cancer patients not on Ic therapy. Similarly, controls for Ta were cancer patients not taking any targeted therapy. The red color indicates a high risk of adverse effect in different cancer drug regimens. This analysis using another control group confirmed the findings in Figure 7 that ADCs had the highest safety concern for sepsis, multiple organ dysfunction syndrome, pseudomonal sepsis, fungemia, and blood culture positive among any other cancer drug regimens (p <0.05).

TABLE 5 Safety signals for sepsis-related toxicities reported with ADCs and colony-stimulating factors (G-CSF/GM-CSF) combination therapy *vs.* the full FAERS database.

	Overall AEs of ADCs+G-CSF/GM-CSF	Full database	ROR_{025}	IC_{025}
Total number of ICSRs available	1914	16,849,672	6.65	2.58
Number of ICSRs by sepsis-related AE subgroups				
Sepsis	96	108277		
Multiple organ dysfunction syndrome	41	17778	15.24	3.52
Septic shock	56	38950	9.98	3.08
Neutropenic sepsis	36	7238	32.21	4.23

ADCs, antibody–drug conjugates; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; and AEs, adverse events.

TABLE 6 Sepsis safety signals for ADCs and proton pump inhibitors combination therapy.

	Overall AEs of ADCs+proton pump inhibitors	Full database	ROR_{025}	IC_{025}
Total number of ICSRs available	3798	16,849,672	7.34	2.75
Number of ICSRs by sepsis-related AE subgroups				
Sepsis	197	1,08,277		
Septic shock	68	38,950	6.20	2.48
Neutropenic sepsis	34	7,238	25.05	3.44
Multiple organ dysfunction syndrome	58	17,778	11.36	3.26
Pseudomonal sepsis	33	1,708	62.36	4.66
Biliary sepsis	19	456	122.30	4.25
Streptococcal sepsis	14	1,238	30.04	3.32

TABLE 7 Sepsis safety signals for ADC and H2-receptor antagonists combination therapy.

	Overall AEs of ADCs+H2-receptor antagonists	Full database	ROR_{025}	IC_{025}
Total number of ICSRs available	1251	16,849,672	15.11	3.66
Number of ICSRs by sepsis-related AE subgroups				
Sepsis	131	108277		
Septic shock	27	38950	6.51	2.38

TABLE 8 Sepsis safety signals for ADCs and CYP3A4/5 strong inhibitors combination therapy.

	Overall AEs of ADCs + CYP3A4/5 inhibitors	Full database	ROR_{025}	IC_{025}
Total number of ICSRs available	341	16,849,672	11.57	3.06
Number of ICSRs by sepsis-related AE subgroups				
Sepsis	33	108277		
Septic shock	17	38950	13.91	3.95

CYP3A4/5 strong inhibitors; we included protease inhibitors, imidazole and triazole derivatives, and macrolides in this study.

TABLE 9 Drug–drug interaction analysis for ADCs and other drugs.

Drug 1	Drug 2	Adverse effect	Ω_{025}
ADCs	Colony-stimulating factors	*Escherichia* bacteremia	0.37
ADCs	Proton pump inhibitors	Enterococcal bacteremia	0.34
ADCs	Proton pump inhibitors	Systemic inflammatory response syndrome	0.09
ADCs	Proton pump inhibitors	Pseudomonal sepsis	1.59
ADCs	Proton pump inhibitors	Streptococcal sepsis	0.95
ADCs	Proton pump inhibitors	Biliary sepsis	3.24
ADCs	H2-receptor antagonists	Sepsis	0.79
ADCs	H2-receptor antagonists	Bacteremia	0.43
ADCs	H2-receptor antagonists	Streptococcal bacteremia	0.09
ADCs	H2-receptor antagonists	Procalcitonin increased	0.41
ADCs	H2-receptor antagonists	*Serratia* sepsis	1.59
ADCs	H2-receptor antagonists	*Salmonella* bacteremia	1.05
ADCs	CYP3A4/5 strong inhibitors	Sepsis	0.28
ADCs	CYP3A4/5 strong inhibitors	Fungemia	0.02

Ω_{025}, the lower limit of the 95% credibility interval of shrinkage observed-to-expected ratio. When $\Omega_{025} > 0$, a significant drug–drug interaction signal was detected.

treatments and not the age itself. Further research needs to be conducted to explore the influence of gender and age on the risk of ADC-related sepsis. We identified that 766 of 2613 (29.3%) cases who developed ADC-related sepsis or related toxicity died during therapy, which reflected a disproportional mortal rate.

Second, we identified a significantly high safety concern of sepsis and multiple organ dysfunction syndrome associated with ADCs compared to other anticancer drug therapies within FDA-approved indications for ADCs. We have not found guidelines related to toxicity management of ADCs. The FDA's drug labels that indicate serious infections and opportunistic infections are likely adverse reactions of brentuximab vedotin. No sepsis-related toxicities were mentioned in ADCs' labels. In contrast, our pharmacovigilance study indicates that ADCs present the strongest safety concern for sepsis and multiple organ dysfunction syndrome among all included anticancer therapies, except for ADCs combined with chemotherapy.

Third, we detected drug interaction signals and found an increased risk of sepsis when ADCs were co-administrated with colony-stimulating factors, proton pump inhibitors, H2-receptor

TABLE 10 Safety signals for sepsis-related toxicities reported with ADC therapy *vs.* the full FAERS database after sensitivity analysis.

	Overall ADCs	Full database	ROR_{025}	IC_{025}
Total number of ICSRs available	12501	16,849,672		
Number of ICSRs by sepsis-related AE subgroups				
Sepsis	650	1,08,277	7.88	2.88
Multiple organ dysfunction syndrome	232	17,778	15.90	3.87
Septic shock	212	38,950	6.53	2.63
Blood culture positive	50	3,668	14.13	3.50
Bacteremia	65	10,139	6.84	2.62
Neutropenic sepsis	95	7,238	14.72	3.68
Staphylococcal sepsis	39	5,561	6.96	2.56
Staphylococcal bacteremia	38	3,880	9.70	2.97
Bacterial sepsis	21	3,217	5.77	2.17
Escherichia sepsis	28	2,915	9.02	2.79
Pseudomonal sepsis	32	1,708	18.15	3.61
Systemic *candida*	22	1,883	10.47	2.85
Systemic inflammatory response syndrome	17	4,066	3.51	1.50
Fungemia	15	1,313	9.36	2.52
Enterococcal bacteremia	12	853	10.88	2.49

antagonists, and CYP3A4/5 strong inhibitors. A previous meta-analysis (Bo et al., 2011) indicated that there is no current evidence supporting the routine use of G-CSF or GM-CSF in patients with sepsis, and G-CSF or GM-CSF could not increase the reversal rate from infection in patients with sepsis. Our pharmacovigilance analysis also detected significant risk of sepsis in cancer patients who received ADCs and colony-stimulating factors (G-CSF/GM-CSF) (IC_{025}=2.58), which further confirmed that G-CSF or GM-CSF could not increase the reversal rate from infection in patients with sepsis when co-administered with ADCs. We also detected DDI signals between ADCs and gastric medications, such as proton pump inhibitors and H2-receptor antagonists, for several subtypes of sepsis. Since most of the ADCs are metabolized by the CYP3A4/5 enzyme, the safety signal for sepsis was elevated when ADCs were co-administrated with CYP3A4/5 strong inhibitors. This result demonstrates that physicians need to be vigilant when ADCs are co-administrated with the aforementioned medications.

In summary, we detected a significant safety concern for ADC-related sepsis in cancer patients. The clinical features and drug interaction signals were explored. Further studies are warranted to describe underlying mechanisms and develop preventive measures of ADC-related sepsis.

Limitations

There are several limitations to this study. First, adverse event reports come from heterogeneous sources, which raise the possibility of incomplete information. Second, detailed clinical information is unavailable from the FAERS database, thus limiting our quality assessment to those reports. Third, we could not definitively confirm the incidence of events using spontaneous reporting systems but only for hypotheses generation. Fourth, we could not combine data from randomized controlled trails with the FAERS database because sepsis cases are rare in ADCs' trials. Fifth, in the time-to-onset analysis, the Weibull distribution does not incorporate the effects of concomitant medications. Sixth, *underreporting* bias is an intrinsic limitation in research using a spontaneous database. Our time trends analysis for IC and credibility intervals in Figure 1 show some peaks and small differences through time, which can be partially explained by differences in reporting rates. However, cases in the FAERS database cover many countries in the world, thus ensuring an unparalleled global assessment of ADC-related sepsis in diverse real-world clinical settings.

Conclusion

Antibody–drug conjugates are promising and cutting-edge anticancer therapies which significantly improve the refractory tumor response and render patients with increased survival. However, severe sepsis-related toxicities are significantly associated with ADCs compared to other common cancer drug therapies. Patients on gemtuzumab ozogamicin and inotuzumab ozogamicin are more prone to develop sepsis than with other ADCs. In this study, males showed a significantly higher safety concern for sepsis than females, while age did not influence the safety signal of ADC-related sepsis. We identified that 766 of 2,613 (29.3%) patients who developed ADC-related sepsis died during treatment. Sepsis,

septic shock, multiple organ dysfunction syndrome, and bacteremia tend to occur in the early stage after ADCs' administration (within a month). G-CSF/GM-CSF, proton pump inhibitors, H2-receptor antagonists, and CYP3A4/5 inhibitors may synergistically increase the risk of sepsis with ADCs. Further studies need to be conducted to uncover the mechanism of sepsis correlated with ADCs. Physicians should be aware of the safety concern of sepsis, and take early recognition and prevention measures when they are treating cancer patients with ADCs.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

SX: formal analysis, data curation, writing—original draft, review and editing, and visualization. MS and YN: software, methodology, and writing—review and editing. LG, Y-CZ, and RM: resources. HG and Y-KW: software. B-KZ and YS: methodology and supervision. MY: conceptualization and methodology. All authors participated in the interpretation of the results. The final manuscript was read, checked, and approved by all authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The supplied data from FAERS come from various sources. The likelihood of a causal relationship is not the same in all reports. The information does not represent the opinion of the FDA Adverse Event Reporting System.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.967017/full#supplementary-material

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Data mining and safety analysis of BTK inhibitors: A pharmacovigilance investigation based on the FAERS database

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Objective: The introduction of Bruton's tyrosine kinase (BTK) inhibitors was a milestone in the treatment of B-cell malignancies in recent years owing to its desired efficacy against chronic lymphocytic leukaemia and small cell lymphocytic lymphoma. However, safety issues have hindered its application in clinical practice. The current study aimed to explore the safety warning signals of BTK inhibitors in a real-world setting using the FDA Adverse Event Reporting System (FAERS) to provide reference for clinical rational drug use.

Methods: Owing to the short marketing time of other drugs (zanbrutinib and orelabrutinib), we only analysed ibrutinib and acalabrutinib in this study. All data were obtained from the FAERS database from January 2004 to December 2021. Disproportionality analysis and Bayesian analysis were utilised to detect and assess the adverse event (AE) signals of BTK inhibitors.

Results: In total, 43,429 reports of ibrutinib were extracted and 1527 AEs were identified, whereas 1742 reports of acalabrutinib were extracted and 220 AEs were identified by disproportionality analysis and Bayesian analysis. Among reports, males were more prone to develop AEs (58.2% for males vs. 35.6% for females treated with ibrutinib, and 55.9% vs. 31.9%, respectively, for acalabrutinib), and more than 30% of patients that suffered from AEs were over 65 years of age. Subsequently, we investigated the top 20 preferred terms (PTs) associated with the signal strength of ibrutinib and acalabrutinib, and our results identified 25 (13 vs. 12, respectively) novel risk signals. Among the top 20 PTs related to death reports, the terms infectious, pneumonia, pleural effusion, fall, asthenia, diarrhoea, and fatigue were all ranked high for these two BTK inhibitors. Further, cardiac disorders were also an important cause of death with ibrutinib.

Abbreviations: BTK, Bruton's tyrosine kinase; FDA, the US Food and Drug Administration; FAERS, the FDA Adverse Event Reporting System; BCM, B-cell malignancy; SRS, spontaneous reporting system; ROR, reported odds ratio method; PRR, proportional report ratio method; PT, preferred term; AE, adverse event; MedDRA, Medical Dictionary for Regulatory Activities; SOC, system organ class; IFI: invasive fungal infection; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; WM, Waldenstrom macroglobulinemia; CLL, chronic lymphocytic leukaemia.

Conclusion: Patients treated with ibrutinib were more prone to develop AEs than those treated with acalabrutinib. Importantly, infection-related adverse reactions, such as pneumonia and pleural effusion, were the most common risk signals related to high mortality associated with both BTK inhibitors, especially in elderly patients. Moreover, cardiovascular-related adverse reactions, such as atrial fibrillation and cardiac failure, were fatal AEs associated with ibrutinib. Our results provide a rationale for physicians to choose suitable BTK inhibitors for different patients and provide appropriate monitoring to achieve safer therapy and longer survival.

KEYWORDS

BTK inhibitors, safety, data mining, non-proportional analysis, B cell malignancies

Introduction

B-cell malignancies (BCMs) include non-Hodgkin's lymphoma, comprising 93% of all cases, and all types of chronic lymphocytic leukaemia, such as mantle cell lymphoma (MCL), follicular lymphoma, marginal zone lymphoma (MZL), diffuse large B-cell lymphoma, Waldenstrom macroglobulinemia (WM), chronic lymphocytic leukaemia (CLL), small lymphocytic lymphoma, which are the most common haematological malignancies (Swerdlow et al., 2016; Teras et al., 2016). Cancer statistics released by the United States in 2021 estimated that the number of new cases of BCM would reached 102,810 and that the number of deaths would reach 25,040 in 2021 (Siegel et al., 2021). Bruton's tyrosine kinase (BTK) is a non-receptor kinase that plays a crucial role in oncogenic signalling and is an essential protein for B cell receptor (BCR) signalling that is critical for the proliferation and survival of leukemic cells in many B cell malignancies (Burger and Wiestner 2018; Pal et al., 2018). As a novel agent approved for BCM treatment, BTK inhibitors were proven to have high efficacy for the treatment of haematological malignancies, such as CLL, WM, MCL, and MZL, as well as chronic graft-versus-host disease (Zelenetz et al., 2019; Wierda et al., 2020). The advent of BTK inhibitors was a milestone in BCM treatment, as the chemotherapy-free era was imminent. To date, there are four BTK inhibitors approved in the United States and China, including the first-generation ibrutinib, the second-generation acalabrutinib and zanubrutinib (Table 1), and orelabrutinib, recently approved by the China Food and Drug Administration (CFDA) and released in clinical practice in December 2021. Although the effectiveness of those BTK inhibitors has been confirmed in numerous clinical trials (Novero et al., 2014; Mercier, Janssens, and Maertens 2019; Byrd et al., 2020; Rogers et al., 2021), approximately 10%–20% of patients remain intolerant to BTK inhibitors owing to serious adverse events (AEs) (Wu, Zhang, and Liu 2016; O'Brien et al., 2018; Byrd et al., 2019; Hillmen et al., 2019; Xu et al., 2020). Therefore, it is of great importance to identify and analyse such potential AEs in real-world practice.

It was reported that the incidence of haematotoxicity (above grade 3) induced by ibrutinib is 41% (Woyach et al., 2018). Owing to its high intolerance rate, various novel BTK inhibitors with fewer safety concerns have been developed. However, due to the strict study entry criteria and relatively small sample sizes, the safety figures released in clinical trials have limitations. A real-world study could thus provide more comprehensive information on drug safety. Consequently, we aimed to evaluate current BTK inhibitors based on a large real-world patient population by analysing AEs in the FDA's Adverse Event Reporting System (FAERS). We also investigated the times to onset and fatality rates associated with different BTK inhibitors, to provide a reference for clinical rational drug use.

Materials and methods

Data source and collection

We performed a retrospective pharmacovigilance study using data from the FAERS database covering the period from January 2004 to December 2021. FAERS contains real-world results from a large population and under conditions that might have been overlooked in controlled studies, which lack the ability to detect the whole spectrum of adverse drug reactions. A deduplication procedure was performed according to the FDA's recommendations, selecting the latest FDA_DT when the CASEIDs were the same and selecting the higher PRIMARYID when the CASEID and FDA_DT were the same. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) (version 25.0) system organ class (SOC) and preferred term (PT) level. The drugs in the FAERS database can be reported using arbitrary drug names; therefore, the MICROMEDEX® (Index Nominum) was utilised as a dictionary for BTK inhibitors (Table 1).

TABLE 1 Summary of FDA-approved BTK inhibitors.

Generic name	Brand name	Target	Approval year
Ibrutinib	Imbruvica	BTK	2013
Acalabrutinib	Calquence	BTK	2017
Zanubrutinib	Brukinsa	BTK	2019

FDA, US Food and Drug Administration; BTK, Bruton's tyrosine kinase inhibitor.

Data mining

Based on disproportionality analysis and Bayesian analysis, the reporting odds ratio (ROR), the proportional reporting ratio (PRR), the Bayesian confidence propagation neural network (BCPNN), and the multi-item gamma poisson shrinker (MGPS) were used to calculate the association between drugs and AEs. The equations and criteria for the four algorithms are shown in Supplementary Table S1. In this study, AEs were extracted based on the circumstances that one of the aforementioned four indices met the criteria (Evans, Waller, and Davis 2001). Reports with input error (EVENT_DT earlier than START_DT) or an inaccurate date of entry were excluded. The proportion of the SOC was calculated as the number of events at the SOC level divided by the total number of events associated with each BTK inhibitor. In addition, reports with fatal events attributed to drug toxicity were counted, and the fatality rate was calculated as the number of fatal events divided by the total number of related events associated with each BTK inhibitor.

Data analysis and statistics

Descriptive analysis was used to summarise the clinical characteristics of the patients treated with BTK inhibitors collected from the FAERS database. Data mining and all statistical analyses were performed using MYSQL software (version 8.0).

Results

Descriptive analysis

During the study period, 1,44,64,087 total reports were retrieved from the FARES database after deduplication. Among them, 43,429 reports were suspected to be related to ibrutinib and 1,742 reports were suspected to be related to acalabrutinib. For zanubrutinib, owing to its short marketing time, there were only 176 reports suspected to be related to the this drug, making it impossible to analyse the data using such a small sample size. The clinical characteristics of events associated with BTK inhibitors are presented in Table 2. According to the results, approximately 70% indications of

TABLE 2 Clinical characteristics of patients at risk of AEs using BTK inhibitors based on the FAERS database (January 2004 to December 2021).

Characteristics	Number of reports, no. (%)	
	Ibrutinib	Acalabrutinib
Country		
United States	33,456 (77.0)	1229 (70.6)
Canada	1267 (2.9)	107 (6.1)
France	1128 (2.6)	19 (1.1)
United Kingdom	788 (1.8)	37 (2.1)
Germany	687 (1.6)	11 (0.6)
Others	6103 (14.1)	339 (19.5)
Reporter		
Medical staff	15,608 (35.9)	707 (40.6)
Non-medical staff	27,754 (63.9)	804 (46.1)
Unknown or missing	67 (0.2)	231 (13.3)
Reporting time		
2013	23 (0.1)	—
2014	1058 (2.4)	—
2015	3413 (7.9)	—
2016	3816 (8.8)	—
2017	4856 (11.2)	18 (1.0)
2018	6385 (14.7)	170 (9.8)
2019	7791 (17.9)	161 (9.2)
2020	9001 (20.7)	451 (25.9)
2021	7086 (16.3)	942 (54.1)
Sex		
Female	15,438 (35.6)	556 (31.9)
Male	25,286 (58.2)	974 (55.9)
Unknown or missing	2705 (6.2)	212 (12.2)
Age (year)		
<18	62 (0.1)	3 (0.2)
18–44	327 (0.8)	6 (0.3)
45–64	4762 (11.0)	180 (10.3)
≥65	17,545 (40.4)	692 (39.7)
Unknown or missing	20,733 (47.7)	861 (49.5)
Indication		
Chronic lymphocytic leukaemia	21,526 (49.57)	778 (44.66)
Mantle cell lymphoma	3573 (8.23)	290 (16.65)
Waldenstrom's macroglobulinaemia	2771 (6.38)	28 (1.61)
Non-Hodgkin's lymphoma	859 (1.98)	14 (0.8)
Lymphocytic leukaemia	812 (1.87)	26 (1.49)
B-cell small lymphocytic lymphoma	805 (1.85)	15 (0.86)
Diffuse large B-cell lymphoma	464 (1.07)	11 (0.63)
Others	3837 (8.83)	161 (9.25)
Unknown or missing	8782 (20.22)	419 (24.05)

Abbreviations: FAERS, Food and Drug Administration's Adverse Event Reporting System; AEs, adverse effects.

TABLE 3 Top 20 preferred terms (PT) for signal strength.

Ibrutinib						Acalabrutinib					
PT	*N*	ROR (95% two-sided CI)	PRR (χ2)	IC (95% two-sided CI)	EBGM (95% one-sided CI)	PT	*N*	ROR (95% two-sided CI)	PRR (χ2)	IC (95% two-sided CI)	EBGM (95% one-sided CI)
Pseudohyperkalaemia	5	1660.4 (194, 14,213.3)	1660.3 (1381.9)	44.1 (5.2, 377.5)	523467361935.8 (61153163443.6)	Richter's syndrome	4	7881.5 (2114.3, 29,380.3)	7860.1 (17,462.5)	39.5 (10.6, 147.2)	9462727139.6 (2538460424.9)
Prostatic mass	6	664.2 (166.1, 2655.9)	664.1 (1324.2)	44.9 (11.2, 179.7)	418773889548.7 (104728347121.5)	Oral blood blister	4	772.7 (278.9, 2140.7)	770.6 (2850.8)	42.1 (15.2, 116.6)	1548446259.2 (558925560)
Mastoid effusion	3	498.1 (83.2, 2981.2)	498.1 (595.3)	43.1 (7.2, 257.9)	376896500593.8 (62973802024.1)	Skin mass	4	205.2 (76.2, 553.2)	204.7 (794.2)	43.9 (16.3, 118.4)	434512980.9 (161216696.2)
Nail growth abnormal	36	291.8 (186.5, 456.6)	291.6 (5550.6)	50.6 (32.4, 79.2)	293685584878.3 (187680362933.1)	Lymph node pain	4	177.5 (66, 477.8)	177.0 (687.8)	44.1 (16.4, 118.8)	376834266.6 (140007155.9)
Blood blister	146	209.7 (170.4, 258)	209 (18,546.6)	54.9 (44.7, 67.6)	242622967754.4 (197204407472.7)	Tumour lysis syndrome	19	163.7 (103.7, 258.4)	161.6 (2984.2)	48.8 (30.9, 76.9)	344575455.9 (218343141.2)
Immunoglobulins abnormal	8	204.4 (84.7, 493.1)	204.3 (1002.1)	46.6 (19.3, 112.4)	239299365456.4 (99178600804.4)	Decreased immune responsiveness	3	154.6 (49.4, 484.3)	154.3 (449.9)	43.5 (13.9, 136.2)	329244372.1 (105129752.4)
Ear haemorrhage[a]	100	195.8 (152.9, 250.7)	195.3 (12,172.8)	53.9 (42.1, 69)	232652160860.4 (181699662242.4)	Onychoclasis	4	137.8 (51.3, 370.1)	137.4 (534.2)	44.5 (16.6, 119.5)	293670842.3 (109322808.4)
Nail bed bleeding[a]	16	171.4 (93.8, 313.5)	171.4 (1787.6)	48.7 (26.7, 89.1)	213841986152.5 (116958710680.9)	Melanocytic -naevus	4	89.8 (33.5, 240.6)	89.5 (347)	45.1 (16.8, 120.9)	192245020.9 (71735647)
Ear neoplasm	8	166.1 (71.1, 388)	166 (874.8)	46.8 (20, 109.3)	209386944774.3 (89606402780.7)	Malignant -neoplasm progression	69	83.7 (65.6, 106.6)	79.8 (5328.6)	53.5 (42, 68.2)	171522287 (134570765.6)
Scrotal haematocoele	6	166 (62.3, 442.4)	166 (656.1)	45.9 (17.2, 122.4)	209386944774.3 (78581686678.9)	Pseudomonal bacteraemia[a]	3	79.4 (25.5, 247.6)	79.2 (229.9)	44.4 (14.3, 138.6)	170329088.5 (54621178.7)
Full blood count abnormal[a]	389	162.5 (143.9, 183.5)	161.1 (41,669.5)	58.0 (51.4, 65.5)	205167560496.8 (181699883256)	Prostatomegaly	3	77.7 (24.9, 242.3)	77.6 (225)	44.5 (14.3, 138.7)	166771300.8 (53485378.5)
Capillary fragility	12	159.4 (80.1, 317.3)	159.4 (1276.1)	48.0 (24.1, 95.5)	203727838158.8 (102349265405.1)	Immunodeficiency	4	70.2 (26.2, 188.1)	70.1 (270.4)	45.5 (17, 121.7)	150733706.6 (56300169.2)
Cerebral aspergillosis[a]	24	150.4 (92.9, 243.7)	150.4 (2451)	50.0 (30.9, 81.1)	195790389918.9 (120870358877.4)	Contusion[a]	52	59.6 (45.2, 78.7)	57.6 (2875)	53.1 (40.3, 70.1)	124007512.9 (93955922.1)
Prostatic specific antigen decreased	5	138.4 (48.7, 392.8)	138.4 (481.3)	45.6 (16.1, 129.4)	184753186565.6 (65084642410.7)	Sinus headache[a]	3	56.0 (18, 174.5)	55.9 (160.9)	44.9 (14.4, 140)	120515864.5 (38698730.9)
Skin mass[a]	57	136.3 (100.2, 185.6)	136.2 (5423.8)	52.6 (38.7, 71.7)	182679426308.2 (134197567691.5)	Haemorrhage subcutaneous[a]	3	52.9 (17, 164.8)	52.8 (151.7)	45.0 (14.5, 140.2)	113856342.6 (36566816.4)
Blood iron abnormal	10	132.9 (63.8, 276.6)	132.8 (934.5)	47.6 (22.9, 99.2)	179474524092.3 (86194633637.9)	Full blood count abnormal[a]	6	49.9 (22.4, 111.6)	49.7 (285.2)	47.1 (21.1, 105.3)	107260131.3 (48011250.2)
Nail cuticle fissure	4	120.8 (38.4, 379.3)	120.7 (348.3)	45.1 (14.4, 141.6)	167509555819.5 (53335821793.2)	Lung opacity	4	49.0 (18.3, 130.9)	48.8 (186.4)	46.0 (17.2, 122.9)	105271377.3 (39361127.5)
	5					Head discomfort[a]	3				

(Continued on following page)

TABLE 3 (*Continued*) Top 20 preferred terms (PT) for signal strength.

Ibrutinib						Acalabrutinib					
PT	***N***	**ROR (95% two-sided CI)**	**PRR (χ2)**	**IC (95% two-sided CI)**	**EBGM (95% one-sided CI)**	**PT**	***N***	**ROR (95% two-sided CI)**	**PRR (χ2)**	**IC (95% two-sided CI)**	**EBGM (95% one-sided CI)**
Renal cyst haemorrhage[a]		118.6 (42.7, 329.3)	118.6 (429.6)	45.8 (16.5, 127.1)	165305482716.6 (59538020198.8)			43.8 (14.1, 136.2)	43.7 (124.5)	45.3 (14.6, 141)	94208566.7 (30272516)
Hair texture abnormal	84	108.7 (85, 139.1)	108.5 (6745)	54.0 (42.2, 69.1)	154737566226.2 (120930338214.7)	Skin cancer[a]	7	38.3 (18.2, 80.6)	38.1 (252.1)	47.9 (22.8, 100.9)	82295942.8 (39107374.3)
Dyschezia[a]	10	107.1 (52.5, 218.5)	107.1 (794.8)	47.9 (23.5, 97.6)	153209959591 (75109564045.8)	Blood urine present[a]	8	32.5 (16.2,65.3)	32.4 (242.5)	48.5 (24.2,97.4)	69921629.1 (34861457.3)

[a]AEs mentioned in the instructions.

Abbreviations: N, the number of reports of BTK-associated AEs; ROR, reporting odds ratio; CI: confidence interval; PRR, proportional reporting ratio; χ2, chi-squared; BTK, Bruton's tyrosine kinase inhibitor; FAERS, the FDA's Adverse Event Reporting System.

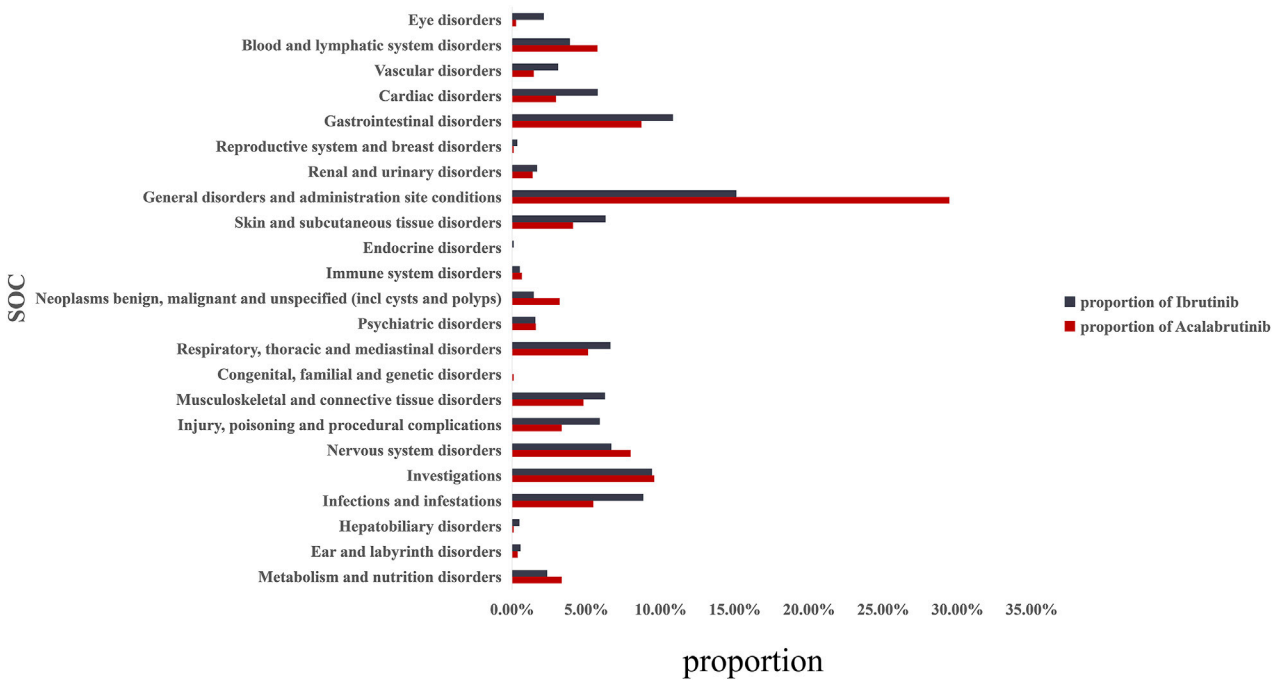

FIGURE 1
Proportion of BTK inhibitor-related AEs at the System Organ Class (SOC) level. AEs, adverse effects; BTK, Bruton's tyrosine kinase.

BTK inhibitors analyzed in this study were lymphocytic malignancies (Chronic lymphocytic leukaemia, mantle cell lymphoma and waldenstrom's macroglobulinaemia ranked the top three indications of the two BTK inhibitors induced AEs), which was also in accordance with the clinical practice.

The results indicated males were more prone to be affected by AEs than females in both ibrutinib and acalabrutinib (58.2% vs. 35.6%, 55.9% vs. 31.9%, respectively). The age of the majority of patients reporting AEs with ibrutinib or acalabrutinib was ≥65 years (40.4% and 39.7%, respectively). Most AEs were reported from the United States (77.0% and 70.6%, respectively), and the number of

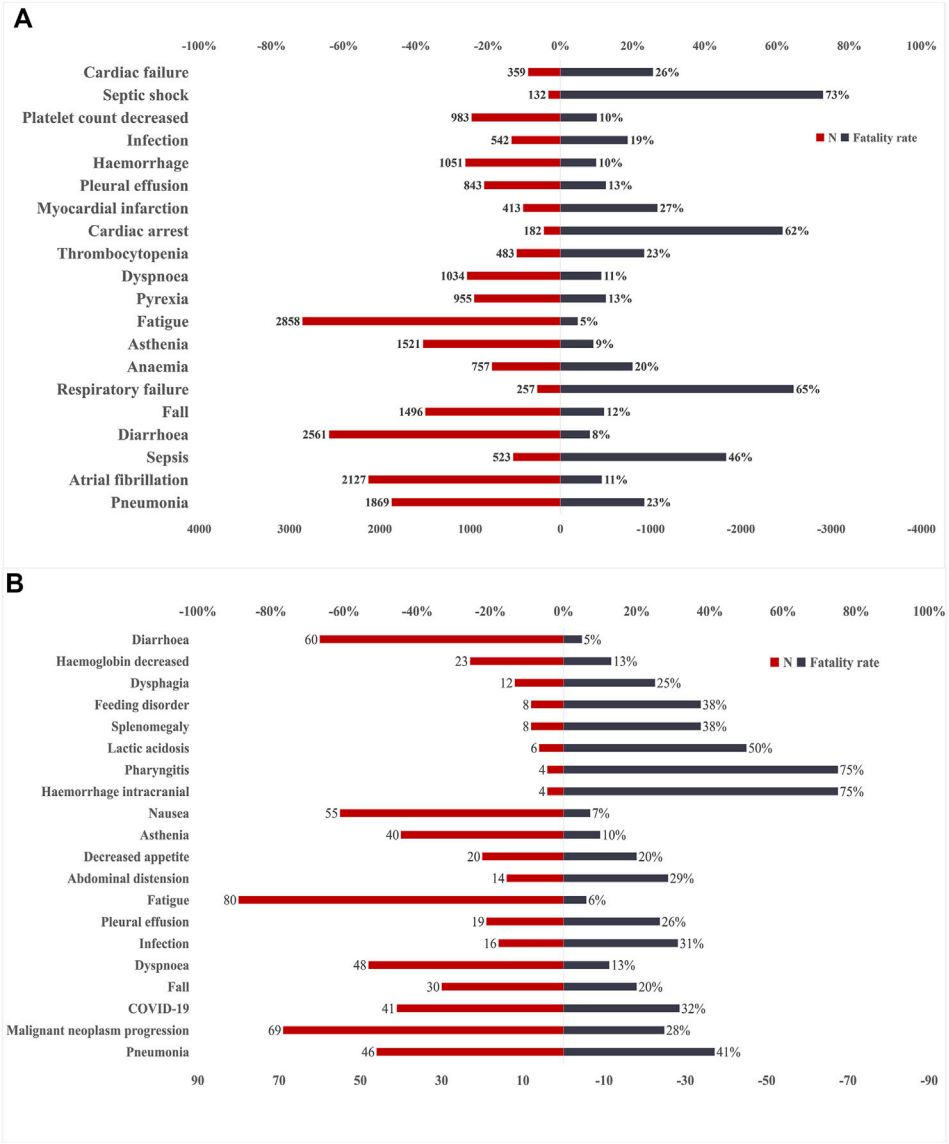

FIGURE 2
Number of reports and fatality rates for BTK inhibitor-associated AEs according to death reports. **(A)**Number of reports and fatality rates for ibrutinib-associated AEs. **(B)** Number of reports and fatality rates for acalabrutinib-associated AEs. AEs, adverse effects; BTK, Bruton's tyrosine kinase; N, indicates the number of reports of BTK inhibitor-associated AEs.

AE reports increased year over year. Cases were mainly submitted by non-healthcare professionals (63.9% and 46.1%, respectively) for both ibrutinib and acalabrutinib.

Disproportionality analysis

Overall, based on the criteria of the four algorithms, we found 1527 AEs related to ibrutinib and 220 AEs related to acalabrutinib excluding product problems, various injuries, and other irrelevant signals. Table 3 shows the top 20 PTs ordered by signal strength referring to ROR, PRR, BCPNN, and MGPS. Among them, we identified some PTs that were not recorded in the instruction manual of BTK inhibitors as follows: 13 AEs for ibrutinib, including pseudohyperkalaemia (PT: 10052185), prostatic mass (10064022), nail growth abnormal (10064764), blood blister (10005372), immunoglobulins abnormal (10021497), ear neoplasm (10055016), capillary fragility (10007191), prostatic specific antigen decreased (10036972), nail cuticle fissure (10079216), hair texture abnormal (10019049) mastoid effusion (10069008), and scrotal haematocoele (10061517); 12 AEs for acalabrutinib, including oral blood blister (10076590), skin mass

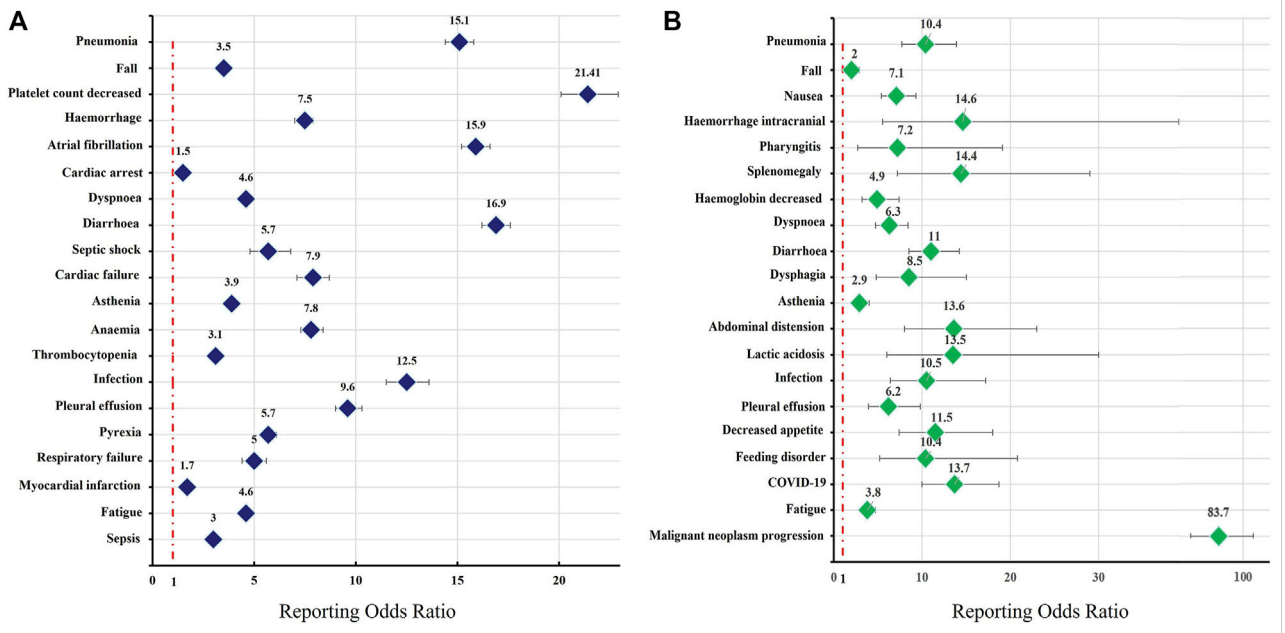

FIGURE 3
Reporting odds ratios (RORs) of the top 20 AEs associated with BTK inhibitors according to death reports. The graph presents the RORs of BTK inhibitor-associated AEs compared to reports in the full database. Colours represent different drugs: **(A)** ibrutinib; **(B)** acalabrutinib. Error bars represent the 95% confidence interval (CI). A lower limit of the ROR 95% CI above 1 was considered significant. AEs, adverse effects; BTK, Bruton's tyrosine kinase.

(10067868), lymph node pain (10025182), tumour lysis syndrome (10045170), decreased immune responsiveness (10011968), onychoclasis (10048886), melanocytic naevus (10027145), prostatomegaly (10051482), immunodeficiency (10061598), lung opacity (10081792), malignant neoplasm progression (10051398), and Richter's syndrome (10058728). Figure 1 shows the proportions of SOCs and Supplementary Table S2 shows the signal strengths of SOCs; significant signal overlap emerged for the SOCs of the BTK inhibitors. The proportion of general disorders and administration site conditions (15.1%, 29.5%, respectively), investigations (9.4%, 9.6%, respectively), and gastrointestinal disorders (10.9%, 8.7%, respectively) were all higher than other SOCs for ibrutinib and acalabrutinib. When compared with each other, the proportions of cardiac disorders (5.8%) and eye disorders (2.1%) were relative higher with ibrutinib than with acalabrutinib, whereas blood and lymphatic system disorders (5.8%) and neoplasms benign, malignant, and unspecified (3.2%) were relative higher with acalabrutinib than with ibrutinib.

Analysis of adverse events associated with Bruton's tyrosine kinase inhibitors in terms of infection, cardiovascular toxicities, and haemorrhage

Infection, cardiovascular toxicities, and haemorrhage were the most common AEs mentioned in the instruction manuals for BTK inhibitors. Therefore, we performed an analysis of those AEs, and the result is shown in Supplementary Table S3. From the data, even with the same SOC, there were differences in PTs between ibrutinib and acalabrutinib. According to the results, for the SOC of infections and infestations, ibrutinib was mainly centred on fungal infections, such as *Aspergillus* and *Cryptococcosis*; however, acalabrutinib was mainly centred on bacterial infections, such as *Pseudomonas* and *Clostridium difficile*. For the SOC of cardiac disorders, blood, and lymphatic system disorders, ibrutinib seemed to cause more AEs than acalabrutinib based on the data.

Fatalities due to Bruton's tyrosine kinase inhibitor-associated adverse events

The safety issues of BTK inhibitors have hindered their clinical application, but BTK inhibitors could change the era of BCM treatment; thus, we further analysed the potential fatality rates associated with BTK inhibitor regimens. Figure 2 shows the fatality rates and the number of reports, and Supplementary Figure S1 shows the SOC distribution of the top 20 AEs associated with BTK inhibitors according to the number of death reports. Figure 3 and Supplementary Table S4 show the signal strength according to ROR. In general, this frequency was much higher for ibrutinib than for acalabrutinib, which could be due to the shorter marketing time and improved safety of acalabrutinib. In addition to

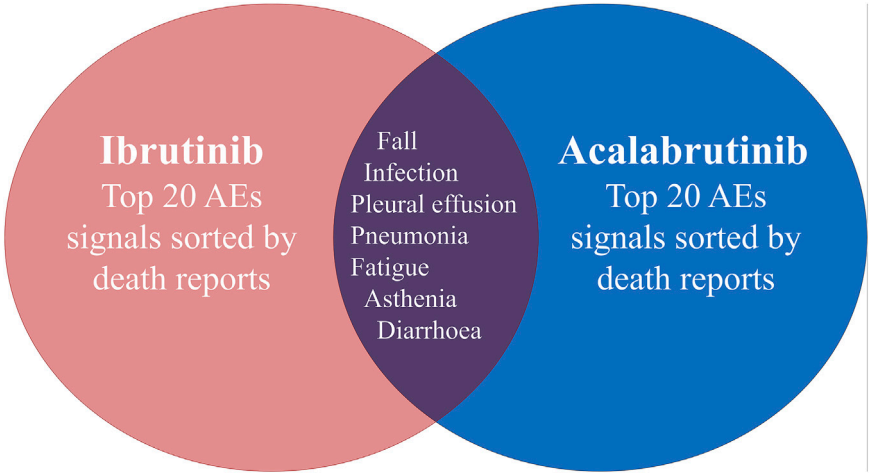

FIGURE 4
Common preferred terms (PTs) for both ibrutinib and acalabrutinib according to death reports.

infections, cardiac disorders were an important death cause with ibrutinib, whereas neoplasms benign, malignant, and unspecified was an important cause of death with acalabrutinib. It was observed that the incidences of pneumonia (434, 19, respectively), pleural effusion (106, 5, respectively), a fall (181, 6, respectively), infection (101, 5, respectively), asthenia (139, 4, respectively), diarrhoea (209, 3, respectively), and fatigue (137, 5, respectively) were relatively higher with both ibrutinib and acalabrutinib (Figure 4). We further analysed the population characteristics in Table 4 and found that the deaths caused by pneumonia, pleural effusion, a fall, infection, asthenia, diarrhoea, and fatigue more often occurred in males and elderly people.

Occurrence time of ibrutinib-related adverse events

As ibrutinib was associated with high frequencies of both AEs and related deaths, we analysed the time to onset for ibrutinib-related AEs. Pneumonia, pleural effusion, atrial fibrillation, diarrhoea, and infection were the top 5 PTs, ordered by signal strength, which were important causes of death, excluding investigations (Supplementary Table S4). The occurrence times of ibrutinib-related AEs are shown in Figure 5. From the data, the AEs diarrhoea and infection both occurred mainly in the first 30 days after the first dose of ibrutinib. In contrast, pneumonia, pleural effusion, and atrial fibrillation mainly occurred after a longer period of ibrutinib use (>180 days).

Discussion

Due to the limited pre-clinical data, it was under experts' opinion that pharmacovigilance data mining from the post-marketing adverse event reporting system could provide useful supplements to the drug instruction. Targeted therapy in hematology got a rapid development in recent years. When it comes to lymphocytic malignancy, the application of BTK inhibitors was a milestone especially in the fields of chronic lymphoma leukemia (CLL) therapy as well as graft versus host diseases after allogeneic hematopoietic stem cell transplantation. However, the safety issues hindered its application and affected the prognostic outcome of the patients. Based on the above concerns, our study focused on analyzing and comparing the association of patients prognosis and AEs induced by BTK inhibitors based on the FAERS pharmacovigilance database in the real-world practice, in order to provide novel perspective in lymphocytic malignancies treatments.

According to the results, there were 13 and 12 novel AEs signals were not included in drug labels of ibrutinib and acalabrutinib, respectively. It was estimated that approximately 40% of patients treated with ibrutinib suspend targeted therapy owing to serious intolerances (some even fatal), which suggests that more attention should be paid to BTK inhibitor safety issues.

Due to their structural differences, the potential AE signals and types vary for ibrutinib and acalabrutinib; further, the AE frequencies for ibrutinib seemed to be higher than those for acalabrutinib. This may result from the higher bruton's tyrosine kinase (BTK) selectivity and target specificity of acalabrutinib, thus reducing the occurrence of off-target effects (Byrd et al.,

TABLE 4 Details concerning patients with deaths associated with ibrutinib and acalabrutinib -related AEs in the FAERS database (January 2004 to Dem 2021).

	Pneumonia	Pleural effusion	Fall	Infection	Asthenia	Diarrhoea	Fatigue
Gender							
Femle	119(27.4) /3(15.8)	31(2.8)/2(40)	64(35.4)/1(16.7)	16(15.8)/0	43(30.9)/0	54(25.8)/0	43(31.4)/1(20)
Male	296(68.2)/16(84.2)	72(29.3)/3(60)	113(62.4)/5(83.3)	43(42.6)/5(100)	91(65.5)/4(100)	104(49.8)/3(100)	82(59.8)/(80)
Unknown or missing	19(4.4)/0	3(67.9)/0	4(2.2)/0	42(41.6)/0	5(3.6)/0	51(24.4)/0	12(8.8)/0
Age(year)							
0-18	2(0.5)/0	/	/	/	/	/	/
18-64	63(14.5)/4(21.0)	13(12.3)/1(20)	12(6.6)/0	10(9.9)/0	14(10.1)/0	29(13.9)/0	16(11.7)/0
≥65	274(63.1)/11(58.0)	64(60.4)/4(80)	128(70.7)/5(83.3)	34(33.7)/5(100)	98(70.5)/3(75)	110(52.6)/3(100)	87(63.5)/5(100)
Unknown or missing	95(21.9)/4(21.0)	29(27.3)/0	41(22.7)/1(16.7)	57(56.4)/0	27(29.4)/1(25)	70(33.5)/0	34(24.8)/0
Reporter							
Medical staff	284(65.5)/7(36.8)	46(43.4)/2(40)	98(54.1)/2(33.3)	71(70.3)/3(60)	72(51.8)/0	148(70.8)/0	60(43.8)/1(20)
Non-medical staff	149(34.3)/7(36.8)	60(56.6)/1(20)	83(45.9)/(33.3)	30(29.7)/1(20)	67(48.2)/2(50)	60(28.7)/2(66.7)	77(56.2)/1(20)
Unknown or missing	1(0.2)/5(26.2)	0/2(40)	0/2(33.3)	0/1(20)	0/2(50)	1(0.5)/1(33.3)	0/3(60)
Reporting time							
2014	28(6.5)/0	7(6.6)/0	6(3.3)/0	2(2.0)/0	9(6.5)/0	16(7.7)	6(4.4)/0
2015	56(12.9)/0	17(16.0)/0	11(6.1)/0	6(5.9)/0	23(16.5)/0	22(10.5)	14(10.2)/0
2016	54(12.4)/0	14(13.2)/0	20(11.0)/0	6(5.9)/0	17(12.3)/0	28(13.4)	23(16.8)/0
2017	47(10.8)/0	13(12.3)/0	28(15.5)/0	15(14.8)/0	25(18.0)/0	33(15.8)	25(18.2)/0
2018	59(13.6)/3(15.8)	22(20.7)/1(20)	39(21.5)/0	18(17.8)/0	12(8.6)/0	21(10.0)	15(10.9)/0
2019	71(16.4)/1(5.3)	14(13.2)/0	26(14.4)/1(16.7)	24(23.9)/0	12(8.6)/0	30(14.4)/1(16.7)	20(14.6)/1(20)
2020	66(15.2)/6(31.6)	13(12.3)/2(40)	32(17.7)/2(33.3)	11(10.9)/5(100)	25(18.0)/0	44(21.0)	17(12.4)/0
2021	53(12.2)/9(44.3)	6(5.7)/(40)	19(10.5)/3(50.0)	19(18.8)/0	16(11.5)/4(100)	15(7.2)/2(66.7)	17(12.4)/4(80)

Values are n (%), unless otherwise indicated.ibrutinib vs. acalabrutinib.

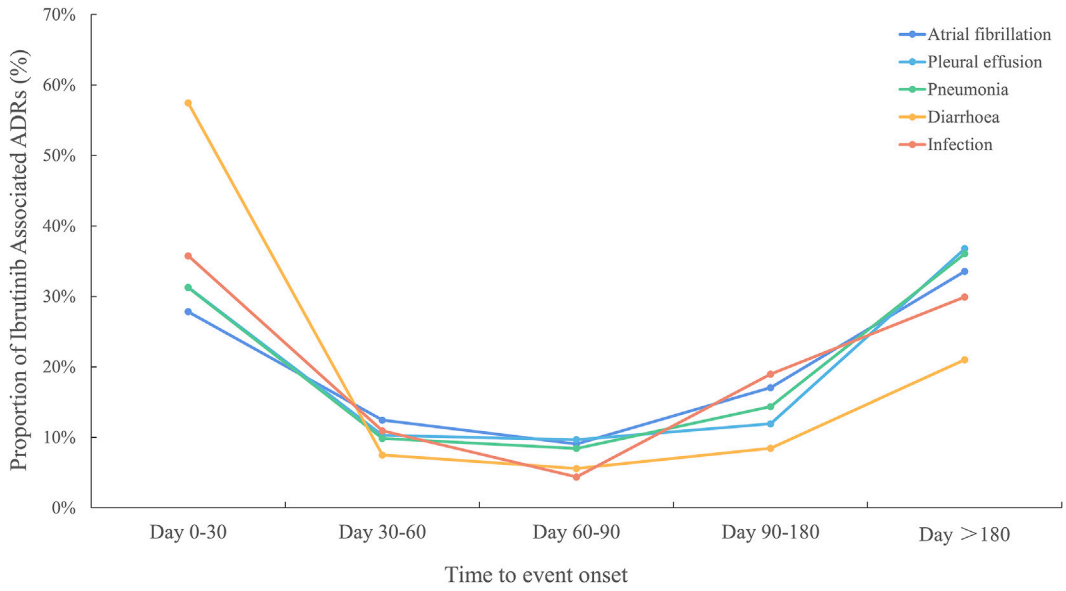

FIGURE 5
Time to event onset caused by infections, pneumonia, pleural effusion, atrial fibrillation, and diarrhoea following BTK inhibitor regimens. BTK, Bruton's tyrosine kinase.

2016; Wen et al., 2021). Studies have found the AEs of ibrutinib mainly due to its blocks of BTK activity, *via* the irreversible binding to Cys481 in the kinase domain and also covalent or non-covalent binding to other homologous kinases regardless of cysteine residues, including TEC family kinases (TEC, ITK, RLK, BMX), epidermal growth factor receptor (EGFR), ERBB2/HER2 human epidermal growth factor receptor 2, ERBB4/HER4 human epidermal growth factor receptor, B-lymphoid kinase (BLK), and Janus kinase 3 (JAK3), thereby resulting in off-target effects (Paydas 2019; Liu et al., 2021). When it comes to acalabrutinib was shown to have 323-, 94-,19-, and 9-fold higher selectivity for the TEC family kinases ITK, TXK, BMX, and TEC, respectively, compared to that of ibrutinib, and shows no activity toward EGFR. Moreover, compared with ibrutinib, acalabrutinib shows almost no inhibitory effect on the activity of ITK, EGFR, ERBB2, ERBB4, JAK3, LYN, LCK, SRS, and YES1, even at a higher half-inhibitory concentration (>1000 nM) (Wu, Zhang, and Liu 2016).

Moreover, some AEs of BTKs inhibitors were fatal, which was also a critical concerns in clinical practice. When concerning the mortality related to AEs, infection, pneumonia, haemorrhage, pleural effusion, and diarrhoea ranked in the top 20 for both ibrutinib and acalabrutinib according to the FARES database death reports. Infection, pleural effusion, and pneumonia may result from the inherent humoral immunosuppression of haematological diseases and treatment-related immunosuppression, which were also the vital reasons underlying the morbidity and mortality associated with BCM. Studies indicated that 4.1% of patients treated with ibrutinib suffer from opportunistic infections, and among them, invasive fungal infections (IFIs) account for approximately half the AE reports, indicating that ibrutinib might be related to early IFIs and likely induce myelosuppression (Ghez et al., 2018). This result was consistent with our results indicating that the ROR of fungal infection was relatively stronger for infectious pneumonia with ibrutinib. The mechanism of IFIs were complex and the involvement of detrimental effects on phagocytes was on of the important factors, possibly caused by neutropenia induced during the progression of malignancies as well as the off-target effects of BTK inhibitors on intracellular signalling components (Estupinan and Berglof et al., 2021). Besides, haemorrhage was also a common AE associated with BTK inhibitors, which mainly caused by inhibition of the TEC family kinases BTK and TEC and the fatality was also ranked high in our study. Researchers have found BTK and TEC could regulate platelet activation through collagen receptor glycoprotein VI (GPVI) *via* the phosphorylation of phospholipase Cγ2 (PLCγ2), and with arterial shear, the interaction between platelets and the damaged vessel wall is mainly mediated by the combination of von Willebrand factor (VWF) and its receptor GPIb-IXV complex (Quek, Bolen, and Watson 1998; Atkinson, Ellmeier, and Watson 2003). Diarrhoea was another AE found to be associated with the use of BTK inhibitors, which is also often related to inhibition of the EGFR. However, studies have found that the EGFR off-target effect alone is not sufficient to explain the mechanism underlying diarrhoea in patients treated with BTK inhibitors, which suggests that mechanisms other than binding to kinases harbouring a cysteine in the active site contribute to diarrhoea (Byrd et al., 2016; Estupinan and Berglof et al., 2021). Further, another fatal AE signal, atrial fibrillation (AF), was reported to be associated with a higher mortality rate and was considered one of the main reasons for patient intolerance (Salem et al., 2019). Our study found that the incidence of atrial fibrillation-related fatality was relatively higher in ibrutinib after its long-term time use; the underlying pathogenetic mechanism may be due to its inhibitory effect on multiple kinases, including ERBB2 and the PI3K-AKT pathway, resulting in the off-target effects of ibrutinib at its therapeutic concentration (McMullen et al., 2014; Tenin et al., 2014). Besides, it was found that mutations in the *Erbb2* gene could impaire atrial electrical signalling; when PI3K activity is decreased, the susceptibility to atrial fibrillation was found to be increased in mouse model. Moreover, ibrutinib also acts specifically on atrial myocytes and shorten the action potential time course of atrial myocytes, thereby shortening the non-return period and increasing the risk of atrial fibrillation, which was fatal especially in some elderly patients (Jiang and Li et al., 2019).

Based on its efficacy of BCM treatment, the development of novel BTK inhibitors was under rapid increase. Zanubrutinib and Orelabrutinib were the latest approved BTK inhibitors by CFDA, which were a new generation of BTK inhibitors marked with higher target selectivity and thus less safety concerns. However, due to the short marketing time as well as the limited market access, it was impossible to make a credible pharmacovigilance study at present.

According to the public data available, zanubrutinib has a higher target occupancy rate and exhibits less off-target binding than ibrutinib, and clinical trials indicated that it is generally well-tolerated in patients (Tam, Quach, et al., 2020; Tam, Robak, et al., 2020; Xu et al., 2020). Besides, orelabrutinib has high selectivity, irreversible binding ability to BTK, and little activity on other kinases (ITK, EGFR, ERBB2, etc.). Studies have shown that at a dose of 50 mg or higher dose, orelabrutinib could almost completely bind to BTK, with a binding rate greater than 99% and low inter-variability (Dhillon 2021). Meanwhile, the off-target effects of orelabrutinib were minimized even in a long-time use after researchers' years dedication (Song et al., 2020).

However, although data mining techniques have advantages in analysing clinical safety issues in real-world, there were also certain limitations. Firstly, the FAERS database is a spontaneous reporting system (SRS), and the data mining technology that was used in this study could not improve its inherent

limitations, such as false reporting, uneven information quality, under-reporting, and inaccuracy, all of which might result in reporting bias. Secondly, the SRS, which is used in qualitative research, cannot be used to quantify adverse reaction signals based on the total number of adverse reactions, which made it impossible to calculate the incidence of each adverse reaction. Thirdly, although data mining techniques could provide a profile of BTK inhibitor-associated adverse reaction signals through signal detection, this is insufficient to prove a causal relationship. Last but not least, due to their short marketing time, our study only analyzed and compared the safety signal of ibrutinib and acalabrutinib, future studies would be made to design a larger pharmacovigilance research. Above all, though the apparent defects and limits, real-world data mining and pharmacovigilance study could provide rational considerations for physicians to choose the suitable BTK inhibitors for different patients to obtain desired tolerance and efficacy.

Consequently, our pharmacovigilance analysis of the FAERS database identified various novel AE signals for BTK inhibitors in a real-world practice setting. We made a comprehensively study and compared the AEs signals of iburintib and acalabrutinib, our results indicated that patients taking ibrutinib are more prone to induce AEs than those taking acalabrutinib. When it comes to fatality, it was found that infection, pneumonia, pleural effusion, and diarrhoea were prevalent both in patients' taking ibrutinib and acalabrutinib, especially in elderly patients. Furthermore, atrial fibrillation was a fatal AE associated with ibrutinib, which exhibited strikingly higher death rates. Our results indicated that more attention should be paid to cardiovascular toxicities with ibrutinib, which suggest that more careful pharmaco-monitoring, such as precise pharmacokinetic analysis and therapeutic drug monitoring, could be implemented in the future in order to provide a more sustainalbe and safety therapy in lymphocytic maligancies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the ethics committee of First Affiliated Hospital of Nanchang University.

Author contributions

Concept and design the work: HP, JH. Acquisition, analysis, or interpretation of data: QW, QL, XL, and TX. Management and checking of all data: HP, QL, JH, and XL. Drafting the article: WQ and HP. All authors critically reviewed the manuscript and interpreted the results. The final manuscript was read, checked, and approved by all authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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Ocular adverse events associated with anti-VEGF therapy: A pharmacovigilance study of the FDA adverse event reporting system (FAERS)

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Background: The purpose of this study is to identify and characterize ocular adverse events (AEs) that are significantly associated with anti-VEGF drugs for treatment of neovascular age-related macular degeneration and compare the differences between each drug, and provide clinical reference.

Methods: Ocular AEs submitted to the US Food and Drug Administration were analyzed to map the safety profile of anti-VEGF drugs. The Pharmacovigilance tools used for the quantitative detection of signals were reporting odds ratio and bayesian confidence propagation neural network.

Results: A total of 10,608,503 AE reports were retrieved from FAERS, with 20,836 for ranibizumab, 19,107 for aflibercept, and 2,442 for brolucizumab between the reporting period of Q1, 2004 and Q3, 2021. We found and analyzed the different AEs with the strongest signal in each drug—ranibizumab-macular ischaemia (ROR = 205.27, IC-2SD = 3.70), retinal pigment epithelial tear (ROR = 836.54, IC-2SD = 7.19); aflibercept-intraocular pressure increased (ROR = 31.09, IC-2SD = 4.61), endophthalmitis (ROR = 178.27, IC-2SD = 6.70); brolucizumab-retinal vasculitis (ROR = 2930.41, IC-2SD = 7.47) and/or retinal artery occlusion (ROR = 391.11, IC-2SD = 6.10), dry eye (ROR = 12.48, IC-2SD = 2.88).

Conclusion: The presence of AEs should bring clinical attention. The use of anti-VEGF drugs should be based on the patient's underlying or present medical condition to reduce any adverse event associated with the treatment.

KEYWORDS

adverse events, pharmacovigilance, ranibizumab, aflibercept, brolucizumab, safety signals

Introduction

Age-related macular degeneration (AMD) is an acquired disease of the macula, a progressive visual impairment caused by late-onset neurodegeneration of the photoreceptor-retinal pigment epithelial complex (Waseem and Sanaa, 2017). AMD is the leading cause of severe and irreversible vision loss for people aged 55 years and over in developed countries (Congdon et al., 2004), and it becomes more serious with the aging of population, with an anticipated rise to 288 million cases worldwide by year 2040 (Wong et al., 2014). AMD can be classified into dry and neovascular (wet) according to the absence or presence of new choroidal blood vessels that invade the retina, respectively (Ambati and Fowler, 2012). Anti-VEGF drugs have set the benchmark in the treatment of neovascular AMD (Velez-Montoya et al., 2013), due to its ability to suppress choroidal neovascularization (CNV), reduce retinal fluid leakage and improve visual impairment (Campochiaro et al., 2016).

Currently, intravitreal injection of anti-VEGF drugs includes ranibizumab, aflibercept, off-label bevacizumab, and brolucizumab (Arepalli and Kaiser, 2021). Ranibizumab is a recombinant humanized IgG1monoclonal Fab fragment, which binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). It can improve average visual acuity, and ameliorate classic CNV remarkably (Brown et al., 2009). Aflibercept is a recombinant fusion protein with the Fc portion, has high affinity to all VEGF-A and VEGF-B isoforms and placental growth factors. It was approved by the FDA in 2011 to treat neovascular AMD (Heier et al., 2012). Bevacizumab originally developed as a chemotherapeutic drug, mainly for the treatment of colorectal cancer, non-small cell lung cancer and other forms of cancer. Its off-label use for the treatment of neovascular AMD, due to the lack of specificity to conditions associated with inhibition of VEGF, has been linked to the incidence of serious AEs and thus, has not been approved by the FDA (Grzybowski et al., 2018).

The new anti-VEGF drug brolucizumab is composed of a single-chain antibody fragment structure, which is the smallest anti-VEGF antibody tested in humans and can inhibit all isoforms of VEGF-A (Holz et al., 2016). The HAWK and HARRIER clinical trials reached the primary end point of noninferiority in best corrected visual acuity after the comparison of brolucizumab and aflibercept and thus, approved by the FDA and European Medical Agency in 2019 and 2020 respectively. Phase III clinical trials are well underway in China (Dugel et al., 2020).

Although anti-VEGF drugs are currently recognized as the first-line treatment for neovascular AMD, repeated injections of anti-VEGF drugs can still cause some ocular complications, such as eye pain (Biagi et al., 2014), conjunctival hemorrhage etc. (Dugel et al., 2020). Due to the small difference in the efficacy of the three drugs (Heier et al., 2012; Dugel et al., 2020), clinicians and patients may pay more attention to safety issues. The overall

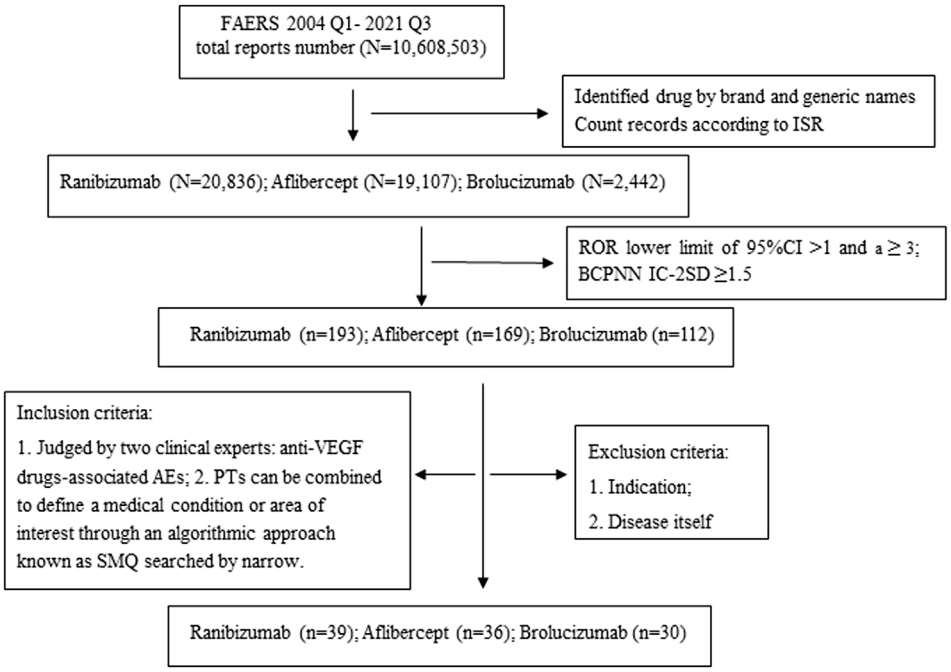

FIGURE 1
The workflow of data mining. Abbreviations: N, Total number of adverse drug events; n, Safety signals; ISR, Number that uniquely identifies an AERS report; PTs, Preferred Terms; SMQ, Standardized MedDRA Queries.

safety of these drugs is satisfactory, but literature review found that there are differences in AEs reported by different drugs. Although they are available, the absence of systematic reports including comparisons of adverse reactions of these drugs in the literature give no conclusive summary of AEs.

Adverse events spontaneous reporting system is currently one of the most important methods in monitoring the safety of medicinal products. FDA Adverse Event Reporting System (FAERS) is a public database designed to support the FDA's post-marketing safety surveillance program for drug and therapeutic biologic products through a system of spontaneous reports by consumers, health professionals, drug manufacturers, and other non-healthcare workers. Based on the needs of clinical, rational and precise drug use and protection of patients' rights and interests, we evaluated and compared the AE reports of anti-VEGF drugs using FAERS database. Findings of this study create real-world evidence for risk signal detection and guide future comparative effectiveness and post-marketing surveillance research for anti-VEGF drugs.

Methods

Data source

The pharmacovigilance tools used in this study to extract data is OpenVigil, an experimental research application, which availed researchers of directly extracting structured AE report information from the FAERS database through the docking application program interface (API). With the additional drug mapping and duplicate detection functionality, OpenVigil is used in many pharmacovigilance studies. We performed a retrospective pharmacovigilance study based on data from Q1 of 2004 to Q3 of 2021 in the FAERS database. AEs in the FAERS are coded by the preferred-terms level of the Medical Dictionary for Regulatory Activities (MedDRA) classification. Due to a large number of preferred terms and their lack of specificity, Standardized MedDRA Queries (SMQs) were developed. SMQs are standard sets of MedDRA terms that are related to the same medical condition, thereby facilitating data retrieval and signal detection.

Ethics approval

De-identified public data was used in this study, not requiring any form of ethics approval.

Adverse events and drug identification

Reports involving three kinds of anti-VEGF drugs for neovascular AMD treatment (including ranibizumab, aflibercept and brolucizumab) were identified using text string searches for each drug by brand and generic names through the FDA public database during the data mining process. Then, we extracted AEs marking "ranibizumab", "aflibercept", "brolucizumab" and brand name "Lucentis", "Byooviz", "Susvimo", "Zaltrap", "Eylea", "Beovu" as the primary suspected object. AEs can be specified at different levels of the MedDRA terminology.

We searched with preferred term (PT) as primary term and counting records according to Individual Safety Reports (ISR). As a result, the safety profile of each of the anti-VEGF drugs was examined through SMQ analysis. Two researchers, including a chief pharmacist and a professor of Ophthalmology classified the AE reports in terms of SMQs and collected clinical characteristics of the patient, including gender, age, and AE outcomes, respectively. Unexpected adverse drug reaction was defined as any significant AE that was uncovered and was not listed in the FDA drug labelling. To minimize the existence of an "indication bias" (i.e., the indication for which the prescribed drug is reported as an AE), PTs and SMQs associated with AMD-related signs and complications were removed for analysis. The workflow of the study as shown in Figure 1.

Data mining

One of the most frequently used methods of safety signal detection is disproportionality analysis, which consisted of two categories: Frequentist Statistics and Bayesian Statistics. Frequentist Statistics included reporting odds ratio (ROR), and proportional reporting ratio (PRR). Bayesian Statistics on the other hand, included bayesian confidence propagation neural network (BCPNN) and multi-item gamma poisson shrinker (MGPS). The frequentist method had its characteristics: the sensitivity of frequency method was high, but it was easy to produce false positive signals when the number of reports was small. The specificity of Bayesian method was good; however, the signal detection time was relatively delayed. In order to minimize the result bias caused by using a certain algorithm alone, two methods, ROR and BCPNN, were used for signal detection in this study. When both algorithms were positive, they were judged as suspicious signals. The ratio imbalance measurement algorithm was shown in Table 1. The principle of disproportionate measure and standard of signal detection were shown in Table 2.

Statistical analysis

Using the ROR and BCPNN, when the lower limit of the 95% confidence interval (CI) of ROR exceeds 1.0 and the information component value minus two standard deviations (IC-2SD) of BCPNN is greater than zero, with at least three records, it is an indication of a safety signal. In addition, the time scan map of

TABLE 1 Ratio imbalance measurement algorithm.

Item	Reports with the target AEs	All other AEs	Total
Reports with the target drug	a	b	a+b
All other drugs	c	d	c + d
Total	a+c	b + d	a+b + c + d

TABLE 2 Principle of dis-proportionality measure and standard of signal detection.

Algorithms	Calculation formula	Criteria
ROR	$ROR = \frac{a/c}{b/d} = \frac{ad}{bc}$	(1) a ≥3;
	$95\%CI = e^{\ln(ROR)\pm1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}}$	(2) 95%CI > 1
BCPNN	$E(IC) = log_2\frac{(C_{xy}+\gamma_{11})(C+\alpha)(C+\beta)}{(C+\gamma)(C_x+\alpha_1)(C_y+\beta_1)}$	(1) a ≥3;
		(2) IC-2SD > 0;
		(3) IC-2SD ≥ 1.5 (medium and strong signals)
	$V(IC) = 1/(In2)^2\{(\frac{C-C_{xy}+\gamma-\gamma_{11}}{(C_{xy}+\gamma_{11})(1+C+\gamma)}) + (\frac{C-C_x+\alpha-\alpha_1}{(C_x+\alpha_1)(1+C+\alpha)}) + (\frac{C-C_y+\beta-\beta_1}{(C_y+\beta_1)(1+C+\beta)})\}$	
	$\gamma = \gamma_{11}\frac{(C+\alpha)(C+\beta)}{(C_x+\alpha_1)(C_y+\beta_1)}$	
	$IC - 2SD = E(IC) - 2\sqrt{V(IC)}$	
	$\alpha_1 = \beta_1 = 1; \alpha = \beta = 2; \gamma_{11} = 1;$	
	$C = a + b + c + d; C_x = a + b; C_y = a + c; C_{xy} = a$	

Abbreviations: ROR, Reporting odds ratio; BCPNN, Bayesian confidence propagation neural network; CI, Confidence Interval; IC, Information Component.

safety signal was shown reflecting the trend of a drug paired with AE in FAERS based on the IC 95% CI. When the time scan map is in a steady upward trend and the 95% CI is narrowed, the signal is stable and the association between the drug and the AE is strong. According to BCPNN signal strength standard, medium and strong signals with signal value IC-2SD ≥ 1.5 were selected for analysis and discussion (Sharwan and Bhaswat, 2015). All analyses were performed using Microsoft EXCEL 2019. Figures were illustrated using GraphPad Prism (v8.2) or R (v4.1.2).

Results

In this study, data mining was performed to obtain the safety signals of anti-VEGF drugs from Q1 of 2004 to Q3 of 2021. A total of 10,608,503 AE reports were retrieved from FAERS, with 20,836 for ranibizumab, 19,107 for aflibercept, and 2,442 for brolucizumab. Based on the geographical perspective, majority of the reports were from America. In gender, reports for females were approximately 10%–20% more than males for both ranibizumab and brolucizumab. For aflibercept, the highest tallied reports fell under unknown gender. For age composition, bulk of the reports were from people aged 50–79 across all three drugs, followed closely by respondents aged 80 and above. The serious outcomes related to aflibercept accounted for a relatively high proportion (11,356 cases, 59.4%). On the other hand, hospitalization, disability and other life-threatening events were unlikely as the numbers were relatively low. The demographic characteristics of AE reports associated with Anti-VEGF drugs are shown in Table 3.

A total of 43 moderate to strong signals with an IC-2SD ≥ 1.5 were identified under 3 kinds of anti-VEGF drugs in Table 4. Some were presented in the instructions while marked signals in the table were found from FAERS database. For instance, macular ischaemia was not indicated in ranibizumab's drug label and yet, was found to have a strong signal. The following is classified as the top AEs in each drug: ranibizumab-macular ischaemia, retinal pigment epithelial tear (RPE tear); aflibercept-intraocular pressure increase, endophthalmitis; brolucizumab-retinal vasculitis and/or retinal vascular occlusion, dry eye. We listed the moderate to strong signals in Table 4, and selected three PTs with the strongest safety signals of each drug that are more clinically concerned to draw IC time scan picture.

In order to investigate the changes of each signal over time, this study drew time scans of safety signals of RPE tear, subretinal fibrosis and macular ischaemia for ranibizumab; endophthalmitis, hypopyon and intraocular pressure increase for aflibercept; and retinal vasculitis, retinal artery occlusion and

TABLE 3 Characteristics of reports associated with Anti-VEGF from Q1 of 2004 to Q3 of 2021.

	Ranibizumab (%)	Aflibercept (%)	Brolucizumab (%)
Number of events	20836	19107	2442
Gender			
Female	9855 (47.3)	1463 (7.7)	1298 (53.2)
Male	7677 (36.8)	1529 (8.0)	808 (33.1)
Unknown	3304 (15.9)	16115 (84.3)	336 (13.8)
Age			
<18	52 (0.2)	3 (0)	1 (0)
18–49	316 (1.5)	165 (0.9)	4 (0.2)
50–79	4702 (22.6)	1644 (8.6)	674 (27.6)
≥80	4056 (19.5)	712 (3.7)	577 (23.6)
Unknown	11710 (56.2)	16583 (86.8)	1186 (48.6)
Serious outcomes			
Death	4958 (23.8)	7947 (41.6)	150 (6.1)
Disability	596 (2.9)	1269 (6.6)	107 (4.4)
Life-threatening	309 (1.5)	143 (0.7)	5 (0.2)
Hospitalization	3572 (17.1)	1997 (10.5)	106 (4.3)
Total	9435 (45.3)	11356 (59.4)	368 (15.1)
Reporter country			
USA	7497 (36.0)	12731 (66.6)	1143 (51.0)
Japan	1296 (6.2)	871 (4.6)	232 (10.3)
Germany	713 (3.4)	438 (2.3)	92 (4.1)
Other countries	11330 (54.4)	5067 (26.5)	775 (34.6)

keratic precipitates for brolucizumab. Each graph shows a steady or upward trend and the confidence interval gradually narrows (as shown in Figure 2), which indicates that the signal is stable and strongly correlated with the use of the anti-VEGF drug. The abscissa was the year of the report, and the ordinate was the Information Component (IC) value. IC values of anti-VEGF drugs induced various AEs from 2008 to 2021. As the years went by, the number of reports increased. Moreover, IC values accumulates gradually and the range of confidence interval continues to narrow across all three anti-VEGF drugs.

All moderate and strong signals associated with anti-VEGF drugs are shown in the Supplementary Data, Supplementary Tables S1–S3. We compared the AE signals mentioned in the instructions and found that the signals of different drugs had their individual characteristics as shown in Figure 3. In general, the manifestation is that AE signals related to retina tallied higher ROR figures than those of retina-unrelated AEs except for vitritis, which totaled 1469.04 RORs for brolucizumab, the highest in its class. In the same category, endophthalmitis had 178.27 RORs for aflibercept. For retina related AEs, brolucizumab collected 391.11 and 2930.41 RORs for retinal artery occlusion and retinal vasculitis respectively while aflibercept had the two lowest RORs for retinal tear at 9.79 and retinal detachment at 14.58. RPE tear ranked the highest ROR of ranibizumab in both groups at 836.54. We found that all three drugs have their individual AEs.

Discussion

To the best of our knowledge, this study is the first to identify and characterize ocular AEs that are significantly associated with anti-VEGF drugs. Based on the database, we carried out 7 SMQs of ocular related AEs, and put emphasis on those safety signals that were classified as strong signals in the AE reports of anti-VEGF drugs in FAERS. After consulting with ophthalmologists and combining medical knowledge, we analyzed the unexpected adverse drug reactions that may or may not be listed in the instructions but were of clinical concern, and compared the characteristics of different drugs. We found statistically-significant signals for anti-VEGF drugs in the visual system for ranibizumab (macular ischaemia, RPE tear), aflibercept (intraocular pressure increased, endophthalmitis), and brolucizumab (retinal vasculitis and/or retinal vascular

TABLE 4 Moderate and strong signals of anti-VEGF drugs in ocular adverse events.

SMQs/PTs	Ranibizumab			Aflibercept			Brolucizumab		
	N	ROR (95%CI)	IC (IC-2SD)	N	ROR (95%CI)	IC (IC-2SD)	N	ROR (95%CI)	IC (IC-2SD)
Retinal disorders									
Retinal pigment epithelial tear	356	836.54 (706.72, 990.22)	7.39 (7.19)	55	58.67 (44.42, 77.50)	4.78 (4.37)	10	77.05 (41.18, 144.16)	3.28 (2.40)
Detachment of retinal pigment epithelium	292*	440.01 (376.16, 514.69)	7.03 (6.82)	63	61.35 (47.27, 79.61)	4.90 (4.52)	18	127.85 (79.88, 204.63)	4.05 (3.38)
Vitreous haemorrhage	333*	133.70 (118.43, 150.94)	6.32 (6.14)	157*	60.00 (50.86, 70.78)	5.33 (5.09)	14*	38.10 (22.48, 64.57)	3.45 (2.70)
Retinal haemorrhage	660*	97.72 (89.83, 106.31)	6.16 (6.04)	307*	44.37 (39.47, 49.88)	5.17 (5.00)	72	77.97 (61.55, 98.76)	5.21 (4.87)
Retinal scar	122*	377.92 (298.95, 477.77)	6.30 (5.98)	25*	52.95 (35.12, 79.83)	4.10 (3.51)	/	/	/
Macular hole	117*	95.96 (78.73, 116.97)	5.59 (5.30)	101*	88.06 (71.35, 108.68)	5.45 (5.14)	/	/	/
Vitreous floaters	401	50.89 (45.89, 56.44)	5.36 (5.21)	386	53.28 (47.96, 59.20)	5.41 (5.26)	453	599.32 (538.71, 666.74)	7.80 (7.65)
Subretinal fibrosis	59*	375.82 (268.40, 526.22)	5.56 (5.11)	/	/	/	6*	196.41 (86.59, 445.50)	2.76 (1.64)
Retinal tear	132*	62.55 (52.18, 74.98)	5.30 (5.03)	21	9.79 (6.36, 15.08)	2.79 (2.17)	9	32.64 (16.92, 62.95)	2.97 (2.05)
Retinal ischaemia	56*	89.16 (67.10, 118.47)	5.03 (4.62)	18*	27.89 (17.37, 44.79)	3.50 (2.82)	23*	285.66 (187.01, 436.37)	4.46 (3.85)
Retinal detachment	261	30.46 (26.86, 34.53)	4.69 (4.50)	119	14.58 (12.15, 17.50)	3.67 (3.40)	8	122.31 (60.51, 247.23)	3.08 (2.10)
Vitreous detachment	72	37.96 (29.87, 48.25)	4.58 (4.23)	31	17.07 (11.94, 24.41)	3.48 (2.96)	9	38.09 (19.74, 73.50)	3.01 (2.09)
Retinal artery occlusion	76*	29.52 (23.41, 37.21)	4.37 (4.03)	74*	31.31 (24.76, 39.58)	4.42 (4.08)	110	391.11 (320.55, 477.19)	6.39 (6.10)
Retinal depigmentation	27*	112.60 (74.20, 170.88)	4.43 (3.84)	9*	35.64 (18.16, 69.95)	2.98 (2.03)	/	/	/
Macular ischaemia	23*	205.27 (126.46, 333.18)	4.37 (3.70)	/	/	/	/	/	/
Retinal vascular thrombosis	24*	36.12 (23.87, 54.66)	3.87 (3.27)	/	/	/	/	/	/
Vitreous haze	17*	154.38 (89.70, 265.70)	3.97 (3.22)	29*	365.83 (228.87, 584.76)	4.73 (4.10)	25	2285.46 (1407.06, 3712.23)	4.68 (4.02)
Retinal vasculitis	28*	23.82 (16.30, 34.81)	3.70 (3.15)	51*	49.29 (37.00, 65.65)	4.61 (4.19)	237	2930.41 (2480.17, 3462.39)	7.70 (7.47)
Photopsia	68*	10.81 (8.50, 13.75)	3.21 (2.86)	28*	4.79 (3.30, 6.94)	2.07 (1.53)	35*	47.60 (34.03, 66.57)	4.36 (3.87)
Vitreal cells	/	/	/	28*	361.41 (224.49, 581.84)	4.68 (4.05)	37*	4799.11 (3007.42, 7658.20)	5.22 (4.64)
Ocular infections									
Endophthalmitis	590	109.11 (99.74, 119.36)	6.26 (6.13)	805	178.27 (164.51, 193.19)	6.82 (6.70)	38	49.60 (35.94, 68.46)	4.45 (3.98)
Vitritis	97	67.92 (54.93, 83.99)	5.22 (4.91)	237*	225.79 (194.06, 262.71)	6.58 (6.37)	196	1469.04 (1244.90, 1733.54)	7.37 (7.13)
Hypopyon	41*	44.51 (32.34, 61.26)	4.39 (3.93)	66*	82.67 (63.81, 107.10)	5.12 (4.75)	18*	160.07 (99.84, 256.63)	4.09 (3.41)
Eye infection	160*	19.64 (16.76, 23.01)	4.08 (3.85)	127*	16.85 (14.12, 20.12)	3.86 (3.60)	18*	18.26 (11.47, 29.06)	3.25 (2.58)
Blepharitis	22	7.77 (5.10, 11.84)	2.57 (1.96)	17*	6.53 (4.04, 10.53)	2.31 (1.62)	/	/	/
Glaucoma									

(Continued on following page)

TABLE 4 (*Continued*) Moderate and strong signals of anti-VEGF drugs in ocular adverse events.

SMQs/PTs	Ranibizumab			Aflibercept			Brolucizumab		
	N	ROR (95%CI)	IC (IC-2SD)	N	ROR (95%CI)	IC (IC-2SD)	N	ROR (95%CI)	IC (IC-2SD)
Ocular hypertension	102*	68.17 (55.42, 83.85)	5.25 (4.95)	62*	42.87 (33.10, 55.53)	4.62 (4.24)	7*	35.49 (16.85, 74.75)	2.74 (1.71)
Intraocular pressure increased	310	21.58 (19.24, 24.19)	4.27 (4.10)	402	31.09 (28.10, 34.41)	4.76 (4.61)	80	47.01 (37.58, 58.81)	4.87 (4.54)
Glaucoma	182*	9.85 (8.50, 11.42)	3.20 (2.98)	100*	5.83 (4.79, 7.11)	2.46 (2.17)	27*	12.32 (8.43, 18.01)	3.12 (2.57)
Lens disorders									
Cataract	430	9.41 (8.54, 10.36)	3.16 (3.01)	261	6.14 (5.43, 6.94)	2.56 (2.38)	62	11.47 (8.91, 14.76)	3.27 (2.90)
Posterior capsule opacification	19	90.31 (55.43, 147.14)	4.00 (3.31)	7*	32.61 (15.21, 69.90)	2.70 (1.65)	6*	217.69 (95.79, 494.71)	2.77 (1.64)
Posterior capsule rupture	8*	63.54 (30.46, 132.53)	2.98 (1.96)	/	/	/	/	/	/
Lenticular opacities	9*	23.34 (11.96, 45.54)	2.83 (1.90)	/	/	/	/	/	/
Toxic anterior segment syndrome	/	/	/	20*	24.17 (15.45, 37.83)	3.49 (2.85)	/	/	/
Corneal disorders									
Corneal abrasion	41*	33.83 (24.65, 46.42)	4.19 (3.73)	28*	24.67 (16.89, 36.03)	3.73 (3.18)	/	/	/
Corneal erosion	22*	37.93 (24.59, 58.51)	3.82 (3.20)	10*	18.06 (9.62, 33.91)	2.81 (1.92)	/	/	/
Corneal oedema	35*	14.46 (10.33, 20.24)	3.37 (2.88)	53	24.26 (18.42, 31.95)	4.04 (3.64)	9*	31.18 (16.17, 60.14)	2.95 (2.03)
Keratic precipitates	14*	44.49 (25.76, 76.84)	3.48 (2.70)	36*	144.85 (100.34, 209.09)	4.81 (4.29)	121*	10432.45 (7534.69, 14444.65)	6.87 (6.53)
Corneal opacity	/	/	/	14*	12.58 (7.41, 21.38)	2.81 (2.06)	9*	63.07 (32.62, 121.94)	3.13 (2.20)
Conjunctival disorders									
Conjunctival haemorrhage	70	16.73 (13.18, 21.23)	3.74 (3.39)	53	13.70 (10.43, 17.99)	3.44 (3.04)	/	/	/
Conjunctival hyperaemia	22	6.64 (4.36, 10.11)	2.40 (1.79)	19*	6.24 (3.97, 9.81)	2.29 (1.64)	21	54.52 (35.39, 84.00)	3.98 (3.36)
Conjunctivitis	63*	4.38 (3.42, 5.62)	2.05 (1.68)	/	/	/	/	/	/
Lacrimal disorders									
Lacrimation increased	183	8.04 (6.95, 9.31)	2.92 (2.71)	136	6.48 (5.47, 7.68)	2.62 (2.37)	70	26.57 (20.94, 33.73)	4.25 (3.90)
Dry eye	/	/	/	/	/	/	46*	12.48 (9.32, 16.71)	3.30 (2.88)

Abbreviations: PTs, Preferred Terms; SMQs, Standardised MedDRA Queries; N, Number of target adverse events of target drug.
/indicates that IC-2SD value of the adverse event is less than 1.5.
*indicates that this adverse reaction is not in the instructions.

occlusion, dry eye). We analyzed the different adverse reactions and they may be due to molecular weight, structure, mechanism of action and pharmacokinetics of the drugs (Avery et al., 2014; Ferro Desideri et al., 2021).

Macular ischaemia has always been a great concern in medical practice. However, ranibizumab drug instructions do not include such, which possess an even greater risk. This study shows a disproportionate association with macular ischemia of ranibizumab, from 2008 to 2021, and the gradual increase was shown in the IC time scan. VEGF has been known to carry the capacity to promote formation of collateral vessels, which is essential for recovery after ischaemic events (Clayton et al., 2008). In addition, the upregulation of VEGF expression in ischaemic retinal conditions can reduce neuroretinal cell apoptosis that may enhance neuroprotection (Kazuaki Nishijima, 2007). As these agents may downregulate normal physiological functions of VEGF, VEGF blockade-induced vasoconstriction in an already compromised macular capillary bed could further increase hypoxic damage with a potentially devastating effect on macular function and visual outcome. Ranibizumab, compared with aflibercept and brolucizumab, blocks all isoforms of VEGF, and has a Fab fragment that penetrates better through all the retinal layers, thus making the effects stronger (Ferrara et al., 2006). Clinicians have noted and closely monitored macular

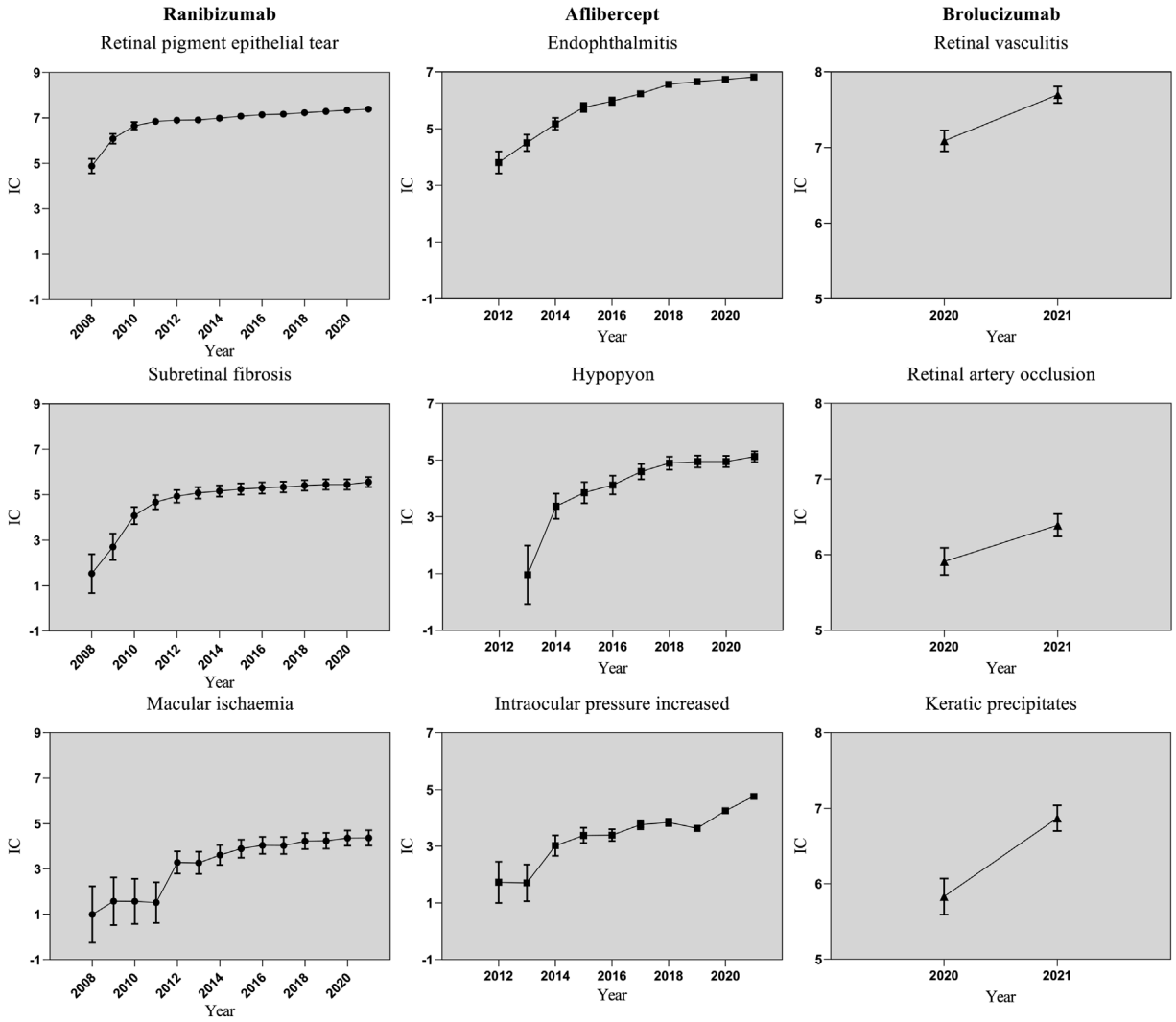

FIGURE 2
Information component and its 95% credibility interval over time for different types of anti-VEGF-associated ocular adverse events. Abbreviations: ●, Ranibizumab; ■, Aflibercept; ▲, Brolucizumab; IC, information component; CI, credibility interval. The error bars show the 95% credibility interval (CI) of the information component (IC), when the IC curve is steady upward trend and the 95% CI narrowed, the signal is stable and strong association.

ischemia after initial and subsequent intravitreal ranibizumab treatment and have recommended the addition of dexamethasone therapy if the condition worsens (Verma and Khetan, 2018). If a patient has symptoms related to macular ischemia at baseline, treatment with ranibizumab should be selected with caution.

Post-injection endophthalmitis is a rare but devastating complication after intravitreal injection of anti-VEGF drugs, and can cause significant vision loss (Mccannel, 2011; Fileta et al., 2014). The most common presenting symptom of endophthalmitis is reduced visual acuity, followed by pain/photophobia, redness, floaters, lid swelling and discharge, and the most common signs are vitritis, hypopyon, hyperemia, corneal edema and increase in intraocular pressure (Lyall et al., 2012; Haddock et al., 2014). The main factors, which play a role in intraocular endophthalmitis after anti-VEGF injection are patient-specific, medication-specific and delivery-specific (Anderson et al., 2021). It has been presented that some patients have anti-idiotype antibodies against anti-VEGF antibody (Sanjeewa et al., 2008). This anti-drug antibody (ADA) titers are associated with inflammation, which may cause endophthalmitis (Baumal et al., 2020). Noninfectious contamination (e.g., endotoxins) and administration formulation during drug manufacturing can also lead to endophthalmitis (Heier et al., 2006; Gasparin et al., 2012; Goldberg et al., 2013; Anderson et al., 2021). The anti-VEGF

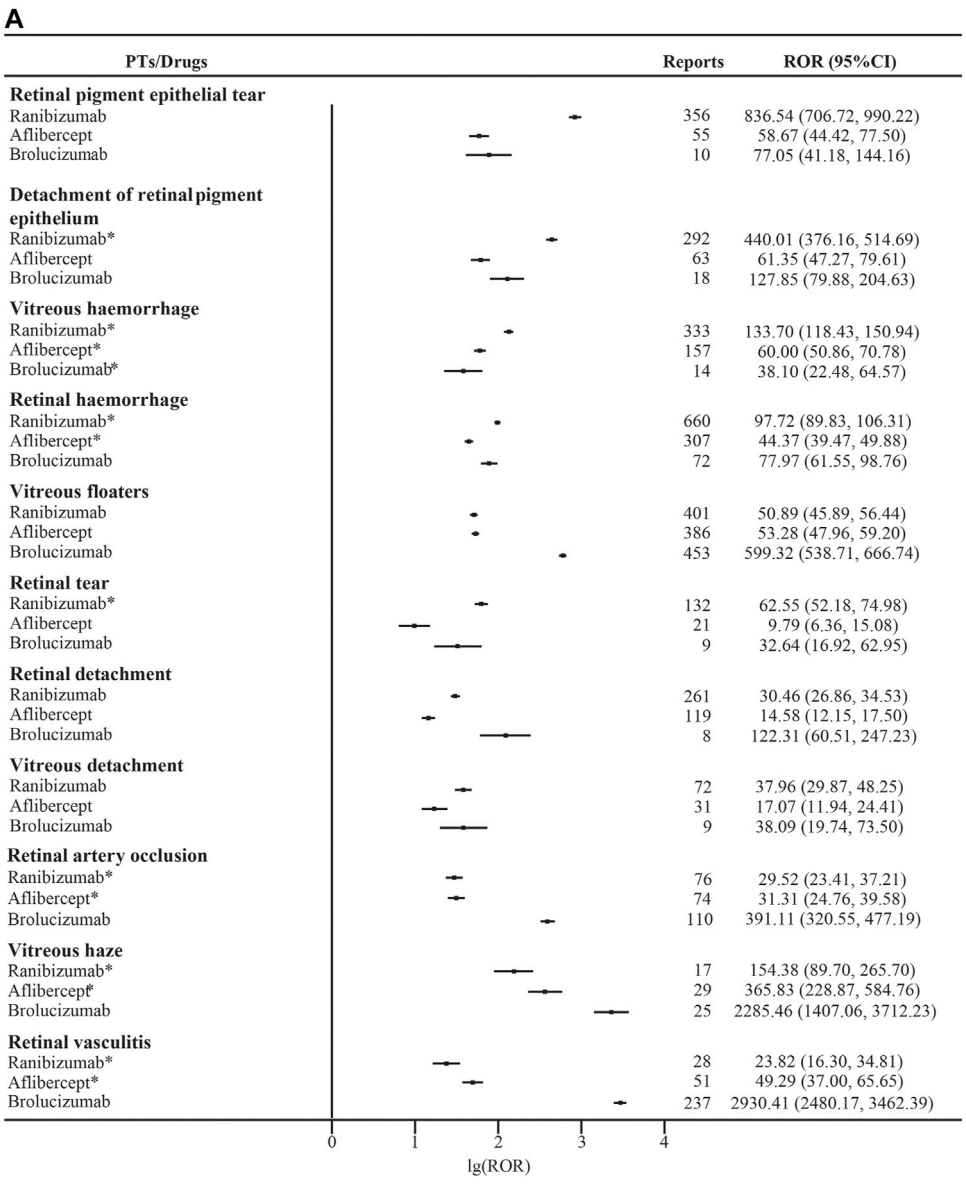

FIGURE 3
(Continued).

antibody itself may have immunogenic properties, such as the Fc portion interacting with intraretinal Fc receptors, triggering an inflammatory reaction that may cause endophthalmitis (Murinello et al., 2014; Anderson et al., 2021). In addition, protein aggregation or change in conformation may also cause endophthalmitis (Melo et al., 2019; Anderson et al., 2021; Melo et al., 2021), due to the delivery-specific constraints.

A large retrospective research report shows that the incidence of endophthalmitis after aflibercept injection is higher than that of ranibizumab (Souied et al., 2016). We identified significant disproportionality of endophthalmitis and its related signs, such as vitritis, anterior chamber empyema, corneal edema, congestion and floaters in three anti-VEGF drugs, which is consistent with literature reports (Haddock et al., 2014). Physicians must be familiar with the clinical manifestations of endophthalmitis after administration in order to make a prompt diagnosis. It is worth noting that this study has unearthed the safety signal of toxic anterior segment syndrome (TASS) of aflibercept as well. The clinical features of TASS are similar to those of endophthalmitis, except that the time and severity of occurrence are inconsistent. The anterior segment inflammation is severe and usually resulting in hypopyon formation (Sengillo et al., 2020), which should raise clinical concern.

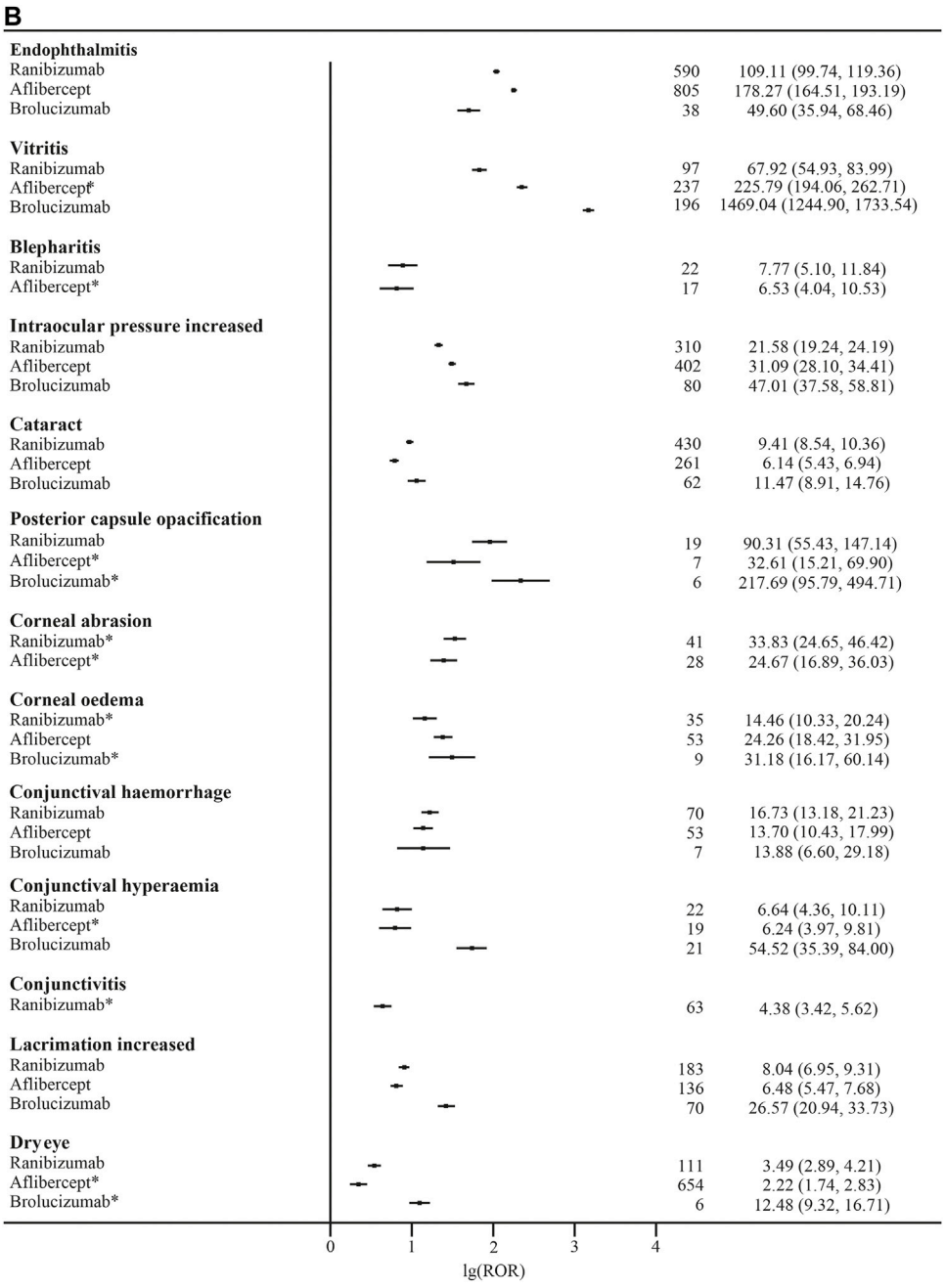

FIGURE 3
(Continued) Reporting Odds Ratios (RORs) for ocular adverse events associated with anti-VEGF. Abbreviations: **(A)**: Retina-related adverse events; **(B)**: Adverse events unrelated to retinas; 95% CI, 95% confidence interval. * indicates that this adverse reaction is not in the instructions.

Intravitreal anti-VEGF therapy may have adverse effects on ocular blood flow. Several cases of retinal vasculitis and/or retinal vascular occlusion were reported following the FDA approval of brolucizumab (Baumal et al., 2020; Haug et al., 2020). In fact, our study identified that all three drugs have the same safety signals yet we affirmed that brolucizumab carries the strongest one, which may be due to its small molecular structure and high affinity (Holz et al., 2016) that induce stronger effect on hypersensitivity, endothelial cells and nitric oxide production. Further knowledge on the retinal vasculitis and/or retinal vascular occlusion associated with brolucizumab may help guide clinicians in their clinical decision making moving forward.

Several recent publications have reported RPE tear associated with the use of intravitreal VEGF antagonists, such as ranibizumab

(Smith et al., 2009; Konstantinidis et al., 2010). Although these reports have raised the question of whether anti-VEGF therapy contributes to the development of RPE tear, the data to date have been anecdotal in nature, making it difficult to assess whether the incidence of RPE tear actually increased in patients receiving intravitreal anti-VEGF therapy. A retrospective analysis of clinical trials of ranibizumab found an overall incidence of RPE tear of 2.4%, which occurs after intravitreal therapy (Cho et al., 2015; Shin et al., 2015). However, a study on an incidence of RPE tear after intravitreal ranibizumab injection for neovascular AMD made no significant difference with the control treatment. This suggests a potential benefit to continuous ranibizumab therapy in patients with neovascular AMD that developed to RPE tear (Cunningham et al., 2011). Currently, there are several mechanisms to explain the development of RPE tear following anti-VEGF injection. One of the most plausible theories is that the anti-VEGF treatment may cause fibrosis contraction of the vascularized tissue underneath the RPE, ripping the overlying RPE (Spaide, 2009) and thus, change of retina during treatment should be closely monitored. In addition, we should take caution in explaining the significant signal, as one of the complications of advanced neovascular AMD.

Glaucoma is currently the leading cause of irreversible blindness worldwide (Quigley, 2006; Miraftabi et al., 2020) due to elevated intraocular pressure (Blumberg et al., 2015). Clinical ophthalmologists are also concerned about the increase of intraocular pressure after the administration of anti-VEGF drugs. A retrospective study estimated the risk of glaucoma or sustained ocular hypertension related to anti-VEGF treatment for neovascular AMD, and found that the rate of injection and lens status are associated with intraocular pressure (Wingard et al., 2019). As the zonular system attached to the lens is fragile, the presumption is that the anterior chamber volume compresses with anterior movement of the lens and iris and thus, may strain the outflow apparatus, and cause increase in intraocular pressure (Kerimoglu et al., 2015). Therefore, eye monitoring should be closely observed for at least 30 min after administration of anti-VEGF drugs.

Dry eye syndrome is defined as chronic inflammatory condition on the ocular surface. Typical symptoms include burning and itchiness, gritty sensation, tearing, redness of the conjunctiva, foreign body sensation, and blurred vision. These have been associated with several clinical markers including tear hyperosmolarity, elevated inflammatory markers, and abnormal tear production (Calonge et al., 2010). Dry eye syndrome is a common complaint among patients undergoing prolonged treatment with anti-VEGF drugs due to repeated exposure to preservatives contained in antibiotic eye drops that causes eye discomfort (Ayaki et al., 2012). As hyperosmolarity is a key event in the pathology of dry eye, it should be used as a marker for testing, diagnosis, and follow-up for chronic ocular treatments to identify the presence of dry eye syndrome (Versura et al., 2010). To prevent any progression, one should focus on measuring and treating the symptoms of tear hyperosmolarity, as initial treatment.

Based on the four-grid table of ratio imbalance, the information about drugs and its AEs are comprehensively considered and the relationship between them is objectively reflected. This provides strong support for the monitoring of adverse drug reactions and rational clinical use of drugs. But spontaneous reporting system has its own limitations. Omission or misstatement could exist and repeated reporting bias. Besides, the number of AE reports is influenced by the time of drug launch, country and region, and the severity of AE. And although brolucizumab in this study has fewer safety signals than the other two drugs, it cannot be inferred that brolucizumab is safer to use. In addition, some AEs may be caused by intraocular injection. Although some have high signal values, we have not analyzed them because no evidence has been found to date, and further research may be needed. Therefore, causality cannot be confirmed based on the FAERS data alone. Moreover, organization of AE reports, rectification of disproportionality analysis at the SMQ level, and application of stricter signal threshold (IC-2SD $\geq$ 1.5) were performed to address the limitations of FAERS (Huang et al., 2020). As a conclusion, this study only suggests the possible AEs and intensity of anti-VEGF drugs, and further clinical studies are needed for higher-level evidence.

Conclusion

In conclusion, our results suggest that ocular AEs associated with anti-VEGF drugs varies, and clinicians should consider specific risk factors based on patients' condition. Our study design does not allow any causality proof, and even though appropriate clinically performed assessment is necessary to validate our claims, it is a step toward understanding the safety profile of anti-VEGF drugs for optimal use.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

Author contributions

PM: Conception and design, analysis and interpretation of the data, drafting of the paper. XP: Drafting of the paper and critical revision for intellectual content. RL: Analysis and interpretation of the data. YQ: Analysis and interpretation of the data. LX: Analysis and interpretation of the data. JX: Analysis and interpretation of the data. LC: Analysis and interpretation of the data. YC: Substantial contributions to the conception or

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Drug-induced liver injury in COVID-19 treatment: Incidence, mechanisms and clinical management

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The COVID-19 outbreak triggered a serious and potentially lethal pandemic, resulting in massive health and economic losses worldwide. The most common clinical manifestations of COVID-19 patients are pneumonia and acute respiratory distress syndrome, with a variety of complications. Multiple organ failure and damage, ultimately leading to patient death, are possible as a result of medication combinations, and this is exemplified by DILI. We hope to summarize DILI caused by the antiviral drugs favipiravir, remdesivir, lopinavir/ritonavir, and hydroxychloroquine in COVID-19 patients in this review. The incidence of liver injury in the treatment of COVID-19 patients was searched on PubMed to investigate DILI cases. The cumulative prevalence of acute liver injury was 23.7% (16.1%–33.1%). We discuss the frequency of these events, potential mechanisms, and new insights into surveillance strategies. Furthermore, we also describe medication recommendations aimed at preserving DILI caused by treatment in COVID-19 patients.

KEYWORDS

COVID-19, drug-induced liver injury, incidence, mechanisms, clinical management

1 Introduction

By July 2022, the outbreak of Corona Virus Disease 2019 (COVID-19) has caused nearly 580 million confirmed diagnoses and over 6.4 million deaths. The most common early clinical symptoms of COVID-19 infection are fever, cough, myalgia, and fatigue. Approximately 15% of patients progress to an advanced stage of respiratory distress and

eventually develop acute respiratory distress syndrome or multi-organ failure (Dong et al., 2020). Due of the scarcity of specific drugs at the onset of the COVID-19 epidemic, repurposed drugs were commonly utilized and mainly divided into antiviral and adjuvant drugs. Antiviral drugs included mainly remdesivir, hydroxychloroquine, lopinavir-ritonavir, azithromycin, oseltamivir, umifenovir, favipiravir, chloroquine, ribavirin, *etc.*; adjunctive drugs included antithrombotics, corticosteroids, antibiotics, metformin, vitamin supplements (C and D), antihypertensives, H2 receptor antagonists, and interleukin inhibitors. There are significant regional and temporal differences in the use of these medications. For example, the use of hydroxychloroquine is 85% in Spain, but less than 2% in China. Lopinavir-ritonavir was only used at the start of the pandemic in Korea and Spain, with a decreasing trend over time. Remdesivir shows a small upward trend from June 2020 (Prats-Uribe et al., 2021). According to clinical guidelines from various countries, antiviral drugs (such as favipiravir, remdesivir, lopinavir/ritonavir and hydroxychloroquine) are the most commonly used for COVID-19 treatment. Patients with comorbidities must also be treated for the underlying disease, and sedatives, anti-inflammatory, antipyretic, and analgesic drugs are used in critical patients. Multiple organ failure and damage, ultimately leading to patient death, are possible as a result of medication combinations, and this is exemplified by drug-induced liver injury (DILI) (Aithal et al., 2011).

Drug-induced hepatocellular injury is identified internationally by alanine aminotransferase (ALT) levels equal to or exceeding 5× the upper limit of normal (ULN) appearing within 3 months of drug initiation after alternative causes are excluded (Lee and Senior, 2005). When the suspect drug is removed, ALT usually drops by 50% or more. With drug re-administration, a positive rechallenge has recently been defined by an ALT level of 3–5× ULN or greater (Lammert et al., 2010). DILI is further affirmed by excluding other causes (e.g., viral hepatitis, biliary obstruction, alcoholic hepatitis, or hypotension), reports of suspect drug hepatotoxicity, and liver injury recurrence upon rechallenge (or re-administration) of the suspect drug, which has traditionally been strongly discouraged (Benichou et al., 1993; Danan and Benichou, 1993). As it is difficult to attribute the liver injury to drugs based on clinical indicators alone, a causality assessment scale is required for a definite diagnosis of DILI. The Council for International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment Method (CIOMS/RUCAM) was the first attempt to standardize the concept of liver injury and is presently the most reliable and commonly used scale. It uses fractions to depict the probability of DILI: definite or highly probable (score>8), probable (score 6–8), possible (score 3–5), unlikely (score 1–2), excluded (score = 0). According to the biochemical mode of injury, drug-induced liver injury is characterized as hepatocellular, cholestatic, or mixed, and as intrinsic DILI and idiosyncratic DILI according to the mechanism of injury. The most frequent cause of intrinsic DILI is acetaminophen toxicity (Fisher et al., 2015). Diverse hypotheses exist about the underlying processes of idiosyncratic DILI, such as drug metabolism, inflammation, mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum stress (Yuan & Kaplowitz, 2013). The treatment of DILI mainly relies on early diagnosis and withdrawal of suspected drugs. Corticosteroids and ursodeoxycholic acid may be used as a supplementary therapy. Plasma replacement and liver transplantation may be the sole remaining choices at the stage of liver failure.

It is undeniable that COVID-19 can also induce liver injury. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may directly bind to angiotensin-2 converting enzyme (ACE2) positive cholangiocytes (Hoffmann et al., 2020). Moreover, activation of the immune system and cytokine storm may contribute to an immune-mediated process of hepatic injury in COVID-19 (Alqahtani & Schattenberg, 2020). Endotheliopathy, altered platelet function, inflammation, and their synergistic effects may lead to liver injury in patients (McConnell et al., 2021). However, this liver injury is not drug-induced and is therefore outside the subject of this article.

Due to a large number of asymptomatic cases and the small number of cases in which rigorous clinical testing for DILI is performed during COVID-19 treatment, it is difficult to determine the absolute incidence of DILI. Therefore, the above technical definition of DILI does not apply to this review. In this article, we take into account data on abnormal liver function tests linked to drugs. We retrieved previously published articles from PubMed in order to analyze the incidence of drug-induced abnormal liver function in patients with COVID-19, in conjunction with drug pharmacokinetics to speculate on possible mechanisms and to provide reasonable clinical management recommendations.

2 High incidence of abnormal liver function during the antiviral treatment of COVID-19 patients

There is a high incidence of liver injury in patients with COVID-19. According to a systematic evaluation performed in September 2020, the cumulative prevalence of acute liver injury among COVID-19 patients was 23.7% (16.1%–33.1%) (Kumar et al., 2020). In another systematic review and meta-analysis, the frequency of liver injury in COVID-19 patients was 19% (1%–53%) (Mao et al., 2020). Several antiviral drugs in the COVID-19 regimen are repurposed drugs that have previously been reported to cause DILI. Lopinavir/ritonavir is primarily used as an HIV therapy medicine in the clinic. The incidence of hepatotoxicity after antiretroviral therapy (ART) in hepatitis B and C patients with HIV was found to be 40.1/100 py in the lopinavir/ritonavir group (Su et al., 2018). In addition, hydroxychloroquine has been

TABLE 1 Incidence of abnormal liver function and medication regimen during the antiviral treatment of COVID-19 patients.

Antiviral drugs	References	Dose	Duration	Frequency	Incidence	Research method	Location
Favipiravir	Tabarsi et al.	1600 mg	1d	Twice daily	9% of abnormal liver function	RCT	Iranian
		600 mg	5d	Twice daily			
	Chen et al.	1600 mg	1d	Twice daily	8.62% of abnormal liver function	RCT	China
		600 mg	6d	Twice daily			
	Udwadia et al.	1800mg	1d	Twice daily	6.8% of abnormal liver function	RCT	India
		800mg	13d	Twice daily			
	Lou et al.	1600mg/ 2200mg	first dose	Not mentioned	11% of elevated AST 44% of elevated ALT 11% of elevated total bilirubin	RCT	China
		600 mg	≤14d	Three times daily			
	Ergür et al.	1600 mg	5-10d	Twice daily	7.28% of elevated transaminases	Retrospective	Turkey
		600 mg		Twice daily			
Remdesivir	Grein et al.	200mg	1d	Not mentioned	23% of increased liver enzymes	Retrospective	USA, Japan, Italy, France
		100mg	9d	Not mentioned			
	Antinori et al.	200mg	1d	Not mentioned	42.8% of elevated transaminases	Prospective	Italy
		100mg	9d	Not mentioned			
	Ader et al.	200mg	1d	Once daily	3% of elevated transaminases	RCT	48 sites in Europe
		100mg	9d	Once daily			
	Goldman et al.	200mg	1d	Once daily	6.5% of elevated ALT	RCT	USA, Italy, Spain, Germany, Hong Kong, Singapore, Korea, Taiwan
		100mg	9d/4d	Once daily	5.8% of elevated AST		
	Beigel et al.	200mg	1d	Not mentioned	3.4% of elevated AST	RCT	USA, Denmark, UK, Greece, Germany, Korea, Mexico, Spain, Japan, Singapore
		100mg	9d	Not mentioned	1.7% of elevated total bilirubin		
	Wang et al.	200mg	1d	Once daily	5% of elevated AST	RCT	China
		100mg	9d	Once daily	10% of elevated total bilirubin		
	Kanai et al.	100mg	1d	Not mentioned	46.2% of abnormal liver function	Retrospective	Japan
		200mg	9d	Not mentioned			
	van Laar et al.	Not mentioned	Not mentioned	Not mentioned	43% of elevated ALT 45% of elevated AST	Retrospective	The Netherlands
	Leegwater et al.	Not mentioned	5d	Not mentioned	elevated ALT (1305 U/L) elevated AST (1461 U/L)	Case	The Netherlands
	McCoy et al.	200mg	1d	Not mentioned	elevated liver enzymes	Case	USA
		100mg	9d	Not mentioned			
	Kaur et al.	5mg/kg/d	1 dose	Not mentioned	elevated ALT (832 U/L) elevated AST (1121 U/L)	Case	India

(Continued on following page)

TABLE 1 (*Continued*) Incidence of abnormal liver function and medication regimen during the antiviral treatment of COVID-19 patients.

Antiviral drugs	References	Dose	Duration	Frequency	Incidence	Research method	Location
Lopinavir/Ritonavir	Fan et al.	Not mentioned	Not mentioned	Not mentioned	63.4% of abnormal liver function	Retrospective	China
	Zhu et al.	400mg/ 100mg	7d	Twice daily	8.8% of elevated ALT(<125U/L)	Retrospective	China
	Cao et al.	400mg/ 100mg	14d	Twice daily	2.1% of elevated AST;1.1% of elevated ALT 3.2% of elevated total bilirubin	RCT	China
Hydroxychloroquine	Cavalcanti et al.	400mg	7d	Twice daily	8.5% of elevated ALT/AST 2.5% of elevated total bilirubin	RCT	Brazil
	Ader et al.	400mg	1d	Twice daily	4% of elevated transaminases	RCT	France
		400mg	9d	Once daily			
	Satlin et al.	600mg	1d	Twice daily	10.7% of elevated AST;8.1% of elevated ALT ;1.6% of elevated ALP	Retrospective	USA
		400mg	4d	Once daily	3.3% of elevated total bilirubin all of which were grade 3 or 4 adverse effects		
	Falcão et al.	400mg	1d	Twice daily	AST from 46 to 469 U/L ALT from 33 to 357 U/L	Case	Brazil
	Hillaker et al.	400mg	1d	Twice daily	elevated ALT, AST	Case	USA
		200mg	4d	Twice daily			

reported to cause severe DILI in the treatment of porphyrias (Sunkara et al., 2018). Therefore, we summarized the incidence of abnormal liver function during the antiviral treatment of COVID-19 patients in Table 1.

2.1 Favipiravir

Favipiravir is a purine analogue and RNA-dependent polymerase inhibitor that has been shown to be effective against a variety of RNA viruses, including the Ebola virus. In two prospective, randomized controlled clinical studies that included patients with moderate to severe COVID-19 but excluded those with severe liver disease, similar incidences of abnormal liver function were found to be 9% and 8.62% in the favipiravir group, respectively (Chen et al., 2021; Tabarsi et al., 2021). Another clinical trial that included only patients with mild to moderate (including asymptomatic) COVID-19 obtained an incidence of 6.8% (Udwadia et al., 2021). Interestingly, in a small exploratory randomized controlled trial comparing baloxavir and favipiravir, one case (11%) of elevated aspartate aminotransferase (AST), four cases (44%) of elevated ALT, and one case (11%) of elevated total bilirubin were found in the favipiravir group, a significantly higher proportion compared to other trials. We believe this is related to the small number of patients included in the favipiravir group (9 cases), as patients with abnormal baseline liver function parameters were not excluded (Lou et al., 2021). An additional retrospective study included 357 favipiravir-treated patients, with 26 (7.28%) having elevated transaminases. The participants were divided into groups based on the existence or absence of side effects, and it was discovered that there was a positive correlation between elevated body mass index (BMI), baseline transaminases, and ferritin levels with the occurrence of side effects (Ergür et al., 2022).

2.2 Remdesivir

Remdesivir is a nucleotide monophosphate analogue prodrug that inhibits viral RNA-dependent RNA polymerase. It has antiviral activity against a broad spectrum of human coronaviruses, including SARS-CoV-2, in cell cultures and mouse models. The Food and Drug Administration (FDA) and European Medicines Agency (EMA) recommend remdesivir for the treatment of COVID-19 infection based on data from three randomized controlled trials (Eastman et al., 2020).

Remdesivir was discovered to be hepatotoxic during clinical trials. An evaluation of the adverse drug reactions associated with remdesivir in the VigiBase database revealed that elevated liver enzymes accounted for 32.1% of the cases (Charan et al., 2021). High incidences of increased liver enzymes were also found in several clinical trials including mechanically ventilated patients,

with rates of 23% and 42.8%, respectively (Antinori et al., 2020; Grein et al., 2020). However, a similar trial with a rate of 3% was also conducted (Ader et al., 2022), and it has been argued that the large difference in rates is due to the fact that the study explicitly excluded people who were taking other antivirals at the same time. In comparison, the incidence of liver enzyme elevations appeared to be lower in patients who did not receive mechanical ventilation, with 26 (6.5%) ALT and 23 (5.8%) AST elevations, respectively, as shown in another clinical trial (Goldman et al., 2020). Similar results were seen in two trials comparing the effects of remdesivir to placebo control, with ASTs raised by 5% and 3.4%, but with total bilirubin raised by 10% and 1.7%, respectively (Wang Q. et al., 2020; Beigel et al., 2020). According to one study, 46.2% of COVID-19 patients aged 80 and older had liver dysfunction, which was significantly higher than the frequency for people under the age of 80 (Kanai et al., 2021). In a retrospective study, 43% and 45% of patients with normal baseline ALT and AST showed elevations after remdesivir treatment, respectively (van Laar et al., 2021). As the study failed to control for variables, there is no assurance that remdesivir was the sole independent factor causing hepatotoxicity in these patients.

Several case reports describe the correlation between remdesivir and liver injury in more detail. A 64-year-old male patient presented with a sharp increase in ALT (1305 U/L) and AST (1461 U/L) after 5 days of remdesivir. After discontinuing the drug immediately, ALT and AST levels decreased rapidly and eventually returned to normal levels. After analyzing the time points of changes in ALT/AST levels, the author ruled out liver injury caused by COVID-19 and amiodarone. He concluded that the patient's elevated liver enzyme levels were most likely caused by remdesivir (Leegwater et al., 2021). Five pregnant women infected with COVID-19 were treated with remdesivir, and four of them developed elevated liver enzymes, prompting one of them to discontinue the medication (McCoy et al., 2020). Another case report describes a newborn with COVID who had a significant increase in ALT/AST (ALT 832 U/L; AST 1121 U/L) after receiving the first dose of remdesivir, which was then discontinued. After 10 days, liver enzyme levels returned to normal. The timing correlation between changes in liver enzyme levels and the use of remdesivir in this case suggested that drug-induced liver injury was more likely (Kaur et al., 2022).

2.3 Lopinavir/ritonavir

Lopinavir/ritonavir is a fixed-dose combination antiretroviral drug that is widely used for HIV/AIDS prevention and treatment. At the beginning of the pandemic, it emerged as a potential candidate for the treatment of COVID-19 (Osborne et al., 2020). Using data from the FDA Adverse Event Reporting System, the incidence of DILI in COVID-19 patients treated with lopinavir/ritonavir was analyzed. The results showed that 313 (37%) of 845 adverse reactions were DILI (Tang et al., 2021).

According to a retrospective study, 63.4% of 41 COVID-19 patients treated with lopinavir/ritonavir had abnormal liver function tests. The study divided 93 patients with normal baseline liver function into two groups based on the presence or absence of abnormalities after therapy. According to the findings, 57.8% of the abnormal group received lopinavir/ritonavir, which was significantly higher than the normal group ($p < 0.01$) (Fan et al., 2020). Furthermore, lopinavir/ritonavir was associated with an increased risk of liver injury, defined as ALT and or AST >3×ULN, ALP, GGT and TBIL (alkaline phosphatase,γ-glutamyltransferase and total bilirubin) > 2×ULN (OR from 4.44 to 5.03, $p < 0.01$) (Cai et al., 2020). The incidence in another study was 8.8%, which is significantly lower than the incidence in the preceding studies. The effect of their included population's younger age (median age = 40 years) and smaller sample size cannot be ruled out (Zhu Z. et al., 2020). To reduce confounding factors, a prospective study included patients with severe COVID-19 and excluded those with severe liver disease and AIDS, which may explain their low percentage of elevated AST, ALT, and total bilirubin (1.1%, 2.1%, and 3.2%, respectively) (Cao et al., 2020).

2.4 Hydroxychloroquine

Hydroxychloroquine is a 4-aminoquinoline compound, a derivative of quinine, previously used for the prevention and treatment of malaria and some rheumatic diseases such as systemic lupus erythematosus (Colson et al., 2020). During *in vitro* testing, hydroxychloroquine effectively inhibited SARS-Cov-2 infection, possibly *via* two main mechanisms (Liu et al., 2020), one of which was the inhibition of SARS-Cov-2 entry into human cells and prevention of its replication, and the other of which was the prevention of fulminant COVID-19, including cytokine release syndrome (Al-Bari, 2017; Zhou D. et al., 2020; Zhou P. et al., 2020). In clinical studies with 665 participants, 199 were given hydroxychloroquine alone; 17 (8.5%) had elevated ALT/AST levels, while 5 (2.5%) had elevated bilirubin levels (Cavalcanti et al., 2020). Two other prospective trials yielded similar incidences of liver enzyme abnormalities (4% and 6.7%, respectively) (Chen J. et al., 2020; Ader et al., 2021). The results of a retrospective cohort study showed that among COVID-19 patients who had received one dose of hydroxychloroquine, 13 (10.7%) had elevated AST, 10 (8.1%) had elevated ALT, 2 (1.6%) had elevated ALP, and 4 (3.3%) had elevated total bilirubin, all of which were grade 3 or four adverse effects (Satlin et al., 2020).

Two case reports also describe liver dysfunction caused by hydroxychloroquine. The first case was a 29-year-old female patient who had just delivered at full term and had an approximately 10-fold increase in transaminase levels after two doses of hydroxychloroquine (AST from 46 to 469 U/L; ALT from 33 to 357 U/L), after which the drug was subsequently discontinued. Transaminase levels returned to near-normal values after 5 days (Falcão et al., 2020). In another case of a 40-year-old male, transaminases were elevated after 5 days of hydroxychloroquine; then stop taking the medication. Unfortunately, the report did not go into detail about the elevated transaminase values (Hillaker et al., 2020).

3 Possible mechanisms of liver injury caused by antiviral agents

The entire process of drug uptake into the liver, metabolism, and final excretion is controlled by the large families of proteins (Yuan & Kaplowitz, 2013). Drugs are passively taken up into hepatocytes or by a series of transporters located in the basolateral plasma membrane, including members of the solute carriers (SLCs), organic anion-transporting polypeptides (OATPs), organic anion-transporter (OAT) family, and organic cation transporters (OCTs) (Burckhardt & Burckhardt, 2011; Hagenbuch & Stieger, 2013). Drugs are metabolized by phase I and phase II enzymatic reactions after ingestion. Phase I metabolism is primarily concerned with the oxidation and reduction of drugs by cytochrome P450 (CYP450) to generate reactive metabolites, whereas phase II metabolism is concerned with the binding of drugs or phase I metabolites to endogenous molecules (Yuan & Kaplowitz, 2013). The ATP Binding Cassette (ABC) transporters then mediate the efflux of the drug and its metabolites from the hepatocytes into the bile or back into the blood sinusoids for subsequent renal excretion (Andrade et al., 2019). Hepatocyte exposure to increased cellular stress is assumed to be the initial step in DILI development. Initial cell damage is induced by drugs and/or their reactive metabolites *via* covalent binding or direct damage to mitochondria, which leads to oxidative stress and the activation of stress-sensing signaling pathways, impairment of the mitochondrial function, and endoplasmic reticulum (ER) stress. The production of reactive metabolites during drug metabolism results in a major rise in mitochondrial oxidative stress, with the aggravation of reactive oxygen species (ROS) further damaging cells and tissues. The production of reactive oxygen species (ROS) from injured hepatocytes increases overall oxidative stress, and the release of damage-associated molecular patterns (DAMP) activates innate immune responses, resulting in the activation of apoptotic and necrotic pathways (Garcia-Cortes et al., 2020; Villanueva-Paz et al., 2021).

According to the drug metabolism process described above, antiviral drug entry into the liver inhibits the activity of CYP enzymes and transporter proteins, which may affect the redox and excretion of drugs metabolized *via* these two pathways (Weemhoff et al., 2003; Ambrus et al., 2021). This may lead to a homeostasis imbalance, which in turn causes DILI (Figure 1). Moreover, according to the National Institutes of Health Guidelines 2021 for the Treatment of Novel Coronavirus Pneumonia, antiviral agents are frequently used to treat COVID-19. The cumulative prevalence of acute liver injury among COVID-19 patients was 23.7% (16.1%–33.1%) (Kumar et al., 2020). Therefore, we hypothesize that antiviral medications may cause DILI in COVID-19 patients by inhibiting CYP enzymes and liver transport proteins.

3.1 Inhibition of CYP by antiviral agents

CYP450 is a class of hemoglobin-coupled monooxygenases found primarily in the endoplasmic reticulum of the liver and other tissues, and it functions with the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen. P450 enzymes are the primary enzyme system responsible for drug metabolism and are primarily involved in oxidative reactions during drug biotransformation (Takahashi et al., 2020). Multiple CYP450 isoforms are present in liver microsomes, with CYP3A4 accounting for 30% of total CYP in the liver. Inhibiting CYP3A4 affects drug metabolism in the liver, causing liver injury. Previous research has shown that remdesivir and lopinavir/ritonavir are CYP3A4 inhibitors, while remdesivir also inhibits CYP1A2, CYP2C9, CYP2C19, and CYP2D6; lopinavir/ritonavir and hydroxychloroquine both inhibit CYP2D6 (Kim et al., 2003; Yang, 2020). Furthermore, favipiravir has also been reported as an inhibitor of CYP2C8 (Deb and Arrighi, 2021).

Despite the fact that many studies have found that the aforementioned drugs inhibit CYP, the strength of their inhibitory effect on CYP remains inconclusive. Lopinavir's inhibition of six CYP enzymes was systematically assessed using a human liver microsomal model, and lopinavir was found to inhibit all five CYP enzymes weakly except CYP3A4, which was moderately inhibited (Weemhoff et al., 2003). Lopinavir/ritonavir is also a weak inhibitor of CYP2C9 (Lim et al., 2004) (Table 2).

3.2 Inhibition of transporters by antiviral agents

Hepatic transporters play an important role in the clearance of endogenous and exogenous substances, and the main common transporter proteins are the following:

The ABCC2/MRP2 transporter (ATP Binding Cassette Subfamily C member 2/Multidrug Resistance-Associated Protein 2) mediates the entry of endogenous and exogenous substances into bile. It is a multi-specific transporter of

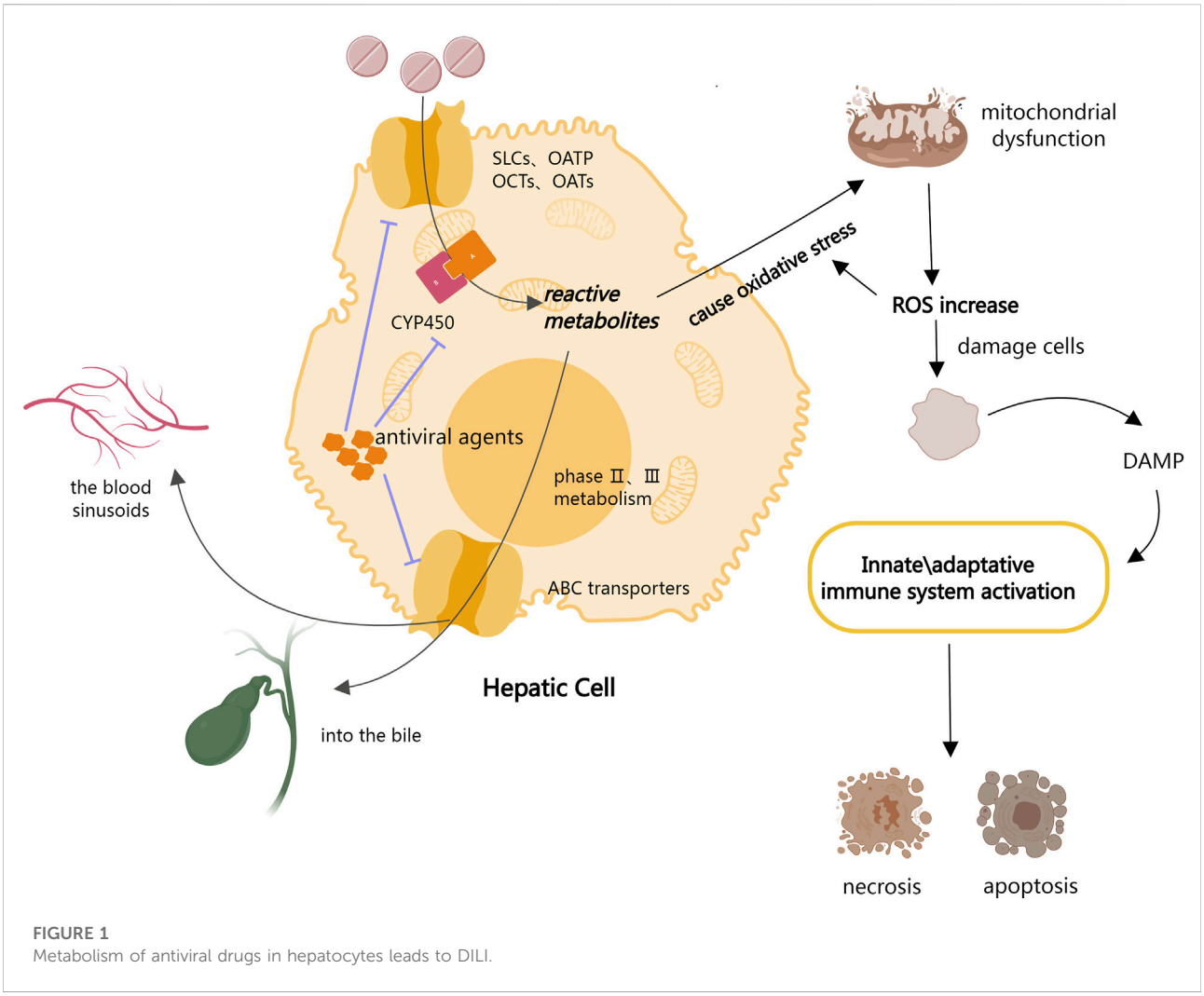

FIGURE 1
Metabolism of antiviral drugs in hepatocytes leads to DILI.

TABLE 2 Relationship between antiviral agents and CYP or transporters.

Antiviral agents	Inhibitors of CYP	Inhibitors of transporters	Substrates of CYP	Substrates of transporters
Favipiravir	CYP2C8	-	-	-
Remdesivir	CYP3A4, CYP1A2, CYP2C9, CYP2C19, CYP2D6	OATP1B1, OATP1B3, OATP2B1, OCT1, ABCC4	CYP3A4, CYP2C8, CYP2D6	-
Lopinavir/ritonavir	CYP3A4, CYP2D6, CYP2C9	ABCC2, ABCB1, ABCB3, ABCB11, ABCG2, OATP1B1, OATP1B3	CYP3A4	OATP1A2, OATP1B1, OATP1B3, ABCB1, ABCC2
Hydroxychloroquine	CYP2D6	ABCB1	CYP3A4, CYP2D6	-

amphiphilic compounds and a key transporter for bilirubin conjugates. OATP1B1 and OATP1B3 are organic anion uptake transport proteins in the liver that are involved in the uptake and elimination of various drugs and toxic compounds from the bloodstream. OCT1 is a highly expressed organic cation uptake transporter in the liver. It allows nutrients to enter cells and can mediate drug uptake in patients. The levels of OCT1 expression correlate with the responses toward many drugs. The interaction between antiviral agents and hepatic transporters may result in hepatotoxicity and adverse drug effects (Giacomini et al., 2010).

The liver drug uptake transporters OATP1B1, OATP1B3, OATP2B1 and OCT1 were found to have low remdesivir uptake

ratios by cellular transfection, indicating that the transporters were not related to remdesivir hepatic uptake (Nies et al., 2021). However, remdesivir inhibits the transporters in a concentration-dependent manner and can function as an inhibitor. And remdesivir has been shown to inhibit at 10 M concentrations, which is close to the peak plasma concentration observed 30 min after receiving 200 mg remdesivir intravenously (Jorgensen et al., 2020). Another study also demonstrated that remdesivir inhibits OCT1 *in vitro*. According to this study, remdesivir inhibited MRP4 but not MRP2 or MPR3 (Ambrus et al., 2021).

Lopinavir was found to have the most significant inhibitory effect on ABCC2/MRP2 by observing bile accumulation of CDF (cumulative distribution function), a visualization fluorescent substrate, while ritonavir's effect was not statistically significant (Holmstock et al., 2018). Some studies support lopinavir's inhibitory effect on ABCC2/MRP2, while other studies suggest that ritonavir similarly inhibits ABCC2/MRP2 (Drewe et al., 1999; Huisman et al., 2002; Agarwal et al., 2007; Ye et al., 2010). Besides ABCC2/MRP2, lopinavir/ritonavir has been shown to inhibit other ABC transporter proteins, including ABCB1 (Perloff et al., 2001), ABCB3 (Ernest et al., 2005), ABCB11 (Pedersen et al., 2013), and ABCG2 (Drewe et al., 1999; Profit et al., 1999; Ernest et al., 2005; Gupta et al., 2004). Favipiravir had no significant effect on any of these transporters (Ambrus et al., 2021).

Hydroxychloroquine inhibited ABCB1/P-gp (P-glycoprotein) at concentrations exceeding 10 μM. Although hydroxychloroquine has a low interaction potential with transporters, it may increase the bioavailability of concurrently administered ABCB1/P-gp substrates (Weiss et al., 2020) (Table 2).

3.3 Interactions between different antiviral agents

Antiviral agents are metabolized as substrates of CYP and transporters in addition to acting as inhibitors of them. Remdesivir has been identified as a substrate for CYP3A4, CYP2C8, and CYP2D6 (Yang et al., 2020); hydroxychloroquine is metabolized by CYP3A4 and CYP2D6 (Deb and Arrighi, 2021); and lopinavir/ritonavir is also metabolized by CYP3A4 (Cao et al., 2020). Lopinavir was confirmed as a substrate for OATP1A2, OATP1B1, and OATP1B3 by examining the substrate specificity of antiretroviral drugs using oocyte cell lines and analyzing the association between SNP and lopinavir plasma concentrations using plasma samples (Hartkoorn et al., 2010). Furthermore, lopinavir is also a substrate for ABCB1 and ABCC2 (Woodahl et al., 2005) (Table 2). CYP and transporter proteins act as mediators of antiviral drug interactions in these cases. This could be one of the mechanisms of liver damage caused by the combination of antiviral drugs used to treat COVID-19 patients.

3.4 Interactions between antiviral agents and other COVID-19 clinical agents

3.4.1 CYP-mediated drug-drug interactions

In the clinical of COVID-19, in addition to antiviral agents, patients may receive medications for sedation during ventilation and medications for comorbidities (e.g., heart disease, diabetes, hypertension, hyperlipidemia, *etc.*). These drugs are metabolized by CYP and may interact with CYP inhibitors to produce DILI.

Propofol, a sedative commonly used in patients with mechanical ventilation, is metabolized by CYP2B6 (Meyer and Maurer, 2011). Fentanyl, benzodiazepine drugs, and midazolam are also commonly used sedatives, all of which are metabolized by CYP3A4 (Meyer and Maurer, 2011; Doi et al., 2020). CYP3A4 inhibitors, such as lopinavir/ritonavir, may affect their pharmacokinetics and toxicity when combined. Epidemiological studies have shown that people with hypertension, diabetes, and hyperlipidemia are more likely to be infected with COVID-19 (Aitken and Morgan, 2007; Croyle, 2009), which means they are more likely to develop drug-drug interactions (DDI) with antiviral agents in the clinical setting and should be taken more seriously. Most dihydropyridine calcium channel blockers (e.g., amlodipine and nifedipine) (Sutton et al., 1997; Katoh et al., 2000), all non-dihydropyridine calcium channel blockers (e.g., verapamil and diltiazem) (Tateishi et al., 1992), and propranolol (Zhang et al., 1998) are metabolized by CYP3A4. Irbesartan and losartan are metabolized by CYP2C9 (McCrea et al., 1999). Cholesterol-lowering drugs, such as statins (except pravastatin and rosuvastatin) are metabolized by CYP3A4 (Neuvonen and Jalava, 1996; Cook et al., 2002). Glimepiride, glipizide, and glyburide are all anti-diabetic drugs that are metabolized by CYP2C9 (Suzuki et al., 2006). Because remdesivir inhibits both CYP2C9 and CYP3A4, extra attention must be paid to the multiple effects of drug-drug interactions when administering remdesivir to the aforementioned patients. It is recommended that blood levels and related indicators of DILI be monitored on a regular basis, or that alternative drugs that are not metabolized by CYP, such as angiotensin-converting enzyme (ACE) inhibitors (Hoyer et al., 1993), thiazide diuretics (Ellison, 2019) be considered.

3.4.2 Liver transporters-mediated drug-drug interactions

In addition to drugs metabolized by CYP, some drugs are metabolized with the involvement of transporters. Transporter inhibitors can also affect such drugs, resulting in drug-drug interactions. Statins make up a large portion of the comorbid medications used in COVID-19 patients, and one of their side effects is hepatotoxicity. A previous study showed that breast cancer resistance protein (BCRP) and P-gp transported atorvastatin, fluvastatin, pitavastatin, and rosuvastatin; MRP2 transported fluvastatin; MRP3 transported atorvastatin,

fluvastatin, pitavastatin, and pravastatin; and MRP4 transported fluvastatin and rosuvastatin (Deng et al., 2021). Lopinavir/ritonavir, as a potent MRP2 inhibitor, can affect the excretion of some of these drugs and may exacerbate their adverse effects. Clopidogrel carboxylate (CPC), the inactive metabolite of clopidogrel, was identified as a substrate of OAT1 through *in vitro* experiments. Additionally, metformin is identified as a substrate of OCT1 (Li et al., 2014). Several macrolides, including clarithromycin, roxithromycin, telithromycin, azithromycin, and erythromycin, have been identified as ABCB1/P-gp substrates (Munić et al., 2010). In the clinical use of COVID-19 patients, other drugs may be metabolized by liver transporters. When these drugs are combined with antiviral agents that inhibit transporters, the possibility of liver injury cannot be ruled out, so caution is advised.

4 Clinical management

4.1 Indicators for monitoring

In COVID-19 hospitalized patients, the incidence of abnormal liver function tests ranges from 10.5 to 69% (Mao et al., 2020). Most studies show that abnormal liver function tests are primarily caused by AST and ALT elevations, with AST elevations being more common than ALT elevations (Cai et al., 2020). Elevations in GGT and total bilirubin are less common than AST and ALT elevations (Lala et al., 2022). According to previous research, the proportion of COVID-19 patients with elevated ALT was 9.6–37.6% (Guan et al., 2020; Bloom et al., 2021), elevated AST was 14.8–36% (Zhang Y. et al., 2020; Xie et al., 2020), abnormal GGT was 13.0–24.4% (Chen N. et al., 2020; Hao et al., 2020), and abnormal total bilirubin was 5.1–18% (Zhang C. et al., 2020; Zhu J. et al., 2020; Huang et al., 2020).

The majority of patients with abnormal liver function have mildly elevated AST/ALT (1–2 times the ULN), with only a minority (<4%) having levels greater than 2 times the ULN. GGT is significantly higher than other indicators and may exceed three times the ULN (Cai et al., 2020). By the time of discharge, the majority of mild COVID-19 patients had normalized their indicators, whereas severe patients were more likely to have not returned to normal levels (Wang Y. et al., 2020). Therefore, all COVID-19 patients should be tested regularly for these biochemical parameters, and re-testing is recommended in severe patients after discharge until liver function levels return to normal. Furthermore, uric acid values should also be monitored with favipiravir, and extra attention should be paid to neutrophils and platelets with remdesivir and lopinavir/ritonavir (Marc et al., 2021). A liver biopsy is recommended for patients with one of the following three conditions:(a) Persistent elevation of hepatic biochemical parameters or signs of deterioration in liver function after discontinuation of the suspected drug; (b) cases of DILI where continued use or re-exposure to the implicated agent is contemplated; (c) liver biochemistry abnormalities persist beyond 180 d, especially if associated with symptoms (e.g., itching) or signs (e.g., jaundice and hepatomegaly) (Chalasani et al., 2021).

4.2 Medication recommendations

4.2.1 General medication recommendations

The dosage and duration of antiviral agents may adversely affect liver metabolism and result in liver injury (Kumar et al., 2021). To minimize liver damage, regular monitoring of the relevant indicators, flexible-dose adjustment, and timely discontinuation of the drug are required. Remdesivir is not recommended for patients with baseline ALT ≥5 x ULN, and should be stopped if any of the following conditions occur during dosing: (I) ALT ≥5 x ULN; (II) ALT elevation accompanied by signs or symptoms of liver inflammation; or (III) ALT elevation accompanied by elevated conjugated bilirubin, ALP or international normalized ratio (INR). When ALT<5×ULN, the drug can be restarted again (Lamb, 2020; Malin et al., 2020). Since hydroxychloroquine accumulates in the liver, it is critical to continuously monitor the patient's liver function throughout clinical treatment and to be cautious when it is combined with other hepatotoxic drugs (Piszczatoski and Powell, 2020). Lopinavir/ritonavir is contraindicated in patients with severe liver injury because it has not been studied in this population (Stower, 2020). While no dose reduction is required for patients with mild to moderate liver damage, liver function tests (LFTs) must be closely monitored (Marra et al., 2021) (Table 3).

4.2.2 Medication recommendations for high-risk groups

4.2.2.1 For patients with hepatic dysfunction

4.2.2.1.1 Favipiravir. The dosage and duration of favipiravir should be modified based on the Child-Pugh Test (CPT). We recommend that CPT A and CPT B patients receive the same dose as the healthy population, while CPT C patients receive a lower dose and a shorter dosing schedule, based on prescribing information (Jafari et al., 2020) and the clinical trial of Preston R.

4.2.2.1.2 Remdesivir. Since remdesivir is primarily metabolized by the kidney, it can be used without dose adjustment in patients with hepatic dysfunction if there are no contraindications and the clinical benefits outweigh the risks (Marra et al., 2021; Sahakijpijarn et al., 2021). The main adverse effect of remdesivir is elevated hepatic transaminases, and this drug is not recommended if the patient's baseline ALT is > 5 x ULN (Sahakijpijarn et al., 2021).

TABLE 3 Medication recommendations of antiviral drugs.

Drugs	Medication recommendations		
	General Recommendations	For Patients with Hepatic Dysfunction	For Patients withComorbidities
Remdesivir	(i) not recommended for patients with baseline ALT ≥5 x ULN	(i)be used without dose adjustment in patients with hepatic dysfunction if there are no contraindications and the clinical benefits outweigh the risks	careful dosage consideration
	(ii)be stopped if any of the following conditions occur during dosing: (I) ALT ≥5 x ULN; (II) ALT elevation accompanied by signs or symptoms of liver inflammation; or (III) ALT elevation accompanied by elevated conjugated bilirubin, ALP or INR	(ii)not recommended if the patient's baseline ALT is > 5 x ULN	close monitoring
	(iii)ALT<5×ULN, the drug can be restarted again		
Lopinavir/Ritonavir	(i)contraindicated in patients with severe liver injury	(i)no dosage reduction is required, but LFTs should be closely monitored	careful dosage consideration
	(ii)no dose reduction is required for patients with mild to moderate liver damage, LFTs must be closely monitored	(ii)not recommended for use in patients with severe hepatic dysfunction on COVID-19	close monitoring
Hydroxychloroquine	continuously monitor and to be cautious	(i)50% loading dose as a maintenance dose and a maximum dosage of no more than 400 mg per day for patients with severe hepatic dysfunction (CPT C)	careful dosage consideration
		(ii)be reduced in patients with mild to moderate hepatic dysfunction if there are other risks of toxicity	close monitoring
Favipiravir	-	(i)CPT A and CPT B patients receive the same dose as the healthy population	
		(ii)CPT C patients receive a lower dose and a shorter dosing schedule	-

4.2.2.1.3 Lopinavir/ritonavir. Although the AUC (Area Under Curve) of lopinavir was 30% higher in patients with mild to moderate hepatic dysfunction than in patients with normal liver function, no clear correlation with clinical treatment was observed (Peng et al., 2006). Consequently, no dosage reduction is required, but LFTs should be closely monitored (Li et al., 2020). Lopinavir/Ritonavir may aggravate liver dysfunction based on the fact that it can cause elevated liver enzymes and bilirubin (Li et al., 2020). Furthermore, no studies in patients with severe hepatic dysfunction have been conducted, so it is not recommended for use in patients with severe hepatic dysfunction on COVID-19.

4.2.2.1.4 Hydroxychloroquine. As hydroxychloroquine accumulates in the liver, it should be used with caution in patients with COVID-19, despite the limited duration of dosing (Ferron et al., 2021). For patients with severe hepatic dysfunction (CPT C), a conservative regimen is to use a 50% loading dose as a maintenance dose and a maximum dosage of no more than 400 mg per day. The dosage should also be reduced in patients with mild to moderate hepatic dysfunction if there are other risks of toxicity. Moreover, baseline monitoring of liver function and ongoing monitoring of toxicity remain essential (Marra et al., 2021).

Other recommendations include that antiviral therapy for HBV should be continued, but antiviral therapy for HCV patients may need to be delayed. Non-emergency patients may postpone liver ultrasounds or biopsies. Strict indications for treatment should be followed when starting immunosuppressive drugs in patients with liver disease such as autoimmune hepatitis or graft rejection. Immunosuppression should be continued in patients with AIH or transplantation (Sucher et al., 2019; Li et al., 2020).

4.2.2.2 For patients with comorbidities

Common comorbidities among COVID-19 patients include coronary artery disease, hypertension, diabetes, and hyperlipidemia, and this population requires an additional targeted drug. The mechanisms by which these drugs interact with antiviral drugs have previously been described. Furthermore, antiviral medications may exacerbate comorbidities. Here, we recommend adjusting the dosage and dosing intervals or switching to a different drug with no interactions.

The clinical medication in elderly COVID-19 patients should be approached with greater caution, due to their diminished physiological functions, decreased liver and kidney metabolic functions, and the more possibility of concomitant coronary heart disease, diabetes mellitus, hypertension, hyperlipidemia, and other underlying diseases. The pharmacokinetic studies of remdesivir and lopinavir/ritonavir did not include elderly patients (age >65), so careful dosage consideration and close monitoring of relevant indicators are advised when administering these antiviral

agents (Zanon et al., 2020; Sahakijpijarn et al., 2021). Hydroxychloroquine has few adverse reactions and can be used in COVID-19 elderly patients, who should be focused on monitoring adverse reactions of cardiac, ocular, and renal.

5 Conclusion

The cumulative incidence of liver injury among COVID-19 patients was alarmingly high at 23.7% (16.1%–33.1%). The incidence of liver function abnormalities linked to favipiravir ranged from 6.8% to 44%, remdesivir from 1.7% to 46.2%, lopinavir-ritonavir from 1.1% to 63.4%, and hydroxychloroquine from 1.6% to 10.7%, according to our review of the data. Antiviral medicines have an inhibiting effect on CYP enzymes and liver transport proteins, which may account for these elevated incidences. Antiviral drugs inhibit CYP enzymes and hepatic transporter proteins, resulting in a buildup of reactive chemicals that initiate a cascade of biochemical stress reactions, finally resulting in hepatocyte necrosis and apoptosis.

The COVID-19 epidemic has been ongoing for 3 years, and clinical guidelines from various countries are continually updated. According to the most recent WHO guidelines, hydroxychloroquine and lopinavir/ritonavir are not recommended, and remdesivir is only recommended for conditional use in serious patients. Two new antiviral drugs are recommended: conditional recommendation against the use of nirmatrelvir-ritonavir (Paxlovid) in patients with non-severe illness at low risk of hospitalization; conditional recommendation for the use of molnupiravir in patients with non-severe COVID-19, at highest risk of hospitalization. A meta-analysis study demonstrated that molnupiravir and Paxlovid were effective in reducing mortality and hospitalization rates in patients with COVID-19 without increasing the incidence of adverse events, thereby demonstrating a good overall safety profile (Wen et al., 2022). However, additional research is required to confirm these findings. Despite no longer being recommended, hydroxychloroquine and lopinavir-ritonavir were frequently utilized during the COVID-19 outbreak. According to the incidence data on liver damage summarized in this research, they have caused severe damage. It may aid in rationalizing the usage of repurposed medications when confronted with a new and severe epidemic.

The history of documented drug use is replete with drug-induced diseases caused by drug exposure and drug resistance owing to drug abuse, ending in the predicament of having no available medications. This rendered the drugs used to treat the sickness hazardous to the health of the patient instead. DILI is a prevalent drug-induced disease, and the authors suggest that it is crucial to use drugs with cautious discretion in dosage, to consider drug-drug interactions if combination, and to strike a balance between the therapeutic effects and toxicity of drugs in order to reduce the incidence of DILI. It is recommended that patients are also closely monitored, with the aim of early detection and treatment to minimize the risk of DILI.

This review summarizes the incidence of liver function abnormalities in COVID-19 patients caused by several antiviral drugs, including favipiravir, remdesivir, lopinavir/ritonavir, and hydroxychloroquine, while also providing thorough speculation of the underlying mechanism and suggesting reasonable clinical management. This advances the systematic understanding of DILI in COVID-19 patients and directs the clinical care of medical practitioners. There are also some limitations, such as the fact that this study's data were not subjected to a systematic analysis because there were insufficient and inconsistently high-quality clinical trials evaluating adverse reactions; additionally, the mechanism hypothesis is largely based on the results of *in vitro* experiments and needs to be confirmed by additional clinical studies.

Author contributions

XL, YS, and CZ designed and directed the studies; WW, SY, and WZ collected data, performed the data and drafted the manuscript; HX and CB participated in the conception and revision of the study; YY and JC contributed to methodology and table design. All authors revised the manuscript critically and approved the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Post-marketing safety of immunomodulatory drugs in multiple myeloma: A pharmacovigilance investigation based on the FDA adverse event reporting system

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Objective: In recent years, the emergence of immunomodulatory drugs (IMiDs) has significantly improved clinical outcomes in patients with multiple myeloma (MM); however, serious adverse events (AEs) have hindered their safe clinical application. This study aimed to characterize the safety profiles and differences in IMiDs through a disproportionality analysis using the U.S. Food and Drug Administration Adverse Event Reporting System (FAERS), a post-marketing surveillance database.

Methods: This study filtered reports of thalidomide, lenalidomide, and pomalidomide as primary suspect drugs in FAERS files from January 2013 to December 2021. AEs in the reports were retrieved according to the preferred terms (PTs) of the Medical Dictionary for Regulatory Activities. Furthermore, we detected safety signals using the reporting odds ratio (ROR), proportional reporting ratio (PRR), and Bayesian belief propagation neural network (BCPNN). When all three algorithms showed an association between the target drug and the AE, a positive signal was generated.

Results: We extracted 9,968 thalidomide, 231,926 lenalidomide, and 55,066 pomalidomide AE reports. AEs were more common in male patients and in those >44 years old. Important safety signals were detected based on the system organ classes (SOC), including thalidomide (cardiac disorders: ROR, 2.87; PRR, 2.79; IC 1.22), lenalidomide (gastrointestinal disorders: ROR, 2.38; PRR, 2.27; IC 0.75), and pomalidomide (respiratory, thoracic, and mediastinal disorders: ROR, 2.14; PRR, 2.09; IC 0.85). Within the PT level, we identified novel risk signals: the thalidomide-induced second primary malignancy (SPM) signal was significant; lenalidomide reduced the success rate of hematopoietic stem cell collection; and three IMiDs may cause human chorionic gonadotropin increase, but this needs to be proven by clinical data. Pneumonia, sepsis, and renal failure are common risk factors for death due to IMiDs. Compared with

thalidomide and lenalidomide, pomalidomide has a lower risk of venous thromboembolism (VTE) and is beneficial to patients with renal insufficiency.

Conclusion: Mining data from FAERS resulted in novel AE signals, including adenocarcinoma of colon, harvest failure of blood stem cells, and increased levels of human chorionic gonadotropin. Further investigation is required to verify the significance of these signals. Moreover, IMiDs showed differences in safety reports, which should be emphasized by clinicians.

KEYWORDS

IMiDs, multiple myeloma, FAERS, adverse event, pharmacovigilance, data mining

1 Introduction

Multiple myeloma (MM) is one of the most common hematological malignancies, accounting for 20% of deaths from hematopoietic cancers and nearly 2% of cancer-related deaths (San Miguel, 2015; Naymagon and Abdul-Hay, 2016; Sonneveld and Broijl, 2016). Clinically, MM is characterized by malignant proliferation of plasma cells in the bone marrow, and monoclonal immunoglobulins in blood or urine, causing anemia, renal insufficiency, extensive bone destruction, hypercalcemia, and repeated severe infections (Fernández-Lázaro, 2020). Currently, MM is incurable (Hemminki et al., 2021). Traditional standard induction therapy for MM includes corticosteroids, melphalan, prednisone, or a combination of vinblastine, doxorubicin, and dexamethasone. However, due to increased resistance and drug-related adverse events (AEs) associated with classical chemotherapy and glucocorticoids, the median overall survival (OS) of MM patients is still not optimistic. Recently, the prognosis of MM patients has dramatically improved with the emergence of immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) as evidenced by the increase in complete remission (CR) rates from 5% to 30% and extension of OS from 3 years to 5–15 years (Rajkumar, 2013; Kyle and Rajkumar, 2014).

Currently, three IMiDs have been approved to treat MM: thalidomide, lenalidomide, and pomalidomide (Palumbo et al., 2008; Scott and Lyseng-Williamson, 2011; Elkinson and McCormack, 2013). IMiDs exert anticancer effects through various mechanisms such as inducing tumor cell apoptosis, disturbing the interaction of tumor cells with stromal marrow cells, and increasing antitumor immune responses (Fernández-Lázaro et al., 2018; Charlinski et al., 2021). IMiDs exhibit moderate cross-reactivity and permissible sequential therapy; therefore, they can be applied to treat all stages of MM (Raza et al., 2017). Meanwhile, IMiDs are also the standard of care for patients who are suitable or unsuitable for induction therapy of autologous stem cell transplantation (ASCT), as maintenance therapy after ASCT, and receive relapsed/refractory MM (RRMM) treatment (Charlinski et al., 2021). Multiagent combinations based on IMiDs can prolong progression-free survival and OS and improve the quality of life (Miguel et al., 2013; Jones et al., 2016a; Garderet et al., 2018; Richardson et al., 2019; Siegel et al., 2020; Charlinski et al., 2021). Due to durable objective response rates, pomalidomide has been recommended as first-line and second-line treatment for lenalidomide-resistant and bortezomib-sensitive patients, respectively, according to the EHA-ESMO guidelines (Dimopoulos et al., 2021). However, further clinical practice and research revealed that IMiDs may cause serious AEs, such as rash, constipation, and venous thromboembolism (VTE) (Lonial et al., 2011; Ocio et al., 2012). Surprisingly, although the chemical structures of IMiDs are similar, their AEs were different. During thalidomide treatment, teratogenicity, sedation, and peripheral neuropathy were observed. Ito et al. identified cereblon as the primary target of thalidomide teratogenicity (Terpos et al., 2015; Holstein and McCarthy, 2017). The incidence of VTE significantly increased when thalidomide and lenalidomide were combined with conventional chemotherapy drugs (Musallam et al., 2009). Studies have demonstrated that patients receiving lenalidomide have an increased risk of second primary malignancies (SPMs), especially hematological malignancies (Razavi et al., 2013). Pomalidomide-associated fatal AEs have also been reported, including pneumonia, cardiac arrest, and progressive multifocal leukoencephalopathy (PML) (Richardson et al., 2019; Health Canada, 2022). Unfortunately, related research that directly compares the safety of the three IMiDs is scarce. Additionally, differences in the safety of IMiDs may affect treatment decisions and medication adherence.

Surveillance of post-marketing adverse drug events is critical for clinically rational drug use, with most IMiD-related AEs coming from clinical trials. However, clinical trials are usually limited by scale and ethics, and it is difficult to conduct large-scale preventive clinical studies to comprehensively analyze all types of patients (Beaulieu-Jones et al., 2020; Roberts and Ferguson, 2021). Therefore, real-world data are needed to supplement or verify clinical trials and to understand the safety profile of IMiDs better. Large real-world databases of AEs are the main data source for safety assessment of marketed drugs with fast-tracking and priority review, such as the U.S. Food and Drug Administration Adverse Event Reporting System (FAERS), the largest publicly available pharmacovigilance database (Health Canada, 2022). It

contains patient data outside clinical trials and can be used for post-marketing surveillance (Raschi et al., 2019; Raschi et al., 2020).

To provide an overview of the safety profiles of IMiDs, we retrospectively analyzed real-world AEs of IMiDs from the first quarter of 2013 (2013Q1) to the fourth quarter of 2021 (2021Q4) by mining data from FAERS.

2 Materials and methods

2.1 Data collection and source

We downloaded all reports from 2013Q1 to 2021Q4 from the publicly available FAERS database (FDA, 2022). Each quarterly report contains seven datasets: patient demographics (DEMO), drug (DRUG), reaction (REAC), outcome (OUTC), report source, therapy, and indications for use; the DEMO, DRUG, REAC, and OUTC datasets were used in this study and are linked by the primary ID that identifies FAERS reports. Following FDA recommendations, we deduplicated the data in two steps: first, by filtering unique row variables; second, by selecting the latest case version with the same CASEID and removing redundant records. Reports for the following terms representing IMiDs were qualified: "Thalomid", "Thalidomide", "Distaval", "Contergan", "Revlimid", "Lenalidomide", "Pomalidomide", and "Pomalyst". Only reports with the drug code "prime suspect" were collected for analysis.

2.2 Definition of adverse events

AEs in the FAERS database were coded according to the preferred terms (PTs) of the Medical Dictionary for Regulatory Activities (MedDRA version 25.0) (Medical Dictionary for Regulatory Activities, 2022). MedDRA is multiaxial in that a PT can be linked to multiple system organ classes (SOCs), but each PT is assigned a single primary SOC. The extracted AEs can be associated with the corresponding SOCs through the hierarchical structure of MedDRA. In this study, we only analyzed the primary SOC associated with PT to avoid repetitive counting. Any significant AE not listed on the label was defined as an unexpected adverse drug reaction. To minimize the risk of indication bias (whereby the drug indication is reported as an AE), we removed PTs associated with the drug indication and complications in MM for analysis (Huang et al., 2020); i.e., we only analyzed drug-induced AEs and not disease states.

2.3 Data mining and analysis

We detected AE signals using three algorithms: the reporting odds ratio (ROR), proportional reporting ratio (PRR), and Bayesian confidence propagation neural network (BCPNN) (Ahmed et al., 2009; Poluzzi et al., 2009; Sakaeda et al., 2013). These methods are based on a two-by-two contingency (Supplementary Table S1) and can be used to investigate the statistical association between a drug and AE to detect potential AE signals. To avoid false-positive signals, the criterion is achieved only when all three algorithms show that the frequency and signal intensity between a drug and AE. Subsequently, it is determined as disproportionality, prompting the generation of a positive signal (Supplementary Table S2) (van Puijenbroek et al., 2002). Microsoft EXCEL 2019 and SPSS 26.0 statistical software were used for data analysis.

3 Results

3.1 Descriptive analysis

During the 9-year study period from January 2013 to December 2021, FAERS received a total of 11,209,429 AE reports, with 9,968 for thalidomide (0.09%), 231,926 for lenalidomide (2.07%), and 55,066 for pomalidomide (0.49%). The characteristics of the IMiD AE reports are described in Table 1. Male patients had a slight advantage compared with female patients, and there was a higher proportion of patients aged >44 years. The majority of reports were from the United States, Japan, and Canada and were submitted by physicians, pharmacists, and other health professionals, which accounted for the highest percentage of reports for thalidomide (31.61%), while pharmacists accounted for the highest percentage of reports for lenalidomide (40.23%) and pomalidomide (44.31%).

3.2 Outcomes and fatality of IMiDs-related AEs

Nearly 50% of AE reports described serious outcomes (Figure 1A), with a higher proportion of hospitalizations (initial or prolonged) and deaths. A peak in the reporting of hospitalization (initial or prolonged) and death was noted for thalidomide (27.70% and 19.65%, respectively), while lenalidomide had the lowest percentage among the drugs studied (26.30% and 11.72%, respectively). To further investigate the AEs leading to death, we separately evaluated the mortality (according to the number of deaths reports) caused by different AEs among the three drugs. Among them, deaths due to pneumonia and sepsis were ranked as the top two reasons for thalidomide (Figure 1B), lenalidomide (Figure 1C), and pomalidomide (Figure 1D). By analyzing the population characteristics, we found that death due to pneumonia and sepsis was more common in middle-aged and elderly male patients, especially those >65 years of age (Table 2).

TABLE 1 Characteristics related to immunomodulatory drugs (IMiDs) safety reports from January 2013 to December 2021.

	Thalidomide	Lenalidomide	Pomalidomide
	N[a] (%)	N[a] (%)	N[a] (%)
Number of adverse events reports	9968 (100)	231926 (100)	55066 (100)
Sex			
Female	4457 (44.71)	111893 (48.25)	26326 (47.81)
Male	4942 (49.58)	116132 (50.07)	28072 (50.98)
Unknown	569 (5.71)	3901 (1.68)	668 (1.21)
Age (year)			
<18	187 (1.88)	131 (0.06)	28 (0.05)
18–44	317 (3.18)	1919 (0.83)	349 (0.63)
45–64	1737 (17.43)	34322 (14.80)	8205 (14.90)
65–74	1724 (17.30)	41986 (18.10)	10540 (19.14)
≥75	1489 (14.94)	43427 (18.72)	8830 (16.04)
Unknown	4514 (45.28)	110141 (47.49)	27114 (49.24)
Reporters			
Consumer	1145 (11.49)	12213 (5.27)	2552 (4.63)
Physician	2877 (28.86)	47425 (20.45)	11014 (20.00)
Other health-professional	3151 (31.61)	58993 (25.44)	12049 (21.88)
Pharmacist	2178 (21.85)	93312 (40.23)	24401 (44.31)
others	447 (4.48)	18949 (8.17)	4768 (8.66)
Unknown	170 (1.71)	1034 (0.45)	282 (0.51)
Reporter country			
United States	8491 (85.18)	212858 (91.78)	50821 (92.29)
Japan	10 (0.10)	4368 (1.88)	1548 (2.81)
Canada	68 (0.68)	2115 (0.91)	722 (1.31)
Others	1399 (14.03)	12585 (5.43)	1975 (3.59)

[a]Number of patients with adverse events.

3.3 Disproportionality analysis

3.3.1 Analysis of AEs at the SOC level

These AEs were classified according to the corresponding SOC of MedDRA involving 27 SOCs. The most frequently reported SOCs for thalidomide and lenalidomide were "infections and infestations," "neoplasms benign, malignant and unspecified (incl cysts and polyps)", and "investigations," while for pomalidomide, the most commonly reported were "infections and infestations," "investigations," and "gastrointestinal disorders" (Supplementary Table S3). Within the SOCs, we conducted disproportionate analysis to assess the association between AEs and organs; the larger the ROR, PRR, and IC values, the stronger the correlation (van Puijenbroek et al., 2002). There are certain differences in the SOC involved with the IMiDs, as shown in Table 3: there were four significant safety signals for thalidomide (cardiac disorders, skin and subcutaneous tissue disorders, metabolism and nutrition disorders, and vascular disorders); AE reports of lenalidomide focused on gastrointestinal disorders and musculoskeletal and connective tissue disorders; and pomalidomide correlated with four SOCs (metabolism and nutrition disorders, respiratory, thoracic and mediastinal disorders, skin and subcutaneous tissue disorders, and musculoskeletal and connective tissue disorders).

3.3.2 Analysis of AEs at the PT level

According to the criteria of the three algorithms, we identified 81, 292, and 189 suspicious signals for thalidomide, lenalidomide, and pomalidomide, respectively. Supplementary Table S4 presents a list of the 20 most frequently reported AEs. We found that the most frequently reported AEs for thalidomide were peripheral neuropathy ($n = 544$), pneumonia ($n = 362$), and unevaluable events ($n = 306$); for lenalidomide, there was a higher percentage of Diarrhea ($n = 15527$), fatigue ($n = 13794$), and pneumonia ($n = 10916$); for pomalidomide, pneumonia ($n = 3,683$), fatigue ($n = 3,299$), and decreased white blood cell count ($n = 1,980$) accounted for a relatively high proportion. Among the AEs, nasopharyngitis caused by pomalidomide was not included in the label. The top 20 PTs associated with statistical significance for IMiDs are shown in Table 4. The

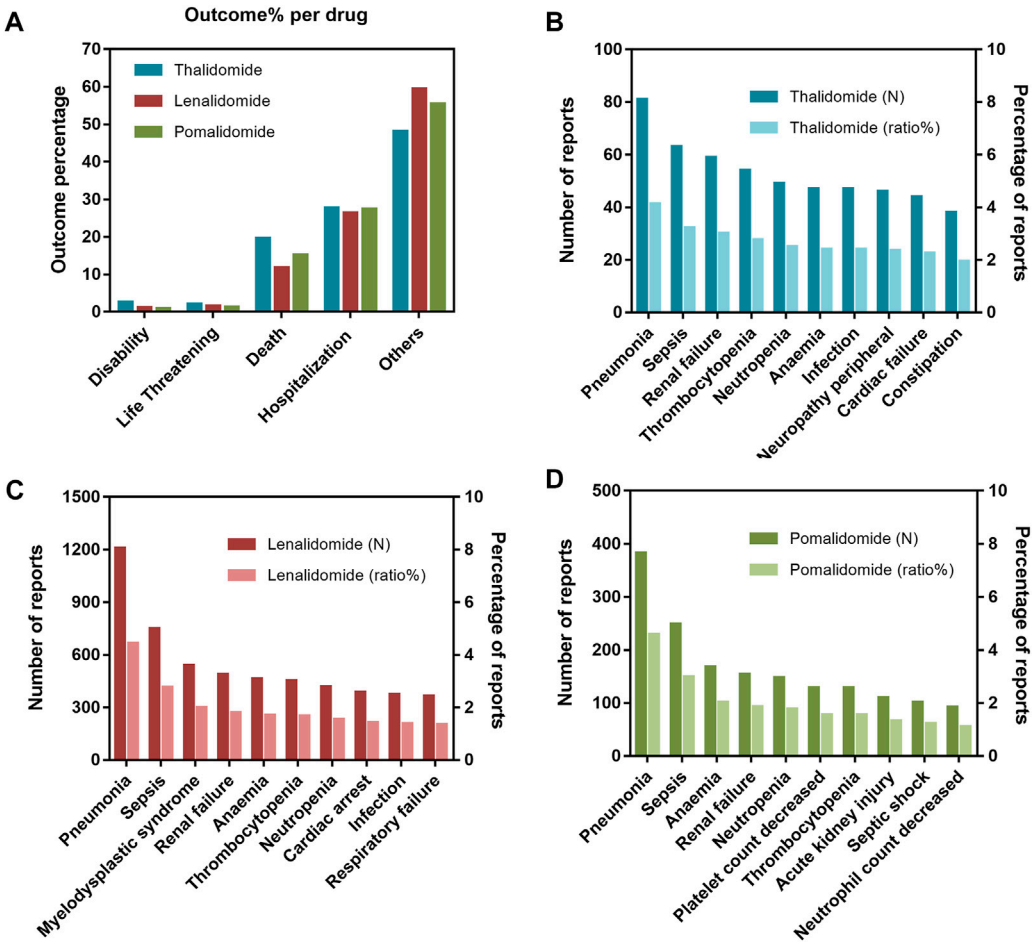


FIGURE 1
(A) Outcomes for adverse events (AEs) associated with immunomodulatory drugs (IMiDs). **(B)** The top 10 AEs leading to death for thalidomide. **(C)** The top 10 AEs leading to death for lenalidomide. **(D)** The top 10 AEs leading to death for pomalidomide.

TABLE 2 Clinical characteristics of deaths due to immunomodulatory drugs (IMiDs) related pneumonia and sepsis in the FAERS database (January 2013 to December 2021).

Pneumonia		Sepsis	
Sex	N[a] (%)	Sex	N[a] (%)
Female	556 (33.27)	Female	392 (36.91)
Male	946 (56.61)	Male	589 (55.46)
Unknown	169 (10.11)	Unknown	81 (7.63)
Age (year)		Age (year)	
<18	4 (0.24)	<18	4 (0.38)
18–44	7 (0.42)	18–44	9 (0.85)
45–64	249 (14.90)	45–64	211 (19.87)
≥65	1038 (62.12)	≥65	597 (56.21)
Unknown	373 (22.32)	Unknown	241 (22.69)

[a]Number of patients with adverse events.

number of AEs not listed on the label were nine for thalidomide: human chorionic gonadotropin increased, medulloblastoma, myelofibrosis, squamous cell carcinoma of the skin, rectal adenocarcinoma, adenocarcinoma of the colon, malignant brain neoplasm, basal cell carcinoma, non-small cell lung cancer; two for lenalidomide: human chorionic gonadotropin increased; blood stem cell harvest failure; and one for pomalidomide: human chorionic gonadotropin increased.

3.4 Changes in the Number of IMiDs AEs reports

Figure 2A shows line graphs with the percentage of AE reports of IMiDs (based on the number of all AEs reported for the drug over 9 years). Of those, thalidomide-related AEs

TABLE 3 Detected significant safety signals based on system organ class (SOC).

SOC	N[a] (%)	ROR (95% CI)	PRR (χ^2)	IC (IC-2SD)
Thalidomide				
Cardiac disorders	86 (4.13)	2.87 (3.67–2.24)	2.79 (76.69)	1.22 (0.43)
Skin and subcutaneous tissue disorders	115 (5.53)	2.56 (3.16–2.07)	2.47 (81.34)	1.09 (0.41)
Metabolism and nutrition disorders	57 (2.74)	2.25 (3.02–1.68)	2.21 (30.92)	0.95 (0.01)
Vascular disorders	63 (3.03)	2.18 (2.88–1.65)	2.14 (31.53)	0.92 (0.02)
Lenalidomide				
Gastrointestinal disorders	434 (7.65)	2.38 (2.71–2.08)	2.27 (174.84)	0.75 (0.37)
Musculoskeletal and connective tissue disorders	216 (3.81)	2.08 (2.49–1.74)	2.04 (66.61)	0.66 (0.13)
Pomalidomide				
Metabolism and nutrition disorders	82 (2.79)	2.45 (3.18–1.89)	2.41 (49.30)	0.99 (0.17)
Respiratory, thoracic and mediastinal disorders	145 (4.94)	2.14 (2.60–1.77)	2.09 (62.97)	0.85 (0.23)
Skin and subcutaneoustissue disorders	133 (4.53)	2.07 (2.53–1.69	2.02 (52.84)	0.81 (0.17)
Musculoskeletal and connective tissue disorders	124 (4.22)	2.06 (2.53–1.67)	2.01 (48.78)	0.81 (0.15)

[a]Number of patients with adverse events.

reports peaked in 2015, contributing to 24.21% of all thalidomide-related AEs reported in the past 9 years, which was followed by a downward trend. However, the number of reports on lenalidomide and pomalidomide increased slowly over time. Compared with lenalidomide and pomalidomide, the thalidomide (as an old drug) related AEs reports was small in quantity in the past 9 years, with only 9968 reports (Figure 2B).

4 Discussion

Although IMiDs share structural similarities, their safety properties differ. However, there is a lack of published studies that evaluate post-marketing real-world AEs of IMiDs. To our knowledge, this is the first such safety study of IMiDs based on data mining of FAERS. Additionally, we focused on the differences in the associations between AEs and real-world prognosis based on the FAERS database.

Our study demonstrated that in terms of SOC, thalidomide was the only drug that showed a significant signal in "cardiac disorders," lenalidomide showed significant signals in "gastrointestinal disorders," and pomalidomide was strongly associated with "respiratory, thoracic, and mediastinal disorders". The safety profiles of the IMiDs in this study were consistent with those of previous reports for individual agents. Several studies have reported that MM patients treated with thalidomide experienced arrhythmias or congenital septal defects (following its administration to pregnant women), which may be related to the interaction of the cardioprotective-related TBX5 transcriptional activator (Basson et al., 1997; Rokicka and Rokicki, 1999; Kropff et al., 2012; Khalil et al., 2017). Meanwhile, lenalidomide was more strongly associated with gastrointestinal AEs, including nausea, vomiting, diarrhea, and constipation, in a meta-analysis by Wang et al. (Wang et al., 2016). Infections were more common in patients receiving pomalidomide, and a few patients discontinued treatment because of pneumonia (Lacy et al., 2009; Leleu et al., 2013; Miguel et al., 2013; Richardson et al., 2014).

MM is associated with a high risk of VTE, and the use of IMiDs further increases this risk (Srkalovic et al., 2004; Kristinsson et al., 2010). The risk of VTE is increased by 28% and 59% when IMiDs are combined with dexamethasone (Zangari et al., 2001; Musallam et al., 2009) and chemotherapy drugs (Baz et al., 2005), respectively. Hence, the choice of drug is a major determinant of VTE risk in patients (Fotiou et al., 2016). Our results showed that pomalidomide had the lowest risk of VTE, consistent with those of previously published studies. Leclerc et al. reported that 14.7% of patients receiving lenalidomide and 13.9% of patients receiving thalidomide experienced VTE; meanwhile, only 7.4% of patients who received pomalidomide experienced VTE (Leclerc et al., 2022). The mechanisms responsible for the increased risk of VTE due to IMiD use are poorly characterized. Thalidomide has been reported to increase the levels of von Willebrand factor and factor VIII, stimulate tissue factors in monocytes, decrease thrombomodulin, and activate platelets, all of which increase the risk of VTE (Palumbo and Palladino, 2012; Abdullah et al., 2013). Lenalidomide-induced upregulation of cathepsin G, which is a platelet activator, has been suggested as a potential mechanism for the increased risk of VTE (Isozumi et al., 2013). Meanwhile, few data are available on the risk of VTE associated with pomalidomide; its incidence appears to be lower than those of thalidomide and lenalidomide, which may be related to the routine inclusion of thromboprophylaxis in the treatment regimen (Scott, 2014).

TABLE 4 Top 20 preferred terms (PT) associated with immunomodulatory drugs (IMiDs) for signal strength.

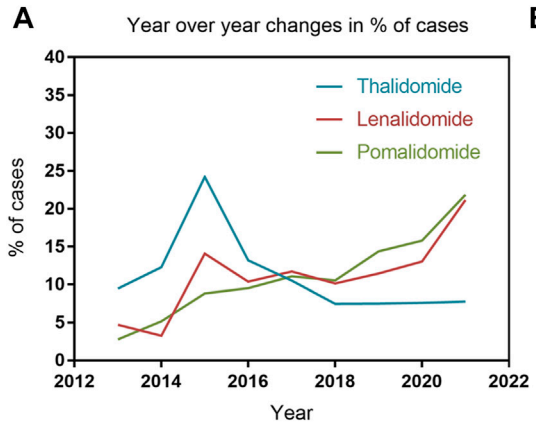
PT	N[a] (%)	ROR (95% CI)	PRR (χ2)	IC (IC-2SD)
Thalidomide	21045 (100)			
Human chorionic gonadotropin increased	34 (0.16)	126.18 (88.92–179.05)	125.98 (3894.97)	4.76 (3.62)
Neuropathy peripheral	544 (2.58)	17.19 (15.78–18.72)	16.77 (7991.69)	4.01 (3.73)
Full blood count decreased	131 (0.62)	17.41 (14.64–20.69)	17.30 (1990.61)	3.93 (3.36)
Medulloblastoma	14 (0.07)	564.16 (305.64–1041.34)	563.79 (5747.54)	3.86 (1.95)
Myelofibrosis	25 (0.12)	22.78 (15.35–33.83)	22.76 (512.47)	3.62 (2.33)
Light chain analysis increased	16 (0.08)	41.74 (25.40–68.60)	41.71 (618.90)	3.61 (2.01)
Squamous cell carcinoma of skin	35 (0.17)	16.57 (11.87–23.12)	16.54 (505.64)	3.52 (2.43)
Unevaluable event	306 (1.45)	10.52 (9.39–11.78)	10.38 (2580.15)	3.32 (2.95)
Rectal adenocarcinoma	11 (0.05)	44.67 (24.52–81.37)	44.65 (456.09)	3.26 (1.35)
Adverse drug reaction	288 (1.37)	9.65 (8.58–10.84)	9.53 (2187.81)	3.20 (2.82)
Acute myeloid leukaemia	52 (0.25)	10.79 (8.21–14.18)	10.76 (457.42)	3.18 (2.28)
Adenocarcinoma of colon	15 (0.07)	18.33 (11.02–30.51)	18.32 (242.71)	3.13 (1.49)
Peripheral sensory neuropathy	24 (0.11)	12.61 (8.44–18.85)	12.60 (254.22)	3.10 (1.79)
Brain neoplasm malignant	22 (0.10)	11.91 (7.83–18.12)	11.90 (217.98)	3.01 (1.64)
Deep vein thrombosis	171 (0.81)	7.72 (6.64–8.98)	7.67 (987.52)	2.88 (2.38)
Myelodysplastic syndrome	40 (0.19)	8.45 (6.19–11.53)	8.43 (260.66)	2.83 (1.81)
Full blood count increased	9 (0.04)	19.85 (10.28–38.32)	19.85 (159.00)	2.78 (0.70)
Basal cell carcinoma	44 (0.21)	7.78 (5.79–10.47)	7.77 (258.33)	2.75 (1.78)
No therapeutic response	34 (0.16)	7.48 (5.34–10.48)	7.47 (189.58)	2.65 (1.55)
Non-small cell lung cancer	10 (0.05)	8.86 (4.76–16.50)	8.86 (69.29)	2.36 (0.39)
Lenalidomide	460923 (100)			
Human chorionic gonadotropin increased	254 (0.06)	90.75 (75.26–109.43)	90.70 (9728.69)	5.11 (4.59)
Full blood count decreased	5301 (1.15)	57.60 (55.53–59.74)	56.95 (159596.97)	4.97 (4.87)
Full blood count increased	290 (0.06)	48.42 (41.67–56.27)	48.39 (7907.35)	4.72 (4.26)
Light chain analysis increased	244 (0.05)	46.86 (39.83–55.14)	46.84 (6516.94)	4.67 (4.17)
5q minus syndrome	68 (0.01)	65.10 (46.73–90.68)	65.09 (2206.79)	4.52 (3.56)
Light chain analysis abnormal	43 (0.01)	45.59 (31.02–67.03)	45.59 (1128.67)	4.10 (2.94)
Protein total increased	320 (0.07)	22.43 (19.77–25.44)	22.41 (4939.82)	4.03 (3.62)
Laboratory test abnormal	3327 (0.72)	15.88 (15.29–16.49)	15.77 (37472.04)	3.70 (3.57)
Refractory anaemia with an excess of blasts	41 (0.01)	21.25 (14.97–30.15)	21.24 (604.59)	3.58 (2.46)
Blood stem cell harvest failure	19 (<0.01)	38.51 (21.97–67.52)	38.51 (445.35)	3.50 (1.79)
Squamous cell carcinoma of skin	470 (0.10)	11.57 (10.49–12.75)	11.56 (3881.44)	3.30 (2.98)
Blood immunoglobulin A increased	38 (0.01)	14.80 (10.42–21.02)	14.80 (402.41)	3.25 (2.12)
Pulmonary thrombosis	796 (0.17)	10.85 (10.07–11.69)	10.83 (6141.71)	3.23 (2.99)
Multiple allergies	526 (0.11)	10.82 (9.87–11.86)	10.80 (4046.14)	3.22 (2.92)
Refractory cytopenia with multilineage dysplasia	18 (<0.01)	22.97 (13.48–39.16)	22.97 (283.71)	3.22 (1.54)
Thrombosis	5506 (1.19)	10.44 (10.15–10.74)	10.33 (40394.22)	3.19 (3.09)
Malignant neoplasm of unknown primary site	43 (0.01)	12.89 (9.30–17.85)	12.88 (397.12)	3.16 (2.10)
Blood immunoglobulin G increased	72 (0.02)	11.41 (8.89–14.64)	11.41 (586.53)	3.14 (2.33)
White blood cell count decreased	6627 (1.44)	9.24 (9.01–9.48)	9.12 (42397.83)	3.03 (2.94)
Neuropathy peripheral	5857 (1.27)	9.21 (8.96–9.47)	9.11 (37384.65)	3.03 (2.94)
Pomalidomide	102810 (100)			
Full blood count decreased	1158 (1.13)	34.65 (32.60–36.83)	34.27 (33717.29)	4.92 (4.71)
Human chorionic gonadotropin increased	60 (0.06)	48.47 (36.93–63.62)	48.44 (2413.59)	4.65 (3.76)
Full blood count increased	78 (0.08)	39.02 (30.84–49.38)	38.99 (2567.19)	4.61 (3.83)
Laboratory test abnormal	908 (0.88)	16.87 (15.78–18.04)	16.73 (12752.72)	3.97 (3.75)
Pneumonia influenzal	32 (0.03)	17.33 (12.14–24.75)	17.33 (466.49)	3.49 (2.32)

(Continued on following page)

TABLE 4 (*Continued*) Top 20 preferred terms (PT) associated with immunomodulatory drugs (IMiDs) for signal strength.

PT	N[a] (%)	ROR (95% CI)	PRR (χ2)	IC (IC-2SD)
Light chain analysis abnormal	14 (0.01)	46.54 (26.54–81.60)	46.54 (542.93)	3.48 (1.68)
White blood cell count decreased	1980 (1.93)	11.51 (11.00–12.04)	11.31 (17985.65)	3.45 (3.30)
Protein total increased	55 (0.05)	13.77 (10.51–18.03)	13.76 (623.31)	3.44 (2.55)
Neutrophil count decreased	608 (0.59)	10.27 (9.47–11.14)	10.22 (4899.05)	3.29 (3.02)
Blood immunoglobulin A increased	15 (0.01)	23.44 (13.87–39.61)	23.43 (299.67)	3.24 (1.56)
Neuropathy peripheral	1306 (1.27)	8.44 (7.99–8.92)	8.35 (8239.76)	3.02 (2.84)
Amyloidosis	30 (0.03)	10.84 (7.53–15.60)	10.84 (258.89)	3.01 (1.82)
Blood immunoglobulin G increased	19 (0.02)	12.17 (7.69–19.24)	12.17 (187.40)	2.93 (1.45)
Paraproteinaemia	10 (0.01)	22.64 (11.92–43.02)	22.64 (192.88)	2.90 (0.86)
Pneumonia respiratory syncytial viral	14 (0.01)	12.83 (7.52–21.89)	12.83 (146.66)	2.81 (1.10)
Multiple allergies	92 (0.09)	7.59 (6.17–9.34)	7.59 (513.68)	2.80 (2.11)
Cardiac amyloidosis	16 (0.02)	11.29 (6.85–18.59)	11.28 (144.75)	2.78 (1.18)
Malignant neoplasm of unknown primary site	11 (0.01)	13.12 (7.18–23.98)	13.12 (118.18)	2.68 (0.76)
Parainfluenzae virus infection	21 (0.02)	8.49 (5.50–13.10)	8.49 (135.06)	2.64 (1.23)
Listeriosis	16 (0.02)	8.99 (5.47–14.78)	8.99 (110.46)	2.59 (0.99)

[a]Number of patients with adverse events.



B

Reports of AE to FAERS from 2013-2021

Year	Thalidomide N (%)	Lenalidomide N (%)	Pomalidomide N (%)
2013	946 (9.49)	10901 (4.70)	1526 (2.77)
2014	1224 (12.28)	7553 (3.26)	2844 (5.16)
2015	2413 (24.21)	32688 (14.09)	4869 (8.84)
2016	1316 (13.20)	24124 (10.40)	5253 (9.54)
2017	1049 (10.52)	27238 (11.74)	6107 (11.09)
2018	744 (7.46)	23543 (10.15)	5806 (10.54)
2019	746 (7.48)	26560 (11.45)	7917 (14.38)
2020	757 (7.59)	30218 (13.03)	8702 (15.80)
2021	773 (7.75)	49101 (21.17)	12042 (21.87)
Total	9968 (100)	231926 (100)	55066 (100)

FIGURE 2
(A) Line graph with the percentage of AE reports of immunomodulatory drugs (IMiDs) published per year. **(B)** The number and percentage of cases reported to the food and drug administration adverse event reporting system caused by IMiDs.

We also found that lenalidomide and pomalidomide reduced the risk of peripheral neuropathy compared to thalidomide (Dalla Torre et al., 2016; Bringhen et al., 2017). Pomalidomide has good safety profiles, but AEs related to the respiratory system, especially pneumonia, cannot be ignored. Health Canada warned of an increased risk of PML in patients treated with pomalidomide, while the disproportionate analysis showed a weaker signal risk in this study with a total of 22 reports received from the FAERS database.

We observed some unexpected AEs that were not listed on the label of the drugs. The disproportionate association with human chorionic gonadotropin (hCG) was observed with all three IMiDs. A non-pregnant premenopausal woman had a positive pregnancy test after thalidomide administration (Slone et al., 2005). Additionally, Tageja et al. (2010) reported a postmenopausal woman who exhibited persistent elevations in hCG levels during lenalidomide treatment for MM. However, only a few cases of IMiDs have been reported. Hence, the risk of increased hCG levels in IMiDs remains to be demonstrated using clinical data. For thalidomide, some AEs were associated with malignancy. The ECOG E1A06 study found ten and four hematologic SPMs in MPT (melphalan, prednisone,

thalidomide) and MPR (melphalan, prednisone, lenalidomide) groups, respectively (Stewart et al., 2015). The Arkansas TT2 (+/−thalidomide) trial found that thalidomide increased the risk of solid tumor SPMs and decreased the risk of hematologic malignancies (Usmani et al., 2012). Although these associations between thalidomide and SPMs are weak and unconvincing due to limited evidence, we still need to pay more attention. Multiple studies have observed an increased risk of SPMs in patients receiving lenalidomide, with the incidence of SPMs ranging from 2.6% to 8.0% (Attal et al., 2013; Holstein et al., 2015). However, this risk appears to be offset by the beneficial effects of lenalidomide on OS. A significant signal for "malignant neoplasm of unknown primary site" was found in pomalidomide, but real-world evidence is lacking. Jones et al. (2016b) reported that IMiDs may reactivate the Epstein–Barr virus (EBV), an oncogenic gamma herpes virus associated with the development and maintenance of various human malignancies, thereby enhancing the EBV lytic cycle and host immune suppression (Jha et al., 2016). Further studies are required to elucidate the molecular mechanisms underlying the association between IMiDs and SPMs. A newly suspected AE signal, Blood stem cell harvest failure, in lenalidomide has garnered our interest. In the era of novel drugs, ASCT remains the first-line treatment despite IMiDs being extremely beneficial for MM patients. However, multiple studies have shown that lenalidomide can cause myelosuppression and modify the matrix environment, thereby affecting the success rate of hematopoietic stem cell collection (Lev et al., 2006; Dupont et al., 2009; Han et al., 2012; Gao et al., 2015; Ma et al., 2016), which are consistent with our findings. Therefore, physicians should consider this risk factor when selecting various chemotherapeutic agents for patients. In summary, the discovery of novel suspected AE signals provides objective evidence for the safe and effective application of IMiDs.

Among the AE-related mortalities associated with IMiD therapy, pneumonia, sepsis, renal failure, and neutropenia ranked as the most common causes. Infectious pneumonia may be related to inherent humoral and therapy-induced immunosuppression of hematological diseases, which is an important cause of morbidity. A systematic review and meta-analysis by Chen et al. revealed that RRMM patients receiving pomalidomide had the highest rate of severe infections in randomized controlled trials and observational studies (Chen et al., 2018), which are consistent with our findings. Sepsis is triggered by infection, and neutropenia increases the risk of infection. Lenalidomide combined with high-dose dexamethasone resulted in grade 3–4 infections in 10%–22% of patients with MM (Dimopoulos et al., 2007; Weber et al., 2007). Meanwhile, grade 3–4 infections occurred in 7%–14% of MM patients treated with thalidomide and glucocorticoid (Palumbo et al., 2006; Facon et al., 2007; Rajkumar et al., 2008). Furthermore, a severe infection rate of 23% was observed among patients with MM undergoing pomalidomide-based regimens (Chen et al., 2018). Moreover, IMiDs also exhibit different pharmacological profiles. The metabolic pathway of lenalidomide is mainly related to renal function (Jelinek et al., 2016). Similarly, the toxicity of thalidomide on renal function is not negligible (Seldin et al., 2003). In contrast, pomalidomide shows promising efficacy and favorable toxicity profiles in patients with renal insufficiency (Jelinek et al., 2016). Renal insufficiency is a typical clinical finding in patients with MM. Therefore, assessment of renal function is recommended before selecting therapeutic regimens to avoid aggravating renal failure and accelerating patient death.

During our study period, the thalidomide-related AEs reports was small in quantity, which may be due to the increased use of lenalidomide and pomalidomide. Additionally, reports of thalidomide causing congenital deformities in neonates have resulted in its decreased use. Although it was approved for treatment of MM, its use has not been widespread compared to the other IMiDs (Millen, 1962). Furthermore, we did not observe the Weber effect in AE reports of IMiDs, and the reason for this is likely multifactorial. First, the pharmaceutical industry and the general public have gradually increased their awareness of drug safety, and AE prevention has received greater attention (Hoffman et al., 2014). Second, institutions engaged in risk evaluation and mitigation strategies, more stringent regulatory authorities, and the convenience brought by the internet have promoted the reporting of AEs (United States Food and Drug Administration (USFDA), 2017; United States Food and Drug Administration (USFDA), 2022; Ilic, 2010; Hart et al., 2004).

Data mining of FAERS can effectively compensate for the shortcomings of clinical trials, such as a small sample size, narrow coverage, and short observation time; however, there are still some limitations regarding this method. First, most reports in FAERS are from the United States, and the results of this study may not be generalizable due to variations in drug usage and ethnicity among different countries (Sakaeda et al., 2013). Second, since the FAERS database is a spontaneous reporting system, some problems inevitably occur, such as underreporting and incomplete or inaccurate reporting. Therefore, bias in the results is expected (Pariente et al., 2007). Finally, the FDA has no requirement for demonstrating the causal involvement of AEs and drugs before reporting. Thus, the risk signals obtained by disproportionality analysis can only indicate statistical significance rather than biological significance (FDA Adverse Events Reporting System (FAERS) Public Dashboard, 2022). Overall, our results do not represent the inevitable causal relationship between a drug and AE. Nonetheless, the FAERS database remains a unique and important tool for post-marketing safety surveillance of approved drugs.

5 Conclusion

We reviewed the safety profiles of thalidomide, lenalidomide, and pomalidomide based on AEs submitted to the FAERS database from 2013Q1 to 2021Q4. According to 296,960 reports, AEs with IMiDs occurred in multiple organs and tissues, including the cardiac, vascular, respiratory, and integumentary systems. IMiDs have different safety profiles that may cause serious AEs, resulting in treatment discontinuation or patient mortality. Clinicians should be aware of these differences and adjust treatment regimens for different patients to improve patient compliance and reduce the risk of AEs. Although several post-marketing safety signals that were off label were found, prospective clinical trials are necessary to confirm these findings.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://fis.fda.gov/extensions/FPD-QDE-FAERS/FPD-QDE-FAERS.html.

Author contributions

YL conceived and supervised the project. YL and TJ contributed to the design, analysis and interpretation of data in the study. TJ and HS contributed to the acquisition of the data. TJ drafted the manuscript. All the authors were involved in the study, critically revised the manuscript, and gave final approval.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.989032/full#supplementary-material

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Treatment for chemotherapy-induced peripheral neuropathy: A systematic review of randomized control trials

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Purpose: Treatment of chemotherapy-induced peripheral neuropathy (CIPN) is challenging for clinicians, and many clinical trials and meta-analyses on CIPN are controversial. There are also few comparisons of the efficacy among drugs used to treat CIPN. Therefore, this systematic review aimed to study the efficacy of drugs in treating CIPN using existing randomized controlled trials.

Methods: Electronic databases were searched for randomized controlled trials (RCTs) involving any pharmaceutical intervention and/or combination therapy of treating CIPN.

Results: Seventeen RCTs investigating 16 drug categories, duloxetine, pregabalin, crocin, tetrodotoxin, venlafaxine, monosialotetrahexosyl ganglioside (GM1), lamotrigine, KA (ketamine and amitriptyline) cream, nortriptyline, amitriptyline, topical *Citrullus colocynthis* (bitter apple) oil, BAK (baclofen, amitriptyline hydrochloride, and ketamine) pluronic lecithin organogel, gabapentin, and acetyl L-carnitine (ALC), in the treatment of CIPN were retrieved. Many of the included RCTs consisted of small sample sizes and short follow-up periods. It was difficult to quantify due to the highly variable nature of outcome indicators.

Conclusion: Duloxetine, venlafaxine, pregabalin, crocin, tetrodotoxin, and monosialotetrahexosyl ganglioside exhibited some beneficial effects in treating CIPN. Duloxetine, GM1, and crocin showed moderate benefits based on the evidence review, while lamotrigine, KA cream, nortriptyline, amitriptyline, and topical *Citrullus colocynthis* (bitter apple) oil were not beneficial. Further studies were necessary to confirm the efficacy of gabapentin in the treatment of CIPN because of the controversy of efficacy of gabapentin. Furthermore, BAK topicalcompound analgesic gel only had a tendency to improve the CIPN symptoms, but the difference was not statistically significant. ALC might result in worsening CIPN. Most studies were not of good quality because of small sample sizes. Therefore, standardized randomized controlled trials with large samples were needed to critically assess the effectiveness of these drugs in treating CIPN in the future.

KEYWORDS

chemotherapy-induced peripheral neuropathy (CIPN), rrandomised controlled trial, drugs, treatment, efficacy, safety, systematic review

1 Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is one of the main dose-limiting side effects of neurotoxic anticancer drugs. The chemotherapy dose needs to be reduced or completely paused when CIPN develops. All of the commonly used chemotherapeutic drugs such as taxanes, platinum derivatives, vinca alkaloids, thalidomide, and bortezomib all can cause CIPN (Staff et al., 2017; Shah et al., 2018; Colvin, 2019). Regarding overall neurotoxic chemotherapy, after completion of chemotherapy, the incidence of CIPN was approximately 68% after 1 month, 60% after 3 months, and 30% after 6 months and above (Colvin, 2019). For a particular chemotherapy drug, the incidence of CIPN varied among many previous reports. However, taxanes and platinum derivatives were the most prone to develop CIPN (Shah et al., 2018; Colvin, 2019). CIPN is mainly characterized by sensory nerve symptoms, presenting with glove and stocking pain, and patients often report numbness, tingling, and pain. CIPN can also be accompanied by motor or autonomic nerve symptoms (Loprinzi et al., 2020). Additional medications or other interventional measures are often required to treat these symptoms that otherwise seriously affect the patient's quality of life, and these remedial measures cause financial burdens on the patients (Miaskowski et al., 2018). The average monthly drug treatment costs for CIPN ranged from USD 15 to USD 1425. Among duloxetine, gabapentin, pregabalin, amitriptyline, nortriptyline, and venlafaxine, the average monthly costs of duloxetine ranged from USD 241 to USD 637 (Gupta et al., 2022). It is worth mentioning that duloxetine is the only drug recommended for painful CIPN (intermediate evidence quality, moderate strength of recommendation), and no agents are recommended for the prevention of CIPN, suggested by American society of clinical oncology (ASCO) guidelines (Loprinzi et al., 2020). The treatment of CIPN is a significant issue, but numerous existing clinical trials and meta-analyses on the treatment of CIPN are still controversial. Furthermore, head-to-head clinical trials are rare. It is urgent to evaluate and find out new and superior drugs in treating CIPN since the evidence is scanty in comparing their efficacy. Therefore, this systematic review aimed to estimate the efficacy of drugs in treating CIPN to provide a reference for clinical practice and future research.

2 Material and methods

This systematic review was performed following the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (Moher et al., 2015). The protocol is available in the PROSPERO database (ID number: CRD42022334388). Although we initially planned to conduct systematic review and meta-analysis, we only conducted systematic review due to the variability in outcome indicators and small sample size in each drug category.

2.1 Search strategies and selection criteria

We searched the PubMed, EMBASE, and Cochrane library databases from their inception to 20 March 2022. Then, additional electronic database searches were conducted to obtain comprehensive and up-to-date information, up to 31 August 2022. We used the Medical Subject Headings (Mesh) and their free words for "chemotherapy", "peripheral neuropathy", and "randomized controlled trial", and their respective subject terms and free words were linked by "OR", followed by "And", and the title and abstract were searched.

Open or blinded studies have been included and all included trials met the following criteria: (1) Adult patients (age of ≥18 years); (2) Patients who developed chemotherapy-induced peripheral neuropathy; (3) CIPN with any single drug intervention and/or combination of drugs administration; and (4) randomized controlled trials (RCTs). The following criteria were used for exclusion: (1) Patients with other neuropathic diseases such as diabetes, acquired immune deficiency syndrome (AIDS/HIV), vitamin B12 deficiency, and serious mental disease; (2) Patients who underwent traditional Chinese medicine decoction and physical therapy; (3) Studies with non-human subjects and non-RCT design; (5) Duplicate studies, reviews, systematic reviews and meta-analyses, and case reports and case series; (6) Publications in non-English. Two authors independently screened and then comprehensively reviewed the titles, abstracts, and articles. Any disagreement between reviewers was resolved by consensus in all cases. The authors of the incomplete studies were contacted by email, but we did not receive a response.

2.2 Data extraction

Two researchers independently extracted relevant data from the RCTs (Table 1). If there was any disagreement with the data, they negotiated to reach a consensus. If the literature was unavailable or the data was lacking, we would try our best to contact the author to obtain related resources. If the outcome indicators were only shown in a graphical presentation, Engauge Digitizer software was used to extract the data.

TABLE 1 Extracted data characteristics.

Basic information of Included Trials	First Author and year of Publication
Characteristics of the research subjects	Total number of participants and the number of each group, gender of patients in each group, and age (mean ± SD)
Intervention	Chemotherapeutic dosage, course of treatment, and others
Key elements of bias risk	RandomACT sequence generation, blinding, allocation concealment, completeness of outcome data, selective reporting, measurement bias, and other bias
Outcome	All outcome indicators in each study such as average change of pain score (mean ± SD), quality of life score, and others

2.3 Risk assessment of bias

Two researchers used the Cochrane collaboration's risk of bias tool to determine the bias risk of all included randomized controlled trials (Higgins et al., 2011). The following seven items were evaluated, including random sequence generation, blinding of participants and personnel, blinding of outcome assessment, allocation concealment, incomplete outcome data, selective reporting, and other bias. The result of the evaluation on each item was "low risk", "unclear risk", or "high risk". In case of a disagreement, the two executors reached a consensus through negotiation and discussion. If the dispute was still unresolved, the decision was made by a third party.

2.4 Assessment of evidence quality

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach was used to evaluate the quality or certainty of each compared evidence (Salanti et al., 2014). The five degraded factors in the GRADE assessment were risk of bias, inconsistency, indirectness, imprecision, and publication bias. The three upgraded factors in the GRADE assessment were large effect, plausible confounding would change the effect, and dose response gradi. The recommended quality of the final report was divided into high, medium, low, and extremely low. Moreover, the recommended strength was divided into strong and weak. The evidence of high quality indicates that the effect value is very close to the actual value, a strong recommendation indicates that the advantages outweigh the disadvantages, and a weak recommendation means that the disadvantages outweigh the advantages.

2.5 Outcome

The changes in pain and neuropathic symptoms were our primary measures of concern, but all outcome measures are summarized for each study due to variations in outcome measures among RCTs.

3 Results

3.1 Study selection

Seventeen RCTs involving 16 medication classes ultimately met the inclusion criteria (Table 2 and Figure 1). Among these RCTs, five studies were conducted with duloxetine, three trials were carried out with pregabalin, and two studies were conducted with gabapentin. Lamotrigine, crocin, tetrodotoxin, nortriptyline, amitriptyline, acetyl L-carnitine, venlafaxine, GM1 (Monosialotetrahexosyl ganglioside), KA (ketamine and amitriptyline) cream, BAK (baclofen, amitriptyline, and ketamine) pluronic lecithin organogel, and topical *C. colocynthis* oil were investigated in one study each.

3.2 Study characteristics

Seventeen RCTs from seven countries were included in the final analysis and the average age was 60 years. The studies were published from 2007 to 2021 (Figure 2), and the lowest score of the impact factor was 1.4 and the highest score was 51 [the first RCT to demonstrate the therapeutic effect of duloxetine for CIPN published in 2013 (Smith et al., 2013)]. The sample sizes ranged from 32 to 462. Additionally, intervention drugs were gabapentin, lamotrigine, duloxetine, venlafaxine, ketamine and amitriptyline (KA) cream, vitamin B12, crocin tablets, tetrodotoxin, nortriptyline, baclofen, amitriptyline hydrochloride and ketamine pluronic lecithin organogel (BAK-PLO), acetyl L-Carnitine (ALC), pregabalin, topical *C. colocynthis* oil, and monosialotetrahexosyl ganglioside (GM1). Most patients developed peripheral neuropathy caused by platinum or taxane. The basic characteristics of patients and reasons for inclusion are detailed in Table 3.

3.3 Risk of bias and quality of evidence assessments

Most of RCTs included in the analysis had a low risk of bias. Two studies showed high-risk bias due to poor blinding of participants and other personnel involved in the trial

TABLE 2 The type of therapeutics and the number of relevant RCTs.

	Type of treatments	The number of relevant RCTs
C	Crocin	1
D	Duloxetine	5
G	Gabapentin	2
L	Lamotrigine	1
KA	Ketamine plus Amitriptyline	1
P	Placebo	13
Pg	Pregabalin	3
T	Tetrodotoxin	1
VB_{12}	Vitamin B_{12}	1
N	Nortriptyline	1
A	Amitriptyline	1
BAK	Baclofen plus Amitriptyline plus Ketamine	1
ALC	Acetyl L-Carnitine	1
V	Venlafaxine	1
TC	Topical *C. colocynthis* oil	1
GM1	Monosialotetrahexosyl ganglioside	1

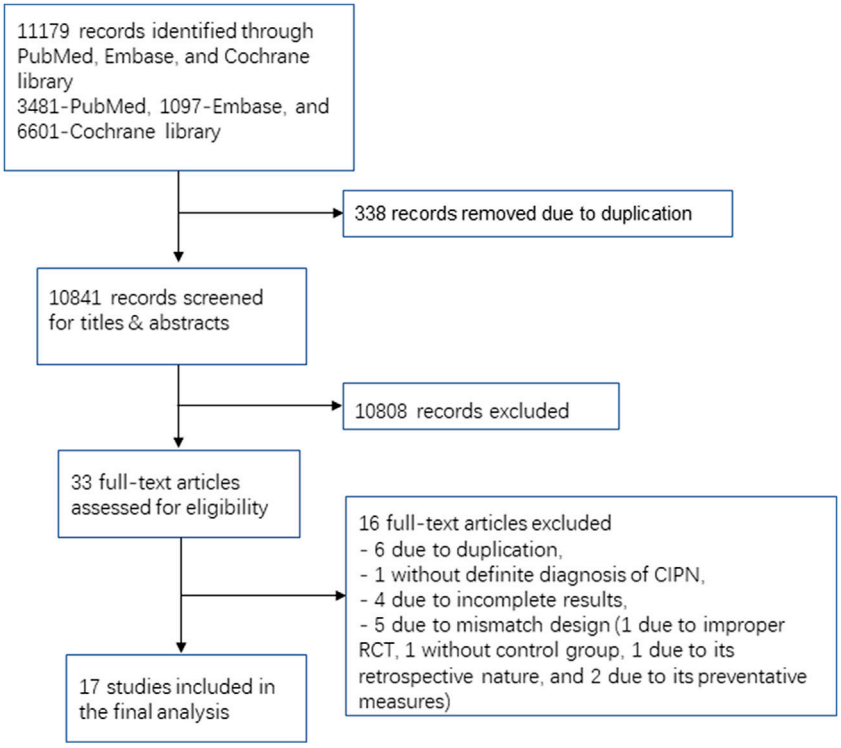

FIGURE 1
Flow diagram.

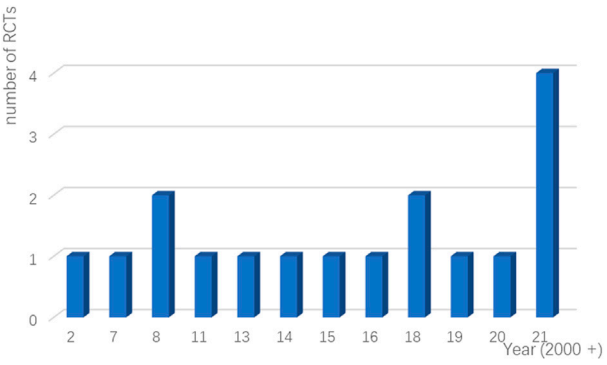

FIGURE 2
Number of RCTs on CIPN treatment published each year from 2007 to 2021.

(performance bias), and only one study had a high risk due to inadequate blinding of outcome assessment (detection bias). The details are shown in Figure 3.

Upon assessing the results for the quality of evidence using the GRADE approach, most of the evidence was of very low or low quality (Table 4).

3.4 Study results

Five studies did not demonstrate any effectiveness for lamotrigine, KA cream, nortriptyline, amitriptyline, and topical *C. colocynthis* oil. A study on BAK (baclofen, amitriptyline, and ketamine) pluronic lecithin organogel found that the sensory neuropathy subscale was improved but without statistical significance. However, duloxetine, venlafaxine, pregabalin, crocin, tetrodotoxin, acetyl L-carnitine, and monosialotetrahexosyl ganglioside were effective for CIPN. A subsequent study on ALC (Hershman et al., 2018) found that long-term (24 weeks) ALC treatment worsened the CIPN over 2 years. Furthermore, the efficacy of gabapentin in the treatment of CIPN was disputed in two studies. The detailed study results such as intervention, duration of intervention, and outcome indicators are summarized in Supplementary Table S1.

3.4.1 Gabapentin

A study conducted in the United States in 2007 (Rao et al., 2007) and a study conducted in India in 2021 (Kim et al., 2018) investigated the effect of gabapentin in treating CIPN. However, the results from these two studies were debatable. The former reported no benefit with gabapentin treatment, while the latter showed benefit with the same treatment. All indicators such as the symptoms of pain, the quality of life, and the WHO neuropathy score did not statistically differ from those of the placebo group in the former study, whereas gabapentin and pregabalin improved the pain caused by CIPN in the latter study. After 8 weeks of treatment of, the VAS decreased from 8.3 ± 1.43 to 1.8 ± 2.51 ($p < 0.0001$) in gabapentin and from 8.2 ± 1.62 to 0.8 ± 0.96 ($p < 0.0001$) in pregabalin. Pain quality assessment scale (PQAS) score reduced from 34.8 ± 6.67 at baseline to 10.2 ± 10.96 after gabapentin treatment and from 36.9 ± 8.5 to 4.5 ± 3.66 in the pregabalin arm ($p < 0.0001$). Furthermore, 2 (6.06%) patients in the gabapentin group and 1 (3.33%) patient in the pregabalin arm required rescue medications. A summary of the information is shown in Table 5. In Rao et al., 2007, no difference in the incidence of adverse events was found between the treatment and placebo groups. In Kim et al., 2018, the incidence of adverse events was higher with gabapentin (21.1%) treatment than with pregabalin (16.6%) treatment. Meanwhile, sedation (6.60%), drowsiness (9.09%), and diplopia and blurring of vision (3.03%) were the common adverse events in the gabapentin arm, whereas adverse events, sedation (13.3%) and drowsiness (3.3%) frequently occurred in the pregabalin group.

3.4.2 Lamotrigine

Lamotrigine was investigated in a 10-week double-blinded RCT with a total sample size of 125, conducted in 2008 in the United States (Rao et al., 2008). CIPN symptoms with ≥ 1-month duration, caused by taxanes, platinum compounds, and vinca alkaloids were treated with lamotrigine, and the dose was gradually increased from 25 mg to 150 mg. However, pain, depression, and quality of life were not improved, and lamotrigine treatment was ineffective for patients with CIPN. There was no statistically significant difference in adverse events between the two arms. The most common adverse events were ataxia, rash, constipation, arthralgia, gastrointestinal reaction, pruritis, fatigue, and headache.

3.4.3 Nortriptyline

Nortriptyline was investigated in a double-blinded, randomized, controlled, crossover study in the treatment of platinum-induced peripheral neuropathy, and this study was conducted in the United States in 2002 (Hammack et al., 2002). The sample size was 91, and the treatment lasted for 9 weeks. Nortriptyline tablets started at a dose of 25 mg/d and increased by 25 mg/d every other week to a maximum dose of 100 mg/d.

In summary, nortriptyline did not improve pain and quality of life in patients with peripheral neuropathic symptoms caused by platinum chemotherapy, but it improved patients' sleep. The changes in daily life scores impacted by pain in the nortriptyline and placebo groups were −0.3 and 0.2, respectively, in phase I of this crossover study ($p = 0.04$), and the change in sleep time in the nortriptyline and placebo groups were 0.5 and −0.3 ($p = 0.02$), respectively, in phase I of this crossover study. This

TABLE 3 Basic characteristics of the subjects and information for inclusion.

Included Studies	Country	Mean/Median Age (years) (Range/SD)		Sex (male/Female)		Patients	Anticancer drugs
		Treatment	Control	Treatment	Control		
Rao et al. (2007)	United States	59 (28–84)	60 (25–80)	15/42	16/42	Patients, with CIPN, whose duration of ≥1 month	Taxanes, platinum compounds, and vinca alkaloids
Rao et al. (2008)	United States	62 (29–84)	59 (34–82)	27/36	24/38	Patients, with CIPN, whose duration of ≥1 month	Taxanes, platinum compounds, and vinca alkaloids
Smith et al. (2013)	Philippines	60 (10.4)	59 (10.6)	38/71	44/67	CIPN patients (sensory neuropathy of ≥grade 1 and pain score of ≥4)	Taxanes, platinum
Gewandter et al. (2014)	United States	NA	NA	73/156	62/171	Patients with CIPN, pain score of ≥4 and Karnofsky performance status of >60	Taxanes 246 (53%)
Hirayama et al. (2015)	JPN	61 (48–7)	64 (49–75)	8/9	9/8	Patients with CIPN, sensory neuropathy of >1 and pain score of ≥4	Taxanes and platinum
Manjushree et al., 2021	India	50.6 (12)	53 (7.6)	9/24	9/21	Patients with CIPN	Paclitaxel, carboplatin, bortezomib, thalidomide, vincristine, oxaliplatin, cisplatin
Bozorgi et al. (2021)	Iran	61 (27–84)	62 (25–89)	41/48	42/46	Patients, with CIPN, pain scores≥4 and duration≥1 month	Taxanes, platinum compounds, and vinca alkaloids
Goldlust et al. (2021)	United States	60.6 (11.1)	59 (1.5)	10/16	10/15	patients with CIPN	Taxanes and platinum compounds
Hammack et al. (2002)	United States	58.7	58.7	NA	NA	CIPN patients with paresthesia and pain for at least 1 month	Cis-platinum
Kautio et al. (2008)	Finland	52 (37–67)	54 (35–67)	3/14	5/11	Patients with neuropathy presenting with numbness, tingling, or with pain score of ≥3	Taxus, platinum, or vinblastine
Barton et al. (2011)	United States	59.9 (10.75)	62.1 (10.27)	35/66	42/60	Patients with CIPN, symptom duration of >1 month	Taxus, platinum, vinblastine, thalidomide and other drugs
SUN et al. (2016)	China	NA	NA	NA	NA	Patients with CIPN, symptom duration of ≥1 month	Paclitaxel, cisplatin, or vinblastine
Avan et al. (2018)	Iran	Pregabalin, NA (29–72)	Duloxetine, NA (30–71)	NA	NA	Breast cancer patients with sensory neuropathy and pain score of ≥4	Taxane
Farshchian et al. (2018)	Iran	Duloxetine, 63.85 (7.58)	Duloxetine 15/37	Duloxetine 15/37	Placebo 10/42	Patients with CIPN	Taxane and Platinum
		Venlafaxine, 57.44 (14.53)	Venlafaxine 7/45	Venlafaxine 7/45			
Rostami et al. (2019)	Iran	59.23 (13.08)	55.25 (11.19)	5/12	6/9	Cancer patients diagnosed by neurologist as peripheral neuropathy and received chemotherapy over the previous 2 months	Taxane/oxaliplatin
Salehifar et al. (2020)	Iran	49.4 (9.67)	48.7 (9.63)	Pregabalin, total 40	Duloxetine, total 42	Patients (sensory neuropathy of ≥grade 1 and VAS score of ≥4)	Paclitaxel or docetaxel

(Continued on following page)

TABLE 3 (*Continued*) Basic characteristics of the subjects and information for inclusion.

Included Studies	Country	Mean/Median Age (years) (Range/SD)		Sex (male/Female)		Patients	Anticancer drugs
		Treatment	Control	Treatment	Control		
Zhou et al. (2021)	China	60 (23–79)	60 (24–75)	52/21	45/27	Patients with chronic OIPN	Oxaliplatin

improvement was most likely due to its adverse effect on sleepiness. The incidence of somnolence was 64% and 41% in the nortriptyline arm and placebo arm, respectively.

Frequently occurred adverse events were sleepiness (64% vs. 41%, p = 0.09), dry mouth (83% vs. 46%, p = 0.001), dizziness (49% vs. 15%, p = 0.002), impaired thinking (23% vs. 12%, p = 0.34), and constipation (54% vs. 34%, p = 0.1) in the nortriptyline arm and placebo arm, respectively.

3.4.4 Amitriptyline

Amitriptyline was explored in an eight-week double-blinded randomized controlled trial conducted in 2008 in Finland (Kautio et al., 2008). The sample size was 33, and patients developed CIPN due to taxanes, platinum, or vinblastine chemotherapy. Amitriptyline capsules started at 10 mg/d and gradually increased to 50 mg/d. Compared with the placebo arm, only the Quality of Life measured by the global health score, EORTC QLQ-C30 was improved (p = 0.038) in the amitriptyline arm. Although amitriptyline reduced the number of times patients woke up during the night (9 vs. 5, amitriptyline vs. placebo, respectively), it was not statistically significant. The duration of sleep did not change significantly in either group. Furthermore, there were no statistically significant differences in the severity of the neuropathic symptoms, physical activity, depression scale and global improvement between the two groups. Similar to nortriptyline, drowsiness was one of the main adverse reactions with amitriptyline. Nortriptyline is an active metabolite of amitriptyline.

3.4.5 KA cream

A study investigated the effect and adverse events of KA (ketamine and amitriptyline) topical application for CIPN in 462 participants (Gewandter et al., 2014). KA cream was topically applied at the maximum dose of 4 g twice daily. The overall course of interventions was 6 weeks. A statistically insignificant effect of KA cream in relieving CIPN-related pain (the change of mean pain score, −0.208, 95% Cl, −0.694 to 0.278, p = 0.4) was observed. However, patients in the taxane arm experienced greater pain relief than those in the non-taxane arm (the change mean pain score: 0.398, 95%Cl, -0.782 to -0.015, p = 0.042). The topical application of KA cream was also well tolerated.

3.4.6 BAK pluronic lecithin organogel

One study investigated the effect and tolerance of BAK pluronic lecithin organogel (BAK-PLO; 1.31 g compound gel containing 10 mg baclofen, 40 mg amitriptyline hydrochloride, and 20 mg ketamine) for CIPN symptoms in 203 participants (Barton et al., 2011). BAK-PLO was applied topically twice daily. The duration of treatment was 4 weeks. Sensory neuropathic symptoms tended to improve. The mean changes in the sensory neuropathy subscale from baseline to 4 weeks were 8.1 ± 15.05 in the BAK arm and 3.8 ± 15.52 in the placebo arm (p = 0.053). However, BAK-PLO did not improve the CIPN-related pain, reduce the incidence of adverse events or improve the Profile of Mood States (POMS) score. The POMS evaluated the current or recent emotional state, such as tension, depression, anger, energy, fatigue, and confusion.

3.4.7 Topical citrullus colocynthis (bitter apple) application

The effect and safety of topical *Citrullus colocynthis* (bitter apple) were evaluated in a double-blinded RCT for CIPN symptoms in 32 participants (Rostami et al., 2019). The topical preparation was applied locally on hands and feet twice daily, 2 ml each time. The study period was 4 weeks, and the Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity (FACT/GOG-Ntx) score was the only curative indicator analyzed. At the end of treatment, no significant improvement was observed in this index and the incidence of adverse events. The total score of the FACT/GOG-Ntx scale was 1.05 ± 1.36 and 2.40 ± 1.90 (p = 0.879) in the intervention and placebo groups, respectively.

3.4.8 Crocin

Crocin was investigated in an 18-week open-labeled, randomized, controlled, crossover study specific to the curative effect of CIPN through the scores of pain, quality of life, ENS, and sensory neuropathy (Bozorgi et al., 2021). The total sample size was 177. Crocin tablets that were derived from traditional Persian medicine were given at a dose of 15 mg twice daily (each crocin tablet contains 15 mg of crocin). Compared with the placebo arm, crocin relieved the symptoms of CIPN, such as pain, paresthesia, and depression. Furthermore, crocin improved the quality of life. Compared with the placebo arm, the changes in mean scores in the crocin arm were -2.5 for NRS mean pain (p = 0.002), -0.4 for BPI (p = 0.009),

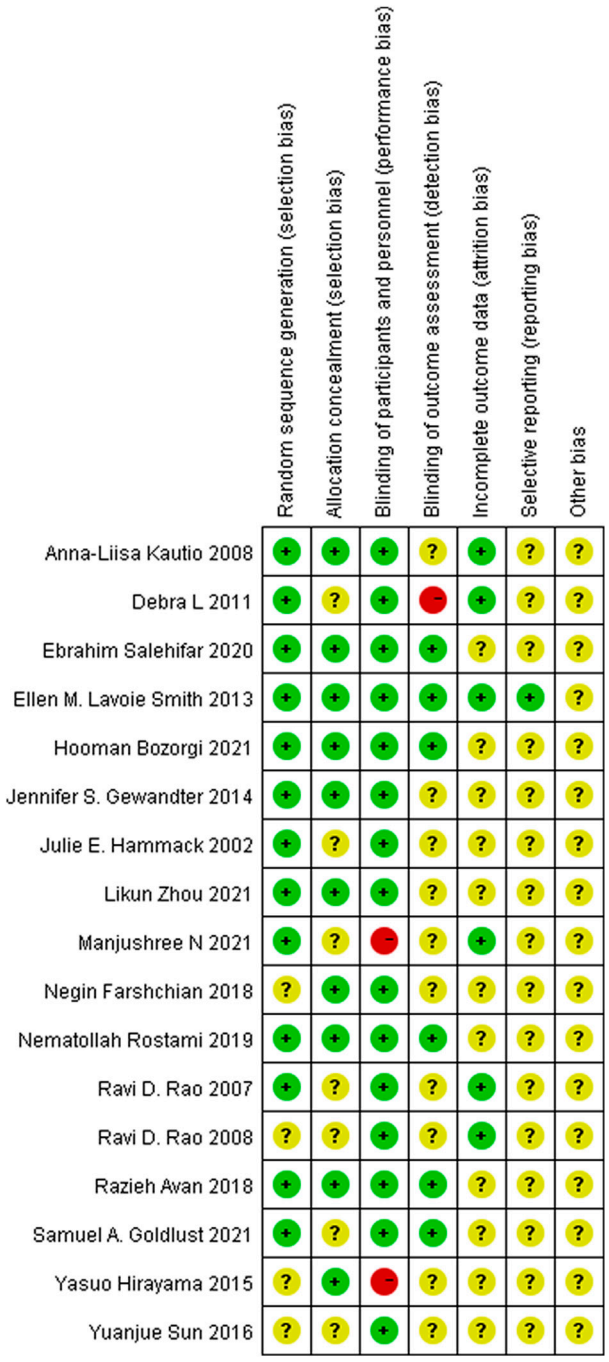

FIGURE 3
Risk of bias summary.

-8.3 for McGill pain rating index ($p = 0.005$), −0.04 for ENS ($p = 0.007$), −0.8 for NCIC-CTC scale ($p = 0.005$), -0.8 for WHO scale ($p = 0.003$), −7.2 for SDS ($p = 0.009$), −0.9 for NPS ($p = 0.005$), +0.6 for SGIC ($p < 0.005$), and +8.1 QOL scales ($p = 0.009$). However, the number of withdrawals and the incidence of adverse events in patients treated with crocin were slightly higher than those treated with a placebo (15.7% vs. 8%, $p = 0.12$). The most common adverse events were grade 1 (except nausea) and were increased appetite (14.7%; 2.9%), sedation (8.8%; 5.8%), headache (8.8%; 5.8%), nausea (8.8%; 2.9%), hypomania (5.8%; 5.8%), stomachache (5.8%; 2.9%), vomiting (2.9%; 2.9%), and swelling of feet (2.9%; 0%) in the crocin and placebo groups, respectively.

3.4.9 Tetrodotoxin

Tetrodotoxin (TTX) was investigated in a double-blinded randomized controlled trial with a sample size of 51, conducted in 2021 in the United States (Goldlust et al., 2021). CIPN symptoms were caused by taxanes and platinum compounds. Tetrodotoxin was given 30 μg subcutaneously twice daily and the duration of treatment was 4 weeks. TTX improved the pain symptoms in patients with CIPN. Compared with the placebo, TTX made a statistically significant improvement in the SF-36 body pain score ($p = 0.004$), the EORTC CIPN20 sensory symptom subscale ($p = 0.091$), and physical component subscales ($p = 0.076$) on day 28. The change in mean pain was a -1.5 ± 1.8 score in the fourth week, and pain relief was best by 3 weeks. Meanwhile, TTX at 30 μg bid achieved 30% pain relief in 30.8% of patients during the first week of treatment and 38.5% of patients on day 28. However, most patients (80%–92.3%) experienced more than one AE due to TTX. The incidence of adverse reactions, such as oral paresthesia, oral hypoesthesia, headache, dizziness, nausea, and limb pain was higher than placebo (oral paresthesia, 42.3% vs. 16.0%; oral hypoesthesia, 38.5% vs. 20%; paresthesia, 26.9% vs. 20%; headache, 34.6% vs. 24%; dizziness, 30.8% vs. 12%; fatigue, 11.5% vs. 16%; nausea, 23.1% vs. 4%; limb pain, 11.5% vs. 4%).

3.4.10 Acetyl L-carnitine

A study investigated the effect and tolerance of acetyl L-carnitine (ALC) in 462 participants who developed CIPN symptoms caused by taxanes, platinum, or vinblastine (Sun et al., 2016). This was an 8-week study in which ALC enteric-coated tablets were given 1 g twice daily. At the end of treatment (8 weeks after the onset of intervention), the neurotoxicity was improved in 50.5% of the patients in the ALC arm, compared with a 24.1% reduction in the placebo arm (95%CI, 14.1%–38.5%, $p < 0.001$). Only the Nerve conductive velocity (NCV) of the sural nerve was significantly different between the ALC and placebo groups. Other neurological NCV tests found no difference between the two groups. Additionally, ALC therapy significantly improved the NCV in the ALC arm (60.7%), compared with the placebo arm (56.9%; $p < 0.05$). ALC treatment also reduced cancer-associated fatigue, and the difference was significant between the two arms on week 8 (33.7% vs. 18.5%, $p = 0.014$) and week 12 (41.1% vs. 25%, $p < 0.015$) through PPS (per protocol set). However, the difference was not statistically significant after 8 weeks of treatment between the ALC (31.2%) and placebo (19.8%) groups ($p = 0.0501$)

TABLE 4 Grade Assessment.

Comparison	Downgrade quality of evidence					Upgrade quality of evidence		
	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Large effect	Plausible confounding would change the effect	Dose response gradi
Very low								
G vs. P	S	N	N	VS	Un	N	N	N
L vs. P	S	N	N	VS	Un	N	N	N
KA vs. P	N	N	N	VS	Un	N	N	N
G vs. Pg	S	N	N	VS	Un	N	N	N
T vs. P	S	N	N	VS	Un	N	N	N
BAK vs. P	S	N	N	VS	Un	N	N	N
D vs. Pg	S	S	S	VS	Un	N	N	N
Low								
D vs. VB12	S	N	N	VS	Un	VL	N	N
N vs. P	N	N	N	VS	Un	N	N	N
A vs. P	N	N	N	VS	Un	N	N	N
TC vs. P	N	N	N	VS	Un	N	N	N
ALC vs. P	S	N	N	VS	Un	L	N	N
V vs. P	N	N	N	VS	Un	N	N	N
Moderate								
D vs. P	N	N	N	VS	Un	L	N	N
C vs. P	N	N	N	VS	Un	L	N	N
GM1 vs. P	N	N	N	VS	Un	L	N	N

N: No; S: Serious; VS: Very Serious; Un: Undetected; L: Large; VL: Very Large.
N: +0; S: 1; VS: 2; Un: +0; L: +1; VL: +2.

through the full analysis set (FAS). Compared with the placebo group (13.0%), ALC caused a statistically significant improvement in Karnofsky physical score (KPS) (29.3%; $p < 0.05$). ALC had no severe adverse reactions. The common adverse events were gastrointestinal reactions such as vomiting, abdominal distension, and diarrhea. Also, no significant difference in the incidence of adverse events was found between the ALC (19.5%) and placebo (15.3%) groups ($p > 0.05$).

3.4.11 Monosialotetrahexosyl ganglioside

Zhou et al.'s study (Zhou et al., 2021) was the first study of the use of GM1 for the treatment rather than prevention of Oxaliplatin-induced peripheral neuropathy (OIPN). GM1 improved the symptoms of OIPN, such as pain and neurotoxicity, and GM1 was well tolerated. MCIPN, a new author-defined patient reported outcome measure indicator (≥30% improvement for the relief of neurotoxicity) modified EORTC QLQ-CIPN20, was used for neurotoxicity in patients with CIPN. A 30% improvement was considered a response and a 30%–50% improvement was considered a high response (MCIPN responders: 53% vs. 14%, $p < 0.0001$; VAS responders: 49% vs. 22%, $p = 0.001$; double responders: 41% vs. 7%, $p < 0.0001$; high responders: 32% vs. 13%, $p = 0.004$).

3.4.12 Duloxetine

The efficacy of duloxetine in relieving CIPN symptoms was studied in 5 RCTs (Smith et al., 2013; Hirayama et al., 2015; Avan et al., 2018; Farshchian et al., 2018; Salehifar et al., 2020). In all five elucidations, duloxetine was effective in the treatment of CIPN. Among these studies, four studies were head-to-head trials in which duloxetine was compared to pregabalin (Avan et al., 2018; Salehifar et al., 2020), VB12 (Hirayama et al., 2015), and venlafaxine (Farshchian et al., 2018). The remaining literature (Smith et al., 2013) was the first to report the efficacy of duloxetine in the treatment of CIPN, and duloxetine relieved pain from CIPN, compared with the placebo arm. Furthermore, duloxetine was more effective for CIPN caused by platinum compounds than taxane compounds. Hirayama et al. (2015) conducted an open-labeled, randomized, crossover study with duloxetine for CIPN, and duloxetine reduced pain caused by CIPN compared with VB12 in Japanese patients. In a study published in 2018, similar to duloxetine, pregabalin improved pain, overall health, and quality of life of patients. Interestingly, pregabalin was more effective than duloxetine in improving pain

TABLE 5 The summary of two studies about gabapentin.

Basic information of included studies	Country	Rao et al. (2007)	Manjushree et al., 2021
		United States	India
Participants	Mean Age (years	Gabapentin: 59 (28–84)	Gabapentin: 50.6 ± 12
	(Range/SD)	Placebo: 60 (25–80)	Pregabalin: 53 ± 7.6
	The total sample size	115	63
	Sex (Male/Female)	Gabapentin: 15/42	Gabapentin: 9/24
		Placebo: 16/42	Pregabalin: 9/21
	Type of chemotherapy	Taxanes (40%), platinum compounds (21%), and vinca alkaloids	Taxanes (79.35%), platinum compounds, vinca alkaloids bortezomib, and thalidomide
		(Combined chemotherapy accounted for 13%)	
Intervention	Dosage of administration	Gabapentin capsules were started at 300 mg/d and increased to 2700 mg/d within 3 weeks	Gabapentin, 300 mg, bid, P.O.
			Pregabalin 75 mg, bid, P.O.
	Intervention time (Weeks)	14 (2W)	8
Comparison	(Washout period)	Placebo	Pregabalin
Outcome		Gabapentin was not beneficial in the treatment of CIPN (See Supplementary Table S1 for details)	Both gabapentin and pregabalin relieved pain symptoms of CIPN(See Supplementary Table S1 for details)
Study design		RCT, open-label, crossover study	RCT, open-label study

($p < 0.001$) and insomnia ($p < 0.001$). However, improvement in the emotional functioning score was found only in the duloxetine arm ($p < 0.001$) (Avan et al., 2018). A RCT published in 2020 similarly concluded that pregabalin was more effective than duloxetine in the treatment of CIPN (Salehifar et al., 2020). Both venlafaxine and duloxetine reduced pain and symptoms of sensory and motor neuropathies in patients with CIPN, and venlafaxine improved hypertension. The hypertension frequency was 51.9% vs. 86.5% vs. 81.4% in venlafaxine, duloxetine, and placebo arms at week 4, respectively ($p < 0.001$). Furthermore, venlafaxine reduced hypertension frequency ($p < 0.05$) (Farshchian et al., 2018). Therefore, venlafaxine might be beneficial in patients with CIPN and hypertension. But generally, duloxetine was more effective than venlafaxine. The detailed results of the above studies are found in Supplementary Table S1.

3.4.13 Venlafaxine

Venlafaxine was investigated in a three-arm, double-blinded, randomized controlled trial of venlafaxine, duloxetine, and placebo with a total sample size of 156 and CIPN caused by taxane or platinum (Farshchian et al., 2018). The study lasted 4 weeks and was published from Iran in 2018. The dose of duloxetine was 30 mg/d, and the dosage of venlafaxine was 37.5 mg/d. Both venlafaxine and duloxetine improved the symptoms of CIPN. But only venlafaxine improved hypertension (Refer to Section 3.4.12 and Supplementary Table S1 for details).

3.4.14 Pregabalin

Pregabalin was investigated in 3 RCTs, and 2 of them compared pregabalin to duloxetine. The dosage and duration of treatment were consistent in both studies (pregabalin 75 mg/d at week 1 and 75 mg bid during week 2–6; duloxetine 30 mg/d at week 1 and 30 mg bid during week 2–6), and the sample size was 82 in both groups (Avan et al., 2018; Salehifar et al., 2020). However, pregabalin was compared with gabapentin in another 8-week study with a sample size of 63, and patients were given 75 mg of pregabalin orally twice daily or 300 mg of gabapentin orally twice daily (Manjushree et al., 2018). Manjushree et al. found that (Manjushree et al., 2018) both pregabalin and gabapentin significantly alleviated the patients' pain caused by CIPN. VAS with gabapentin decreased from 8.3 ± 1.43 to 1.8 ± 2.51 at the end of treatment ($p < 0.0001$) and VAS with pregabalin decreased from 8.2 ± 1.62 to 0.8 ± 0.96 at the end of treatment ($p < 0.0001$). Generally, pregabalin was superior to gabapentin in the treatment of CIPN. However, this conclusion was inconsistent with the conclusion from the study by Rao et al. (2017). Information on the two studies is detailed in Supplementary Table S1. Similarly, two studies on pregabalin

vs. duloxetine (Avan et al., 2018; Salehifar et al., 2020) were summarized in section 3.3.12. In summary, pregabalin was more effective than duloxetine and gabapentin.

4 Discussion

CIPN not only seriously affects the quality of life in patients but also brings additional economic burden to patients. A specific set of goals, transparent and reproducible methods, systematic and comprehensive searches, assessment of the validity of results including the risk of bias, and a systematic presentation of those results are required to evaluate the existing treatments or interventions of CIPN. ASCO presently recommends duloxetine as the sole treatment for painful CIPN. In this systematic review involving 17 RCTs, venlafaxine, pregabalin, crocin, tetrodotoxin, acetyl L-carnitine, and monosialotetrahexosyl ganglioside demonstrated some benefits in treating CIPN. However, only BAK topical analgesic gel improved CIPN symptoms without statistical significance. Before the elucidation on KA topical cream, only BAK topical analgesic gel (0.76% baclofen, 3% amitriptyline hydrochloride, and 1.5% ketamine) exhibited slight benefit, but not enough to conclude (Bozorgi et al., 2021). This might have been attributed to its lower dose and transdermal absorption. Compared with KA cream, BAK topical analgesic gel had an additional component baclofen, which might have contributed to more effectiveness of BAK compared to KA. Further, no subsequent studies on BAK were conducted. And two studies on the efficacy of gabapentin were controversial. Besides, the quality of evidence in most studies was not high, but the smaller sample size was the main problem. Therefore, we are unable to advise based on this evidence, and more future studies are needed to make definitive conclusions. Nevertheless, this systematic review can still provide reference value for conducting subsequent studies.

Based on the evidence to date, duloxetine is still effective and well-tolerated in the treatment of CIPN. However, no established standard for duloxetine in the treatment of CIPN is not presently available. From the analysis of all five RCTs included, duloxetine was beneficial for the treatment of CIPN. The dose of duloxetine ranged from 20 g/d to 60 g/d. Patients received orally 60 mg of duloxetine daily in the first published study in 2013 (Smith et al., 2013). The dosages of duloxetine in the subsequently published studies were 20 mg daily for the first week and 40 mg daily for the remainder of the study (Hirayama et al., 2015), 30 mg daily for the first week and 30 mg twice daily until 6 weeks (Avan et al., 2018), or 30 mg/d orally for 4 weeks (Farshchian et al., 2018). Although duloxetine was well tolerated without severe adverse reactions in the five RCTs analyzed a recent clinical, open-labeled experience identified the poor tolerance of duloxetine, with 20% of subjects dropping out due to lack of efficacy and 37% dropping out due to adverse events (Velasco et al., 2021). The incidence of adverse reactions (47%) and discontinuation rate (54.8%) of duloxetine were also quite high with long-term use. We included studies with treatment durations of 4–6 weeks and no studies with long-term follow-up. Further, nausea was the most commonly reported adverse effect leading to treatment discontinuation. Other common adverse reactions of duloxetine were dry mouth, insomnia, drowsiness, constipation, dizziness, and fatigue. Incidence of hepatic events such as liver injury (Kang et al., 2011; Xue et al., 2011; Malik et al., 2021), hyponatremia (Hu and Wurster, 2018; Wang et al., 2018; Ikeguchi et al., 2020), hyperprolactinemia and galactorrhea (Derle and Can, 2021), rapid eye movement sleep behavior disorder (Tan et al., 2017), weight loss (Poppen et al., 2021), and tachycardia (Stevens, 2008) were also reported due to duloxetine. Although duloxetine was well tolerated for 4–6 weeks of treatment based on the five RCTs analyzed, these adverse effects should be consistently monitored. Importantly, duloxetine needs to be discontinued slowly due to its untoward withdrawal symptoms (Fava et al., 2018). The pharmacokinetics of duloxetine also differ between specific populations. The bioavailability of duloxetine in female non-smokers is greater than that in male smokers, likely due to lower CYP1A2 enzyme activity in females than in males (Duloxetine is mainly metabolized through CYP1A2) (Knadler et al., 2011). The rate of elimination of duloxetine in elderly women (older than 65 years) is slower than that in younger women. Patients with liver insufficiency such as chronic liver disease or cirrhosis should avoid taking duloxetine due to its weak elimination ability (Knadler et al., 2011). Most of the metabolites of duloxetine (70%) were excreted in the urine. On population pharmacokinetic analyses, duloxetine should be avoided in patients with end-stage renal disease and severe renal impairment (CLCR of <30 ml/min), but it does not need to be adjusted in patients with mild-to-moderate renal impairment (CLCR of 30 and 80 ml/min) (Knadler et al., 2011). Duloxetine with FDA Grade C for pregnancy appeared to be safe for pregnant women. Two observational studies conducted in Sweden and Denmark demonstrated no increased risk of congenital malformations or stillbirth (Ankarfeldt et al., 2021a) and spontaneous or elective abortion (Ankarfeldt et al., 2021b), respectively. However, when the advantages outweigh the disadvantages, it can be used in pregnancy. But no pregnant women received chemotherapy because of the high teratogenicity, carcinogenicity, and mutagenicity of chemotherapeutic drugs. Duloxetine is mainly metabolized by CYP1A2 and CYP2D6, and duloxetine enteric-coated tablets can be affected by gastrointestinal PH. Besides, caution should also be taken for the possible occurrence of drug interactions when duloxetine is accompanied by alcohol or high plasma protein-binding drugs. The drug interactions between duloxetine and some specific drug can be referred to in this literature (Knadler et al., 2011).

Pregabalin was more effective than duloxetine in treating CIPN (Salehifar et al., 2020) and improved insomnia in

patients, and duloxetine improved patients' mood (Avan et al., 2018). But only two randomized placebo-controlled trials investigating pregabalin for the prevention of CIPN were identified and no benefits were observed for the prevention of CIPN (Shinde et al., 2016; de Andrade et al., 2017). But two RCTs investigating gabapentin had the opposite conclusion. Furthermore, although ALC was beneficial with an 8-week treatment (Sun et al., 201624 weeks of ALC therapy significantly worsened the CIPN symptoms in a long-term follow-up analysis over 2 years (Hershman et al., 2018). Therefore, future studies should be considered to draw firm conclusions.

Besides, standardized diagnostic criteria, study design, outcome indicators, and outcome measurement methods were lacking in these published studies. Due to variations in chemotherapy drugs used and outcome indicators in every study, it was difficult for us to conduct a quantitative meta-analysis. Thus, long-term studies with larger sample sizes should be implemented following a standardized study design, including the inclusion of patients, setting of outcome indicators, and validating measurement methods of outcome indicators to ensure a high degree of consistency. However, we put forward some advice about the therapeutics for chemotherapy-induced peripheral neuropathy.

First, we should focus on the basic information of patients with peripheral neuropathy, such as the chemotherapy drugs used before developing CIPN, gender, and duration of the CIPN symptoms. Since different types of chemotherapy drugs have different mechanisms of antitumor action, the mechanisms of the development of peripheral neuropathy caused by chemotherapeutics are also different. Hence, clarification of different types of chemotherapy drugs used is important to select drugs for clinical trials to alleviate CIPN. The mechanisms of the development of CIPN were quite complex and herein, a few studies were included for reference (Zajaczkowska et al., 2019; Bae et al., 2021; Burgess et al., 2021; Kang et al., 2021). In one study (Gewandter et al., 2014), although KA cream (2% ketamine and 4% amitriptyline) did not have benefits in patients with CIPN, patients in the taxane arm experienced a larger pain improvement than those in the non-taxane arm by the application of KA cream. At the same time, duloxetine showed a better analgesic effect for peripheral neuropathy induced by platinum compounds than taxane compounds (Smith et al., 2013). The chemotherapeutics that caused CIPN should be focused on the selection of drugs to alleviate CIPN. A recent observational study with a sample size of 100 found that female gender and short-lasting CIPN (<6 months) were independently associated with a favorable response to duloxetine (Velasco et al., 2021). A secondary analysis of a randomized controlled trial found that patients with better emotional states were more likely to report reduced pain from duloxetine ($p = 0.026$) (Smith et al., 2017).

Second, a set of rigorous diagnostic and evaluation criteria should be established. Currently, there are no unified diagnostic and evaluation criteria for CIPN. Similar problems existed in the studies we included in the analysis, and most studies had their own outcome metrics and measuring methods that prevented conducting a quantitative meta-analysis. Additionally, due to a common problem of small sample sizes, studies with larger sample sizes are required. Furthermore, long-term follow-up studies are also vital. Although a 12-week randomized controlled trial conducted in China found that oral administration of ALC (1000 mg three times daily) was effective in improving the symptoms of CIPN and physical conditions, and reducing cancer-associated fatigue (Sun et al., 2016), another randomized, double-blinded, multicenter study (ALC 1000 mg three times daily) in women undergoing adjuvant taxane-based chemotherapy for breast cancer found that 24 weeks of ALC therapy resulted in statistically significant worsening of CIPN over 2 years (Hershman et al., 2018).

Finally, in addition to drug therapy, some non-drug areas, such as physical therapy and traditional natural medicines should be focused to use as potential candidates for the treatment of CIPN. The effectiveness of crocin for the treatment of CIPN also provided us a hint to find some other traditional natural medicines to treat CIPN. A recent systematic review and meta-analysis of Chinese herbal medicine found that topical application of Chinese herbal medicine was effective in treating CIPN as it significantly improved clinical symptoms and quality of life in patients with CIPN (Li et al., 2022). Crocin was derived from Saffron, a traditional Persian medicine (TPM), which had analgesic, antioxidant, anti-genotoxic, anti-tumor, anti-inflammatory, anticonvulsant, anti-depressant, antibacterial, sedative, memory-enhancing, and neuroprotective effects (Bozorgi et al., 2021).

5 Conclusion

The primary objectives for this systematic review were to examine the efficacy of drugs in the treatment of CIPN using existing randomized controlled trials to provide evidence for clinical practice and future studies. The analysis results demonstrated that pregabalin, crocin, tetrodotoxin, venlafaxine, and GM1 may be beneficial for the treatment of CIPN in addition to duloxetine. ALC and gabapentin are somewhat controversial in treating CIPN. However, the number of randomized controlled trials of CIPN treatment is small and most studies are lacking evidence to provide a solid basis for decision-making. Therefore, a standardized study design involving the characteristics of patients, the duration of therapy, and outcome indicators is required. RCTs with larger sample sizes and longer follow-ups are recommended to comprehensively evaluate the efficacy of the drugs in the treatment of CIPN. Finally, some randomized controlled trials investigating the curative effect of peri-neural platelet-rich plasma injection, donepezil, topical menthol application, topical cannabidiol, and single-cycle tetrodotoxin for the treatment of CIPN are expected to be carried out.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

CW, SC and WJ performed the study, acquisition and analysis of data. CW performed literature database searching. CW and SC discussed the data; conceived the idea and revised the manuscript. CW drafted the manuscript. All authors have read the manuscript and provided critical feedback. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1080888/full#supplementary-material

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Immune-mediated hepatitis induced by immune checkpoint inhibitors: Current updates and future perspectives

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In recent years, cancer immunotherapy has made remarkable achievements. Immune checkpoint inhibitors (ICIs) have been used successfully in several types of cancer in the past decade. However, expanded indication and increased use of Immune checkpoint inhibitors have resulted in increased reports of toxicity called immune-related adverse events (irAEs). Due to the unique immunological characteristics of the liver, a hepatic immune-related adverse events has also been reported, which is usually termed Immune-mediated hepatitis (IMH). So far, it is generally considered that the mechanism of IMH induced by Immune checkpoint inhibitors is mainly the overactivation of T cells. It has been reported that the incidence of IMH ranges from 1% to 15%. Because of the lack of specific markers, a diagnosis of exclusion of IMH is critical. Although most IMH is mild and recoverable, several death cases have been reported, which has been increasingly concerned. This review summarizes the current understanding of the pathophysiology, epidemiology, diagnosis, management and prognosis of IMH caused by Immune checkpoint inhibitors. It also discusses the controversial issues in IMH, such as the role of liver biopsy, grading criteria, risk factors, rational treatment strategies with steroids, and the timing of Immune checkpoint inhibitors rechallenging, which may provide helpful information for IMH in future clinical practice.

KEYWORDS

cancer, immunotherapy, immune checkpoint inhibitors, immune-related adverse events, drug-induced liver injury, hepatitis

Introduction

In the past decade, immune checkpoint inhibitors (ICIs) have developed rapidly in the application of advanced malignancies (Bagchi et al., 2021). According to the targets of immune checkpoint molecules which act as negative regulators of T cells function in cancer immunological process, there are three main types of ICIs so far: cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) (Qin et al., 2019; Kotanides et al., 2020). ICIs, the monoclonal antibodies of these molecules, have been exploited to block these immune checkpoint molecules, enhance

T cells function and finally recover anti-tumor activity in the host. Since a CTLA-4 inhibitor, ipilimumab, has been approved by America food and drug administration against advanced-stage melanoma in 2011 (Hodi et al., 2010), ICIs have become a hotspot and have revoluted treatments of various cancers (Table 1).

However, with the wide application of ICIs, several unexpected immunological and inflammatory events, termed immune-related adverse events (irAEs), have been reported (Michot et al., 2016). It has been demonstrated that irAEs result from overactive immune response, which can affect almost any organ, especially skin, liver, endocrine and gastrointestinal tract (Regev et al., 2020). As an essential organ of drug metabolism, liver is one of the frequently affected organs in cancer immunotherapy and its injury caused by ICIs is usually termed immune-mediated hepatitis (IMH). It has been reported that IMH is the third most frequent adverse event (5%–10%), after dermatologic toxicity (44%–68%) and gastrointestinal adverse reactions (35%–50%) (Kroner et al., 2019). In recent years, the incidence of IMH has increased. Although most IMH cases are mild, there is a risk of acute liver failure and even death if the diagnosis or management is not properly (Vozy et al., 2019; Yamamoto et al., 2021), especially in hepatocellular carcinoma (HCC) patients on a background of chronic liver diseases. Furthermore, inappropriate interventions of IMH may cause the failure of cancer immunotherapy. Therefore, IMH has become an increasing concern and a large amount of clinical data has accumulated.

This review aims to discuss the pathophysiology, epidemiology, diagnosis, management and prognosis of IMH caused by ICIs and provide references for the clinical application of ICIs.

Underlying mechanisms of IMH

The critical step for ICIs in cancer immunotherapy is the activation of T cells. As mentioned above, CTLA-4, PD-1 and

TABLE 1 Immune checkpoint inhibitors and their indications.

Target	Drug name	Indications	Time to market
CTLA-4	Ipilimumab (Yervoy)[a,b]	Melanoma, advanced RCC, MSI-H or dMMR CRC, HCC, metastatic NSCLC, MPM, esophageal cancer	2011
PD-1	Nivolumab (Opdivo)[a,b]	Melanoma, NSCLC, MPM, advanced RCC, classical hodgkin lymphoma, HNSCC, urothelial carcinoma, MSI-H or dMMR CRC, HCC, esophageal cancer, gastric cancer, gastroesophageal junction cancer, and esophageal adenocarcinoma	2014
PD-1	Pembrolizumab (Keytruda)[a,b]	Melanoma, NSCLC, HNSCC, classical hodgkin lymphoma, PMBCL, urothelial carcinoma, MSI-H or dMMR CRC, gastric cancer, esophageal cancer, cervical cancer, HCC, MCC, RCC, endometrial carcinoma, TMB-H solid tumors, cutaneous squamous cell carcinoma, TNBC	2014
PD-L1	Atezolizumab (Tecentriq)[a,b]	Locally advanced or metastatic urothelial carcinoma, metastatic NSCLC, SCLC, HCC, melanoma	2016
PD-L1	Avelumab (Bavencio)[a]	Metastatic MCC, locally advanced or metastatic urothelial carcinoma, advanced RCC	2017
PD-L1	Durvalumab (Imfinzi)[a,b]	NSCLC, SCLC	2017
PD-1	Toripalimab[b]	Melanoma, metastatic nasopharyngeal carcinoma, metastatic urothelial carcinoma	2018
PD-1	Sintilimab[b]	Classical hodgkin lymphoma, NSCLC, HCC	2018
PD-1	Cemiplimab (Libtayo)[a]	Cutaneous squamous cell carcinoma, basal cell carcinoma, NSCLC	2018
PD-1	Camrelizumab[b]	Classical hodgkin lymphoma, advanced HCC, advanced or metastatic esophageal squamous carcinoma, nasopharyngeal carcinoma	2019
PD-1	Tislelizumab[b]	Classical hodgkin lymphoma, metastatic urothelial carcinoma, metastatic NSCLC, HCC, esophageal squamous carcinoma	2019
PD-1	Penpulimab[b]	Classical hodgkin lymphoma	2021
PD-1	Zimberelimab[b]	Classical hodgkin lymphoma	2021
PD-L1	Envafolimab[b]	MSI-H or dMMR CRC	2021
PD-L1	Sugemalimab (Cejemly)[b]	NSCLC	2021

[a]Approved by U.S. Food and Drug Adminstration.
[b]Approved by National Medical Products Administration (China).
CTLA-4, cytotoxic T-lymphocyte associated protein four; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; RCC, renal cell carcinoma; MSI-H, microsatellite instability-high; dMMR, deficient mismatch repair; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; MPM, malignant pleural mesothelioma; HNSCC, head and neck squamous cell cancer; SCLC, small Cell Lung Cancer; PMBCL, primary mediastinal large B-cell lymphoma; MCC, merkel cell carcinoma; TMB-H, tumor mutational burden-high; TNBC, triple-negative breast cancer.

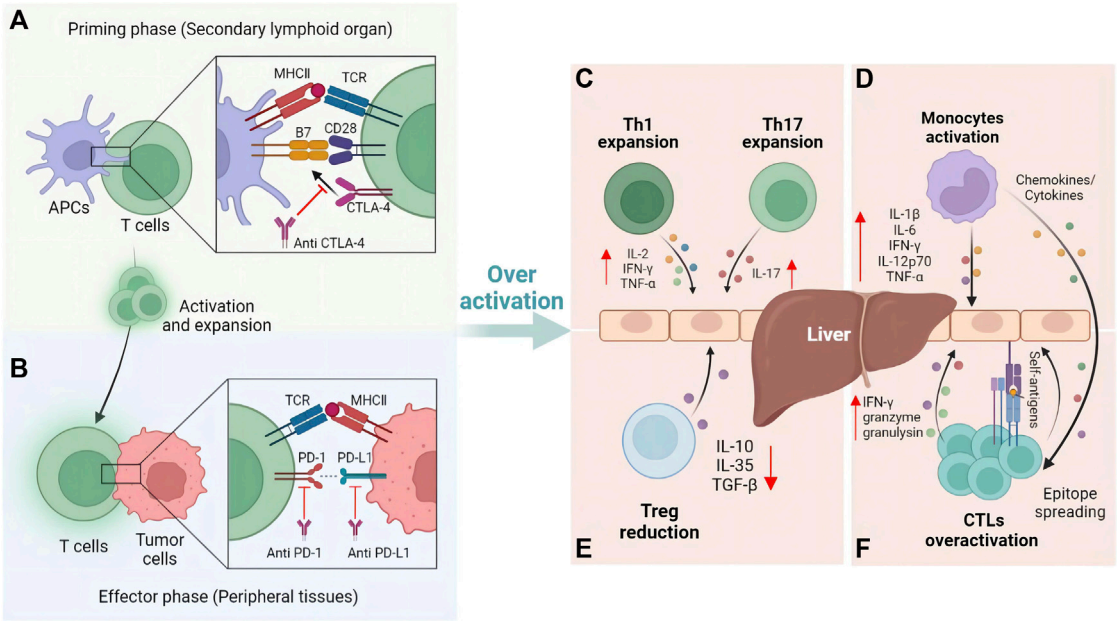

FIGURE 1
Mechanisms of T cells activation and immune-mediated hepatitis caused by ICIs. **(A)** Blockade of CTLA-4 activates T cells at the priming phase. **(B)** Further anti-tumor effect induced by the blockade of PD-1 and PD-L1 occurs in the effector phase. Once liver self-tolerance impairs, immune cells such as **(C)** Th cells, **(D)** Monocytes, **(E)** Treg cells, and **(F)** cytotoxic T cells will be involved in the pathophysiological process of immune-mediated hepatitis.

PD-L1 are three current ICIs targets. However, the mechanisms of these ICIs are different. It has been demonstrated that the CTLA-4 inhibitors play a role in the initial phase, while PD-1 and PD-L1 inhibitors are involved in the effector phase (Buchbinder and Desai, 2016). In the initial stage, CTLA-4 on T cells competitively binds with CD28 to B7-1 and B7-2 on antigen presenting cells (APCs), inhibiting the activation of T cells (Figure 1A). CTLA-4 inhibitors can enhance T cell activation by binding to CTLA-4 and increasing CD28 and B7 costimulatory signals (Yang et al., 2020). In the effector phase, binding of PD-1 on T cells and PD-L1 on tumor cells inhibits T cells activation and allows tumor cells to evade immune surveillance (Figure 1B). Similar to CTLA-4 inhibitors, PD-1/PD-L1 inhibitors can block this binding and enhance the anti-tumor effect of T cells (Peeraphatdit et al., 2020).

To date, the mechanism of IMH caused by ICIs has not been fully elucidated. However, the unique immunological features of the liver are crucial to the pathogenesis of IMH. Portal circulation connects the liver to the intestines, thus making the liver the first site to detoxify the blood entering the portal circulation and to process many antigen exposures. Therefore, the liver has evolved specific immune mechanisms to protect the organism from pathogens while maintaining a state of immunotolerance to harmless antigens from the intestine (Crispe, 2014). As one of the key mechanisms of liver immunotolerance, PD-L1 expressed on hepatic non-parenchymal cells including hepatic stellate cells, Kupffer cells and dendritic cells, together with CTLA-4 expressed on $CD4^+$ Treg cells, protect the liver from autoimmune responses to antigens by downregulating effector T cells (Makarova-Rusher et al., 2015). However, due to the use of ICIs blocking these key modulatory pathways, T cells may be overactive and the immune tolerance of the liver can be broken, making it susceptible to acute inflammatory response, which further induces hepatitis (Gudd and Possamai, 2022).

Current evidence suggests several primary mechanisms of IMH: Firstly, expansion of T helper cells in ICIs therapy such as Th1 and Th17 cells increase the levels of proinflammatory cytokines (IL-2, IFN-γ, TNF) production, which can go on to activate cytotoxic T lymphocytes (Figure 1C), as well as innate immune cells such as macrophages and natural killer cells (Gudd et al., 2021). Secondly, ICIs induce the activation of monocytes and lead to formation of an inflammatory environment related to IMH (Figure 1D) (Gudd et al., 2021). Thirdly, reduction of regulatory T cells (Treg) caused by ICIs can reduce anti-inflammatory cytokines such as interleukin (IL) -10, IL-35, TGF-β and modulate the interaction between adaptive-innate immunity (Figure 1E) (Vignali et al., 2008). Additionally, clonal expansion of $CD8^+$ T cells and epitope spreading is another mechanism of IMH (Vanderlugt and Miller, 2002; Das et al., 2015; Riaz et al., 2017). ICIs could stimulate the proliferation of $CD8^+$ T cells to overcome immune tolerance, which could further upregulate proliferative and cytotoxic genes such as IFN-γ, granzyme and granulysin. At the same time, epitope spreading causes an indiscriminate immune reaction to self-antigens (Figure 1F).

TABLE 2 Incidence of immune-mediated hepatitis according to different treatment regimens with immune checkpoint inhibitors.

Pathway	Agent	Dose	Indication	Patients, n	Incidence of all grades of IMH, n (%)	Incidence of grade 3/4 of IMH, n (%)	Ref
CTLA-4	Ipilimumab	3 mg/kg every 3 weeks for four dosages	Melanoma	151	4 (2.65)	2 (1.32)	Aamdal et al. (2022)
		3 mg/kg every 3 weeks for four dosages	Melanoma	256	3 (1.17)	1 (0.39)	Robert et al. (2015)
		10 mg/kg every 3 weeks for four dosages	Melanoma	57	8 (14.04)	7 (12.28)	Weber et al. (2009)
		10 mg/kg every 3 weeks for four dosages	Melanoma	71	2 (2.82)	2 (2.82)	Wolchok et al. (2010)
PD-1 PD-L1	Nivolumab	3 mg/kg every 2 weeks	Melanoma	313	1 (0.32)	1 (0.32)	Wolchok et al. (2022)
		240 mg every 2 weeks	Advanced NSCLC	391	5 (1.28)	4 (1.02)	Paz-Ares et al. (2022)
		480 mg every 4 weeks	Melanoma	359	9 (2.51)	4 (1.11)	Tawbi et al. (2022)
	Cemiplimab	350 mg every 3 weeks	Advanced NSCLC with PD-L1 of ≥50%	355	2 (0.56)	2 (0.56)	Sezer et al. (2021)
		350 mg every 3 weeks	Recurrent or metastatic cervical carcinoma	300	0 (0.00)	4 (1.33)	Tewari et al. (2022)
		3 mg/kg every 2 weeks	cSCC with or without metastatic	78	1 (1.28)	1 (1.28)	Migden et al. (2020)
	Pembrolizumab	200 mg every 3 weeks	Melanoma	509	9 (1.77)	7 (1.38)	Eggermont et al. (2018)
		10 mg/kg every 3 weeks for four dosages	Melanoma	277	5 (1.81)	5 (1.81)	Robert et al. (2015)
		200 mg every 3 weeks	HCC	104	3 (2.88)	3 (2.88)	Zhu et al. (2018)
		200 mg every 3 weeks	HCC	279	5 (1.79)	4 (1.43)	Finn et al. (2020a)
	Atezolizumab	1200 mg every 3 weeks	Advanced NSCLC	68	1 (1.47)	1 (1.47)	Cho et al. (2022)
		1200 mg every 3 weeks	Muscle-invasive urothelial carcinoma	390	36 (9.23)	9 (2.31)	Bellmunt et al. (2021)
	Avelumab	10 mg/kg every 2 weeks	Clear-cell renal-cell carcinoma	55	3 (5.45)	2 (3.64)	Choueiri et al. (2018)
	Durvalumab	1500 mg every 4 weeks	Urothelial carcinoma	345	1 (0.29)	1 (0.29)	Powles et al. (2020)
Combination Therapy	Nivolumab plus ipilimumab	1 mg/kg nivolumab plus 3 mg/kg ipilimumab every 3 weeks for four dosages	Melanoma	313	7 (2.23)	5 (1.60)	Hodi et al. (2018)
		1 mg/kg nivolumab plus 3 mg/kg ipilimumab once every 3 weeks for four dosages, followed by nivolumab 3 mg/kg once every 2 weeks	Melanoma	313	1 (0.32)	1 (0.32)	Wolchok et al. (2022)

(Continued on following page)

TABLE 2 (*Continued*) Incidence of immune-mediated hepatitis according to different treatment regimens with immune checkpoint inhibitors.

Pathway	Agent	Dose	Indication	Patients, n	Incidence of all grades of IMH, n (%)	Incidence of grade 3/4 of IMH, n (%)	Ref
		nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks for four dosages, followed by nivolumab 240 mg every 2 weeks	HCC	49	10 (20.41)	10 (20.41)	Yau et al. (2020)
		Nivolumab3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks for four dosages, followed by nivolumab 240 mg every 2 weeks	HCC	49	6 (12.24)	5 (10.20)	Yau et al. (2020)
		Nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks	HCC	49	3 (6.12)	3 (6.12)	Yau et al. (2020)
	Pembrolizumab plus ipilimumab	200 mg pembrolizumab every 6 weeks every 3 weeks, followed by ipilimumab 1 mg/kg	Metastatic NSCLC	282	5 (1.77)	4 (1.42)	Boyer et al. (2021)
		2 mg/kg pembrolizumab every 3 weeks, followed by 1 mg/kg ipilimumab every 3 weeks for four dosages, followed by 2 mg/kg pembrolizumab every 3 weeks	Melanoma	153	15 (9.80)	9 (5.88)	Long et al. (2017)

CTLA-4, cytotoxic T-lymphocyte associated protein four; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; NSCLC, non-small-cell lung cancer; HCC, hepatocellular carcinoma; cSCC, cutaneous squamous cell carcinoma.

In addition, due to the high exogenous antigens exposure such as LPS in the liver, Kuffer cells and liver sinusoidal endothelial cells (LSECs) express the adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). These continuously expressed adhesion molecules and the slow blood flow in the hepatic sinusoids promote the interaction of activated $CD8^+$ T cells in the systemic circulation with Kuffer and LSECs, leading to the retention of activated $CD8^+$ T cells in the liver (Mehal et al., 1999; John and Crispe, 2004). Upon retention, these cells bind and secrete IFN-γ through their FasL molecules and Fas expressed by Kuffer cells, inducing TNF-α secretion by Kuffer cells (Murray and Crispe, 2004), which would induce hepatocytes sensitive and susceptible to Fas-induced and IFN-γ-mediated apoptosis (Horras et al., 2011; Faletti et al., 2018), leading to hepatocyte injury. Although this hypothetical mechanism may not answer why IMH occurs in only a subset of patients on ICI treatment and not in most patients, this hypothesis provides a possible mechanism of IMH, further studies are still needed.

Incidence

The incidence of IMH is mainly counted through the reports of irAEs in clinical trials. Up to date, most trials defined the occurrence and grades of irAEs based on the common criteria for adverse events (CTCAE), which was used by referring to the elevations of aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total bilirubin based on the upper limit of normal (ULN).

The reported incidence of IMH varies according to the different agents, dosages and indications (Regev et al., 2020). It has been described that the incidence of all grades of IMH widely ranges from 1% to 15%, and the incidence of grade 3 or four ranges from 1% to 10% (Table 2). The incidence of IMH caused by CTLA-4 inhibitors (2%–15%) usually demonstrates an increased risk compared to those using PD-1 (0%–3%) or PD-L1 (0%–6%) inhibitors (Weber et al., 2009; Robert et al., 2015; Choueiri et al., 2018; Aamdal et al., 2022). Meanwhile, a higher dose of CTLA-4 inhibitors appears to increase the incidence of ICIs induced IMH. For melanoma patients who received ipilimumab, monotherapy with high doses (10 mg/kg) may cause an increased incidence compared to lower doses (3 mg/kg) (Wolchok et al., 2010; Robert et al., 2015). Furthermore, combination therapy seems more likely to cause IMH than monotherapy. In a phase III clinical trial of CheckMate 067, patients with advanced melanoma who received ipilimumab plus nivolumab were reported a higher incidence of IMH than those who received ipilimumab or nivolumab alone (Wolchok et al., 2022). In another clinical trial, KEYNOTE-598, patients with non-small-cell lung cancer who received pembrolizumab plus ipilimumab reported a approximate incidence of all grades of IMH compared to those who received pembrolizumab alone. However, the incidence of

grade 3/4 IMH was higher in the combined treatment group than in the monotherapy group, suggesting that combined therapy of ICIs may be associated with more severe IMH (Boyer et al., 2021). Furthermore, compared to IMH caused by ICIs in other tumors, the incidence of IMH in patients with HCC may be slightly higher (Zhu et al., 2018; Sangro et al., 2020). Similar to other tumors, the incidence of IMH with combined therapy is much higher than those received monotherapy in HCC (Zhu et al., 2018; Finn et al., 2020a; Yau et al., 2020). The background of chronic liver disease and the influence of the primary location of HCC may partly explain the higher incidence of IMH in HCC. However, the incidence of IMH may be overestimated due to causes other than ICIs, such as other drugs, viral reactivation or tumor progression. More rigorous assessment and differential diagnosis need to be developed.

In addition, it is worth noting that there are some commonalities between other irAEs and IMH. The incidence of other irAEs was also associated with different treatment strategies. It has been reported that the incidence of rash, colitis and diarrhea is higher in patients treated with anti-CTLA-4 than in patients treated with PD-1 (33% vs. 26%, 12% vs. 1%, 33% vs. 20%) (Kroner et al., 2019). The risk of non-hepatic irAEs has been demonstrated to be dose dependent in anti-CTLA-4 agents (Ascierto et al., 2017). Meanwhile, compared to monotherapy, combined immunotherapy has higher incidence in most of irAEs and more than 60% of patients treated with combined therapy have been reported to occur severe irAEs (Wolchok et al., 2017; Esfahani et al., 2019). Furthermore, nearly half of IMH patients are reported to have concomitant non-hepatic irAEs such as pneumonia, pituitary inflammation, hyperthyroidism and pancreatitis, which may precede the diagnosis of IMH (De Martin et al., 2018; Huffman et al., 2018).

Although IMH occurs less commonly than some other non-hepatic irAEs, fatal cases have been observed in both clinical trials and post marketing phase. A meta-analysis investigated the fatality rates caused by ICIs, which indicated that in 613 reported fatal cases, 124 were secondary to IMH. Furthermore, in this study, of all fatal cases, 31 (5.1%) in the ipilimumab group, 74 (12.1%) in the anti-PD-1/PD-L1 group and 19 (3.1%) in the combined therapy group were caused by IMH. The study further analyzed the patients with melanoma from seven international academic medical centers and found that 21 fatal cases were reported, of which 5 (23.8%) cases were caused by IMH, followed by myocarditis (28.6%) and colitis/enteritis (28.6%) (Wang et al., 2018). These studies suggest that IMH accounts for a high proportion of fatal irAEs, which is noteworthy and has important clinical significance.

Risk factors

Although certain risk factors have been associated with irAEs during ICIs therapy, the risk factors associated with IMH have not been fully elucidated (Yang et al., 2020). There are several risk factors have been demonstrated until now, such as the therapeutic strategy of ICIs, background in chronic liver diseases, and some other factors demonstrated by several clinical reports.

Therapeutic strategy

From the perspective of the treatment strategy of ICIs, it has been reported that the incidence of irAEs in monotherapy of anti-CTLA-4 is higher than that in anti-PD-1 or PD-L1, which suggests that the types of ICIs may be a risk factor in IMH. Furthermore, the risk of incidence of IMH is correlated with the dosage of ICIs. In a study of ipilimumab for melanoma, serious hepatic adverse events were more common at 10 mg/kg compared to the dosage of 3 mg/kg (30% vs. 0%) (Wolchok et al., 2010). Additionally, ipilimumab plus nivolumab combination therapy and previous ICI treatment are two independent risk factors for IMH, respectively (Kitagataya et al., 2020; Yamamoto et al., 2021).

As for the drugs other than ICIs, it has been reported that acetaminophen was associated with a 2.1-fold increased risk of all grades of IMH and the use of 3-hydroxy-3-methyl-glutaryl-coenzyme reductase inhibitors was associated with a 4.7-fold increased risk of grade 3 or higher IMH compared with untreated (Cho et al., 2021).

Chronic liver diseases

For patients with a background in chronic liver diseases, the incidence of IMH is higher than that of patients without liver dysfunction (Sangro et al., 2020). However, a clinical trial has reported no relation between the occurrence of IMH and the background of viral hepatitis in HCC patients who received nivolumab monotherapy (El-Khoueiry et al., 2017). A retrospective study on a total of 135 patients who received PD-1 inhibitors has reported 8 cases occurred IMH, two cases of combined non-alcoholic fatty liver disease (NAFLD) and one case of combined alcoholic liver disease, which suggests that some liver disease other than chronic viral hepatitis may also increase the risk of IMH (Sawada et al., 2020). Further analysis in the study has shown a significant correlation between NAFLD and IMH (hazard ratio [HR] = 29.34, p = 0.003). Furthermore, several studies have demonstrated that patients with autoimmune disorders such as thyroiditis or rheumatological have a higher risk of IMH during ICIs therapy (Johnson et al., 2016; Abdel-Wahab et al., 2018). However, there is still no available data supporting this tendency in autoimmune hepatitis, which needs further investigation.

Other factors

For sex, a retrospective study confirmed that male (HR = 1.608, $p < 0.05$) was an independent risk factor for IMH (Cho

et al., 2021). However, another study has reported that females are significantly associated with higher grade IMH compared to males, which still exists a divergence and further studies are necessary to draw a definite conclusion (Kitagataya et al., 2020). Furthermore, for age, a study by Cho et al. demonstrated that patients younger than 65 years old (HR = 1.527, $p < 0.05$) was another independent risk factor for IMH (Cho et al., 2021), which may be due to the immunosenescence as people age (Nishijima et al., 2016).

Secondly, as for the types of cancer, a Japanese study reported that malignant melanoma was significantly and independently associated with increased risk of IMH (odd ratio [OR] = 11.6, $p = 0.002$) (Yamamoto et al., 2021), which suggested that comprehensive and systematic evaluation should be carried out in malignant melanoma patients who received ICIs therapy to reduce the risk of IMH. Additionally, the risk of IMH has been reported to be associated with patients with primary liver cancer. It has shown higher elevations of ALT, AST, total bilirubin, and more severe grade of IMH in patients with primary liver cancer compared to patients with other solid tumors (Fu et al., 2021), which suggests that more concern should be paid to the occurrence of IMH in HCC patients during ICIs administration.

Furthermore, fever over 38°C within 24 h of initial ICI treatment was also identified as another risk factor for IMH (HR = 6.21, $p < 0.001$) (Mizuno et al., 2020). In sum of these studies, risk factors of IMH need to be further investigated, which may be helpful to reveal the underlying mechanisms of IMH caused by ICIs and to improve the diagnosis and management of IMH in clinical practice in the future.

Diagnosis

Although most cases of IMH are asymptomatic, a few patients may present with fatigue, abdominal discomfort, fever, rash, and rarely jaundice (Huffman et al., 2018; Riveiro-Barciela et al., 2020). Acute liver failure is also rarely present in the initial stage of IMH. Furthermore, the clinical presentation is demonstrated to vary in different types of ICIs. It has been reported that fever is more prevalent in CTLA-4 inhibitors than in PD-1 and PD-L1 inhibitors (De Martin et al., 2018). The pattern of IMH commonly presents the type of hepatocellular injury, while a cholestatic or mixed liver injury pattern may also be observed, which is more commonly secondary to PD-1 and PD-L1 inhibitors than CTLA-4 inhibitors (De Martin et al., 2018; Imoto et al., 2019).

Abnormal elevations of serum liver enzymes in liver function tests are usually indexed in the diagnosis of IMH. Elevations of ALT or AST more than two times ULN should be concerned. Sometimes it should also be concerned mild to moderate elevation of serum ALP >2.5 × ULN, and abnormal elevation of total bilirubin >1.5 × ULN. Since the IMH is usually asymptomatic or has non-specific symptoms, many cases are diagnosed during monitoring during ICI therapy. Furthermore, specific biomarkers of IMH have not been elucidated. Although recent studies have demonstrated that human leukocyte antigen and IL-6 are susceptible to liver injury induced by ICIs, there is no specificity in IMH, which needs more studies to verify (Chowell et al., 2018; Valpione et al., 2018). For time to onset of IMH, it has been reported that the onset time of IMH is between 4 and 12 weeks or after 3 times of ICIs infusion, the onset time of IMH induced by CTLA-4 inhibitors is sooner than that induced by PD-1 and PD-L1 inhibitors (De Martin et al., 2018).

The 2019 European Association for the Study of the Liver (EASL) guideline for drug-induced liver injury (DILI) classified IMH as a special type of DILI (European Association for the Study of the Liver, 2019). Similar to idiosyncratic DILI, IMH is a diagnosis of exclusion and it is essential to assess the causality in patients with abnormal liver function tests to confirm IMH (Regev et al., 2014). The Roussel Ucalf Causality Assessment Method (RUCAM) scale is a well-established tool to assess the likelihood of DILI and is recommended to assist the diagnosis of IMH by some hepatologic experts (Danan and Teschke, 2015), which includes the assessment of onset time after therapy, the course of liver enzymes after drugs cessation, response to drug re-exposures, alcohol use, age, and concomitant drugs (Hoofnagle and Bjornsson, 2019). However, the RUCAM scale in IMH diagnosis is less application in the diagnosis of IMH. It should be further verified in clinical practice to evaluate whether RUCAM is suitable for the IMH diagnosis.

Differential diagnosis

The differential diagnosis of IMH is still challenging as the existing a lot etiologies of abnormally elevated liver enzymes, which mainly include drugs other than ICIs, viral infection, autoimmune and metabolic diseases, tumor-related causes, biliary diseases, musculoskeletal and cardiovascular system diseases (Shantakumar et al., 2016; Ricart, 2017). Therefore, it is important to comprehensively assess other common differentials to avoid the inappropriate interruption of effective anticancer therapy or unnecessary interventions in patients suspected IMH during ICIs therapy.

Identification of the above differential causes of liver injury during ICIs therapy requires a detailed medication history. It is noteworthy that chemotherapeutic drugs combined with ICIs, such as dacarbazine, carboplatin, and bevacizumab, which may also cause liver injury during cancer immunotherapy (Reck et al., 2013; Fashoyin-Aje et al., 2019; Finn et al., 2020b). Furthermore, dietary supplements, herbal, as well as alcohol can also induce a non-immune mediated hepatitis. Another cause of liver injury that deserves mention is liver metastasis as ICIs are usually for patients with

TABLE 3 Differential diagnosis and recommended tests in immune-mediated hepatitis diagnosis.

Etiology	Differential diagnosis	Related tests
Drugs other than ICIs	Concomitant anti-tumor medications; Complementary and herbal medications; acetaminophen toxicity	Medication history
Viral infection	a) Hepatic virus infection (HAV, HBV, HCV, HEV); b) Reactivation of HBV; c) CMV infection; d) EBV infection; e) HSV infection	a) anti-HAV IgM, HBsAg, anti-HBc IgG, anti-HBc IgM, HBV DNA, anti-HCV, HCV RNA, anti-HEV IgG, anti-HEV IgM, HEV RNA; b) HBV DNA; c) anti-CMV IgM, CMV DNA; d) anti-EBV IgM, EBV DNA; e) anti-HSV IgM, HSV DNA
Alcohol related	Alcoholic hepatitis	Alcohol intake history
Autoimmune disease	Autoimmune hepatitis	ANA, ASMA, anti-LKM-1, anti-LC-1, anti-SLA/LP, pANCA, serum IgG, IgM, IgA
Metabolic disease	Non-alcoholic fatty liver disease	Metabolic risk factor, imaging of hepatic steatosis
Tumor related	Hepatic metastasis or HCC progression	Hepatic imaging (ultrasonography, CT scan, MRI)
Biliary disease	Biliary obstruction; Gallstones; Cholecystitis; Cholangitis	Hepatobiliary imaging (ultrasonography, CT scan, MRI, MRCP)
Genetic disease	Wilson's disease	Blood ceruloplasmin, serum copper, slit lamp eye examination for Kayser-Fleischer rings, genetic testing
Systemic infection	Sepsis	Blood pressure, complete blood count, procalcitonin, blood or urine cultures
Musculoskeletal system	Muscle injury (mostly myositis); Rhabdomyolysis	Serum CK; CK-MB
Cardiovascular system	Myocarditis; Portal-vein/hepatic vein thrombosis; Ischemic or congestive hepatic injury	Imaging and clinical history (Blood pressure, pulse, electrocardiogram, echocardiogram)

ICIs, immune checkpoint inhibitors; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; CMV, cytomegalovirus; EBV, epstein barr virus; HSV, herpes simplex virus; ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; CK, creatine kinase; CK-MB, creatine kinase-MB; CT, computed tomography; MRI, magnetic resonance imaging; MRCP, magnetic resonance cholangiopancreatography; LKM-1, liver kidney microsomal type 1; LC-1, liver cytosol type 1; SLA, soluble liver antigen; LP, liver pancreas; pANCA, perinuclear anti-neutrophil cytoplasmic antibodies; HCC, hepatocellular carcinoma.

advanced malignancies. A cohort of 491 patients who received pembrolizumab reported 14.3% incidence of liver injury, however, more than half patients were found with liver metastasis, which suggests that liver metastasis may be the cause of the liver injury rather than ICIs (Tsung et al., 2019). Other chronic liver diseases such as hepatic viral infection should also be concerned. Additionally, some extra-hepatic causes of elevation of liver enzymes also need to be considered, such as myocarditis, myositis, and bone or other organ metastasis (Regev et al., 2014; Touat et al., 2018). A detailed differential diagnosis and related tests in IMH diagnosis are listed in Table 3.

Pathologic diagnosis

Liver biopsy is commonly unnecessary for diagnosis as the feature that IMH is a diagnosis of exclusion and is often reflected on liver tests. At present, it is recommended that a liver biopsy may reserve for patients with more severe than grade 2 (Sangro et al., 2020). As liver biopsy is unnecessary in most patients, there are few histological appearance data during IMH caused by ICIs. Common histopathology findings from reported cases are mainly mononuclear inflammation, including periportal inflammation with or without interface hepatitis, diffuse panlobular inflammation with prominent perivenular infiltrate, confluent necrosis, and rarely cholestatic injury which appears a mononuclear infiltrate in portal tracts that are centered around bile ducts and bile ductular proliferation (Kleiner and Berman, 2012; Kim et al., 2013; Kawakami et al., 2017; Zhang et al., 2020). Immune cells in liver tissues of patients with IMH consist of predominantly $CD8^+$ T cells and eosinophils, less frequently $CD4^+$ T cells, B cells, and plasma cells (Johncilla et al., 2015).

Although liver biopsy is not necessary for routine diagnosis of IMH, some studies indicate that it may be helpful in patients with atypical presentation or unusual clinical course, as well as a differential diagnosis in patients with viral hepatitis or autoimmune hepatitis (Haanen et al., 2017). It has been reported that liver biopsy was able to differentiate the hepatitis C virus (HCV) or IMH in HCC patients with untreated HCV, as HCV appears to have lymphocytic infiltration. In contrast, IMH appears to involve a mixed inflammatory infiltrate comprising eosinophils, histiocytes, and lymphocytes (Hsu et al., 2020). Furthermore, IMH shares several histopathological similarities with idiopathic autoimmune hepatitis (iAIH), such as panlobular inflammation, necrosis, and lymphocytic infiltrate. However,

TABLE 4 Grading assessment of immune-mediated hepatitis by common terminology criteria of adverse events and drug-induced liver injury network.

Grade	Common terminology criteria of adverse events version 5.0 (NCI, 2017)	Drug-induced liver injury network (Fontana et al., 2009)
Grade 1	ALT>3.0 × ULN if baseline is normal; >1.5–3.0 × baseline if baseline was abnormal	Elevated serum ALT and/or ALP; TBil <2.5 mg/dl; INR <1.5; With or without symptoms (fatigue, weakness, nausea, anorexia, right upper abdominal pain, jaundice, pruritus, rash, or weight loss)
	AST>3.0 × ULN if baseline is normal; >1.5–3.0 × baseline if baseline was abnormal	
	ALP>2.5 × ULN if baseline is normal; >2.0–2.5 × baseline if baseline was abnormal	
	TBil>1.5 × ULN if baseline is normal; >1.0–1.5 × baseline if baseline was abnormal	
Grade 2	ALT>3.0–5.0 × ULN if baseline is normal; >3.0–5.0 × baseline if baseline was abnormal	Elevated serum ALT and/or ALP; TBil ≥2.5 mg/dl or INR ≥1.5 without Elevated TBil; Symptoms may be aggravated
	AST>3.0–5.0 × ULN if baseline is normal; >3.0–5.0 × baseline if baseline was abnormal	
	ALP>2.5–5.0 × ULN if baseline is normal; >2.5–5.0 × baseline if baseline was abnormal	
	TBil>1.5–3.0 × ULN if baseline is normal; >1.5–3.0 × baseline if baseline was abnormal	
Grade 3	ALT>5.0–20.0 × ULN if baseline is normal; >5.0–20.0 × baseline if baseline was abnormal	Elevated serum ALT and/or ALP; TBil ≥ 5 mg/dl with or without INR ≥1.5; Symptoms are further aggravated; Indication for hospitalization or prolonged hospitalization
	AST>5.0–20.0 × ULN if baseline is normal; >5.0–20.0 × baseline if baseline was abnormal	
	ALP>5.0–20.0 × ULN if baseline is normal; >5.0–20.0 × baseline if baseline was abnormal	
	TBil>3.0–10.0 × ULN if baseline is normal; >3.0–10.0 × baseline if baseline was abnormal	
Grade 4	ALT>20.0 × ULN if baseline is normal; >20.0 × baseline if baseline was abnormal	Elevated serum ALT and/or ALP; TBil ≥10 mg/dl or daily elevation ≥1.0 mg/dl; INR ≥1.5 with ascites, encephalopathy, or other organ dysfunction
	AST>20.0 × ULN if baseline is normal; >20.0 × baseline if baseline was abnormal	
	ALP>20.0 × ULN if baseline is normal; >20.0 × baseline if baseline was abnormal	
	TBil>10.0 × ULN if baseline is normal; >10.0 × baseline if baseline was abnormal	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBil, total bilirubin; INR, international normalized ratio; ULN, upper limit of normal.

there are also exist some significant differences between IMH and iAIH, which has been reported that there is an increased presence of $CD8^+$ T cells and fewer $CD20^+$ B cells and $CD4^+$ T cells in IMH compared to iAIH, and the panlobular inflammation is often confined in zone 3 in IMH (Kim et al., 2013; Zen and Yeh, 2018). Those findings may help differentiate IMH from iAIH.

The concern is that IMH caused by different ICIs has distinct histopathological patterns. Anti-CTLA-4 drugs are mainly characterized by specific patterns of granulomatous hepatitis, fibrin deposits, and central vein endothelialitis. However, histological findings in anti-PD-1/PD-L1 drugs are more heterogenous, of which biopsy mainly shows lobular hepatitis, periportal activity, and centrilobular necrosis (De Martin et al., 2018). Further study of the histopathological characteristics of different ICIs may be helpful in elucidating the underlying mechanisms of IMH and finally benefit the clinical practice.

Grading criterion

The criterion of IMH grading is crucial as the severity of IMH corresponds to the management. Currently, two grading criterions, CTCAE and Drug-induced liver injury network (DILIN), are clinically used to evaluate IMH (Table 4). Both grading systems consider the alteration of serum liver enzymes and bilirubin, while most oncology clinical trials prefer to use the

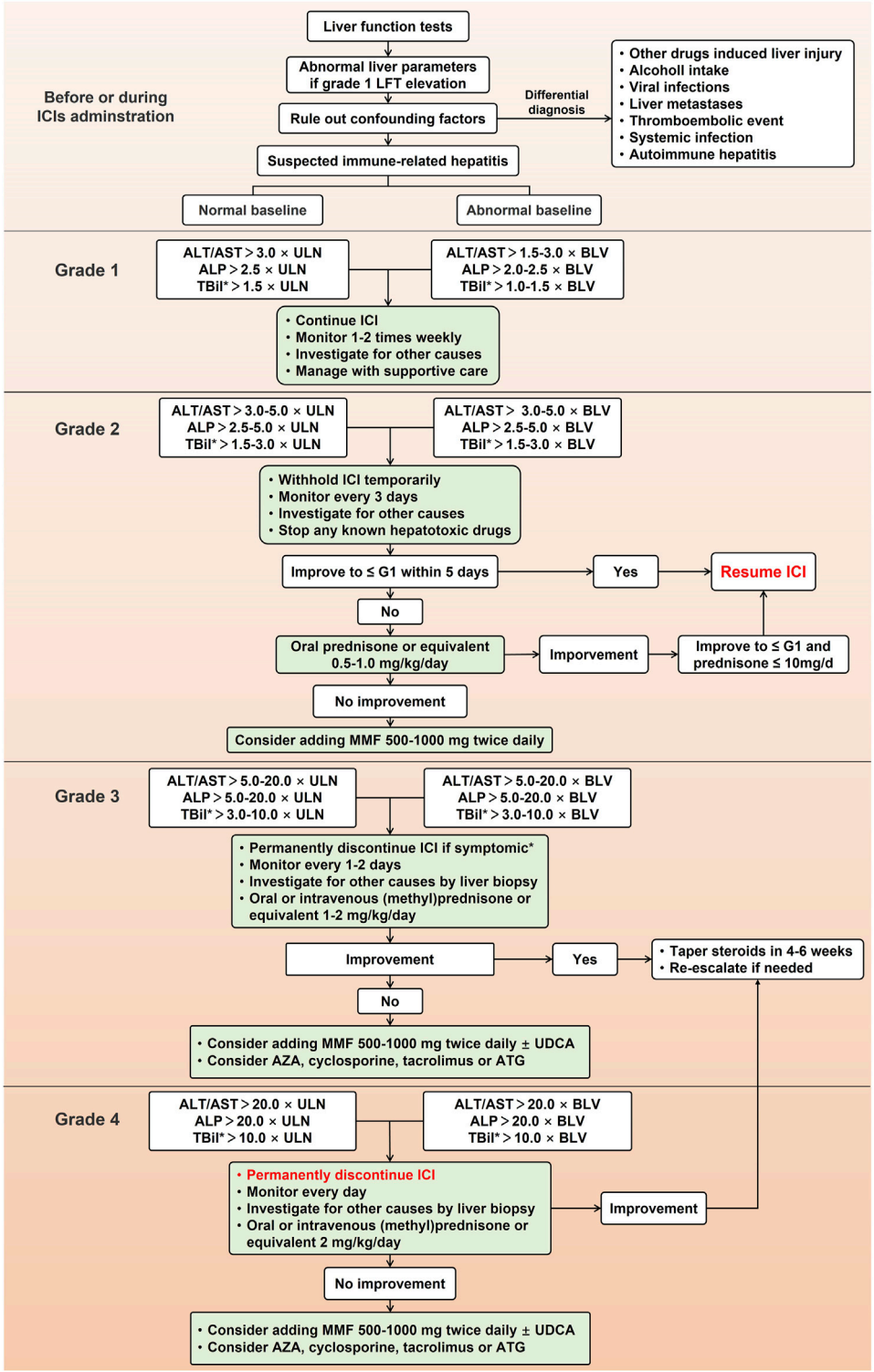

FIGURE 2
Management for immune-mediated hepatitis caused by immune checkpoint inhibitors. LFT, liver function test; ICI, immune checkpoint inhibitor; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBil, total bilirubin; ULN, upper limit of normal; BLV, baseline value; MMF, mycophenolate mofetil; UDCA, ursodeoxycholic acid; ATG, antithymocyte globulin; AZA, azathioprine.

CTCAE grading system to evaluate the irAEs caused by ICIs, which classifies the severity as 5 grades and grade 5 refers to fatal IMH. However, it should be noted that this grading system may sometimes be insufficient to reflect the clinical severity of IMH (Personeni et al., 2021). The CTCAE system may overestimate the severity of IMH compared to DILIN. For example, transaminases >20× ULN without coagulation derangement are considered a grade 4 adverse event, which corresponds to a life-threatening event, while a normal coagulation function may not be considered a severe liver injury clinically. Therefore, compared to CTCAE, the DILIN system seems more comprehensive as it considers the international normalized ratio, symptoms, and other organ failures (Fontana et al., 2009). However, neither criteria are formulated explicitly for IMH grading but rather for elevated liver function induced by any treatment. Furthermore, which criterion is more suitable for predicting the prognosis of IMH is also unknown and still needs further exploration.

Management

Recently, American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO), Society for Immunotherapy of Cancer (SITC), National Comprehensive Cancer Network (NCCN), and EASL have developed guidelines on irAEs, including IMH, to guide the management of irAEs (Haanen et al., 2017; Thompson et al., 2020; Brahmer et al., 2021; Schneider et al., 2021). Due to the lack of prospective clinical trials evaluating the effects of different treatment options, management guidelines of IMH are currently based on practice in case reports and expert consensus. Currently, most clinical practices follow the guidelines issued by ASCO in 2021, which includes the frequency of liver function tests, timing of hold and resume ICIs and corticosteroids administration. The detailed management based on current guidelines is shown in Figure 2.

Most guidelines recommend that before every ICIs administration, all patients should check liver parameters, especially for patients with a background in chronic viral hepatitis, which is recommended an antiviral therapy before the first time of ICIs therapy. To patients with asymptomatic elevations of liver tests and excluded other suspicious causes, IMH induced by ICIs should be considered. Unlike other DILI, it is not enough to discontinue the suspected culprit drugs in the management of IMH as IMH is usually induced by excessive immune response of the liver, so initiation of immunosuppressive therapy is equally necessary. Currently, all recommended management of IMH suggests using corticosteroids such as prednisone, methylprednisone, or equivalent (Miller et al., 2020). Furthermore, although CTCAE may be insufficient to reflect the clinical severity of IMH and a management algorithm based on DILIN and histopathology severity has been proposed (De Martin et al., 2018), management of IMH in most consensus and clinical trials varies with the severity of hepatitis based on CTCAE grading system. Although current guidelines of irAEs have minor differences in the management of IMH, they all follow a gradual treatment process, including continuing or ceasing ICIs, escalated first-line corticosteroids, further use of second-line mycophenolate mofetil (MMF), and the application of third-line immunosuppressive treatment (Haanen et al., 2017; Thompson et al., 2020; Brahmer et al., 2021; Schneider et al., 2021).

Corticosteroids

Although guidelines recommend using a dosage of prednisone from 0.5 to 1 mg/kg/day for grade 2 IMH and initiating methylprednisone 1–2 mg/kg/day in more severe IMH, the timing for corticosteroid administration is still controversial. It has been shown that nearly half of patients with grade 3 or 4 IMH who discontinue ICIs can improve spontaneously without a corticosteroid treatment (Gauci et al., 2018). Another case series also reported that six patients with grade 2 or higher IMH who received no corticosteroid treatment or no escalated dose of steroid showed a sooner resolution of liver injury compared to four patients who received corticosteroids (median time: 4.7 weeks vs. 8.6 weeks) (Gauci et al., 2018), which provide a possible to avoid corticosteroids as an increased risk of severe infections are found in patients received corticosteroids during ICIs therapy (Del Castillo et al., 2016). A recent study demonstrated similar outcomes and reduced risk of corticosteroids-mediated complications of grade 3 or 4 IMH patients who received 1 mg/kg/day methylprednisolone compared to those who received high-dose steroid, which further provides support for the use of lower doses of steroids without compromising the improvement of liver function and a reduced risk of steroid-related complications (Li et al., 2022; Pan and Razumilava, 2022). In addition, budesonide, another corticosteroid used in autoimmune hepatitis, has also been reported to be effective in the treatment of grade 3 IMH and restarting ICI, which has been considered for the treatment of IMH as its metabolism feature and the lower side effects (Ziemer et al., 2017). However, the timing and indication of corticosteroid use need to be clarified further. At present, it is essential to consider an individualized treatment for IMH, and further studies are needed to evaluate the new management strategy for IMH.

Refractory IMH to steroid

Currently, most society guidelines recommend corticosteroids as a first-line treatment for IMH. However, some cases of refractoriness on steroids were reported to not

TABLE 5 Additional treatments for steroids-refractory immune-mediated hepatitis from case reports.

Additional treatments	Time for recovery of liver parameters	Ref
MMF 1 g/day for 1 week plus ATG 1.5 mg/kg/day for 2 consecutive days	1 month from the start of the ATG	Chmiel et al. (2011)
MMF 500 mg twice-daily plus intravenous ATG 1.5 mg/kg/day for 2 consecutive days	After 49 days	Motomura et al. (2020)
MMF 1 g/day plus two intravenous doses of ATG of 100 and 50 mg for 2 consecutive days	After 162 days	McGuire et al. (2018)
MMF 1 g twice daily for 2 weeks plus ATG for 2 dosages	After 2 eeks	Ahmed et al. (2015)
ATG 100 mg/day	After 5 days	Spankuch et al. (2017)
MMF 500 mg plus tacrolimus 500 mg twice daily	After 186 days	McIlwaine et al. (2022)
MMF 1 g twice daily plus tacrolimus 5 mg/kg/day	N/A	Cheung et al. (2019)
MMF 1 g plus tacrolimus 1.5 mg/kg twice daily	After 9 weeks	Ziogas et al. (2020)
Cyclosporine 100 mg twice daily	After 40 days	Huffman et al. (2018)
Oral AZA with 100 mg/day	After 1 month	Iwamoto et al. (2017)
UDCA 600 mg/day plus bezafibrate 400 mg/day	After 35 days	Onishi et al. (2020)
Oral MMF 2 g/day plus UDCA	After 56 days	Doherty et al. (2017)
MMF 1.5 g/day plus plasma exchange (1,500 ml of 5% albumin plus 4 units of plasma, every other day)	After 2 weeks	Riveiro-Barciela et al. (2019)

MMF, mycophenolate mofetil; ATG, anti-thymocyte globulin; AZA, azathioprine; UDCA, ursodeoxycholic acid.

respond to steroids or failure to normalize liver function. It was recommended that if there is no response with intravenous methylprednisolone, second-line treatment of 500–1000 mg of MMF twice daily can be considered. In addition to MMF, ASCO also proposed that azathioprine (AZA) can be used as the second-line agent for steroid-refractory IMH after ruling out the infectious causes (Schneider et al., 2021), and test for thiopurine methyltransferase deficiency is required to avoid life-threatening bone marrow suppression (Ziogas et al., 2020). Although some cases reported the successful use of AZA in patients (Iwamoto et al., 2017; Huffman et al., 2018), it should be noted that the immunosuppressive effect of AZA was exerted later than that of MMF. In addition, AZA metabolites may also cause hepatotoxicity. Therefore, using AZA as a second-line treatment for IMH should be cautious.

Although MMF has been successfully used in many patients with refractory IMH to steroids, some cases still show no response after steroid and MMF treatment (McGuire et al., 2018; Motomura et al., 2020; McIlwaine et al., 2022). Therefore, given the mechanisms underlying IMH, ESMO and EASL have proposed third-line immunosuppressive agents targeting T cells, including the calcineurin inhibitors tacrolimus and cyclosporine, as well as anti-thymoglobulin (Haanen et al., 2017; European Association for the Study of the Liver, 2019). The successful use of these agents has been reported in several cases (Huffman et al., 2018; Motomura et al., 2020; McIlwaine et al., 2022). Some other treatments have also been reported to be used in both steroids and MMF refractory IMH, such as tocilizumab (Stroud et al., 2019) and plasma exchange (Riveiro-Barciela et al., 2019). Furthermore, one study suggested that treatment with ursodeoxycholic acid (UDCA) and bezafibrate should be considered in steroid-refractory IMH cholestatic injury, which may reduce the immune response *via* the proliferator-activated receptor-α-nuclear factor-κB signal pathway (Onishi et al., 2020). Some case reports showed that anti-TNF inhibitor infliximab improved hepatitis in patients with steroid-refractory IMH (Cheung et al., 2019; Corrigan et al., 2019). However, considering the potential hepatotoxicity, all guidelines do not recommend its use in IMH. A detailed additional treatment for steroid-refractory IMH in case reports showed in Table 5.

Withhold and resume ICIs

Another controversial point in the treatment of IMH is whether to permanently cease or resume ICIs in patients with grade 3 or 4 IMH. As current guidelines recommend, ICIs should be permanently ceased in patients with more severe IMH (grade 3 or 4). However, according to a systemic review, grade 3 or 4

IMH should be considered to resume ICIs therapy or switch CTLA-4 inhibitors to PD-1/L1 inhibitors when hepatitis severity improves to grade 2 (Peeraphatdit et al., 2020). Furthermore, another study reported that four patients with grade 3 or 4 IMH were successfully given further immunotherapy after improved liver function, which provides the possibility of resuming ICIs in patients with more severe IMH (Cheung et al., 2019). However, a prospective multicenter study reported that retreatment with ICIs in patients with previous grade 3 or 4 IMH led to 8 of 23 recurrences (Riveiro-Barciela et al., 2022). Moreover, the administration of budesonide during resuming ICIs was considered another promising treatment in patients with severe IMH (Ziemer et al., 2017). In summary, some arguments still exist in the management of IMH. With the understanding of IMH evolved over the years, individualized management should be considered, and the underlying mechanisms of IMH should be further explored to set out a more appropriate management guideline.

Prognosis

Most patients with IMH can recover spontaneously or after corticosteroid administration. For the recovery time, it has been reported that IMH usually resolves in 5–9 weeks (Gauci et al., 2018). However, extended time of ALT levels returned to normal have also been reported in several cases, especially in steroids refractory IMH (Matsubara et al., 2018; McGuire et al., 2018; McIlwaine et al., 2022), which may due to a more severe IMH in these cases. Considering this, a timely diagnosis and management of IMH are critical for prognosis. Nonetheless, there are few studies to validate the recovery time of IMH with different severity and treatments, which may be a direction for selecting treatment and prognosis prediction of patients with IMH in the future. Furthermore, for the mortality of IMH, a retrospective multicenter review showed that the incidence of fatal IMH was 0.01% (5/3345) of all patients treated with ICIs. However, IMH accounted for a high proportion of fatal cases (23.8%, 5/21) (Wang et al., 2018). Moreover, other studies also reported a high mortality rate for IMH (Vozy et al., 2019). These results suggest that attention should be paid to IMH, especially fatal cases, and with the development of diagnosis and management of IMH, the mortality of IMH should be reevaluated.

As to the oncology outcomes, fewer studies have focused on the clinical outcomes of IMH compared with other irAEs. Despite this, a study showed an excellent outcome and overall survival in patients with IMH (Patrinely et al., 2021), which is consistent with the results from studies in extra-hepatic irAEs (Abu-Sbeih et al., 2018; Das and Johnson, 2019; Quach et al., 2019). Although another study indicated that patients with previous IMH showed a lower tumor response and poorer survival outcomes, these results may be due to liver metastases and the administration of other treatments for advanced cancer rather than ICIs (Tsung et al., 2019). A retrospective study reported that IMH was not associated with anti-tumor efficacy and overall survival in patients treated with ICIs (Yamamoto et al., 2021). Moreover, studies have shown no difference in survival outcomes between IMH patients with and without steroid treatment (Gauci et al., 2021). Therefore, given that the oncology outcome of IMH is controversial, more extensive prospective studies are needed to evaluate the prognostic impact of IMH.

Future prospectives

With the success of ICIs in several types of cancer, more and more patients are being treated with ICIs. However, ICIs therapy also causes a variety of irAEs. Due to the immunological characteristics of the liver, ICIs also cause liver-related adverse events, usually termed “immune-mediated hepatitis”. Although IMH is not common compared with other irAEs, with the expanded indications of ICIs therapy, an increasing number of cases diagnosed with IMH are reported. IMH has become increasingly concerned about its potential influence on anti-tumor therapy and lethality. However, the diagnosis and management for IMH are based on found in retrospective case series experience, so there is an urgent need for some randomized clinical trials to clarify the current debate in the IMH, such as further exploring the molecular mechanisms and identifying the prediction markers of IMH as well as evaluate the role of liver biopsy in IMH causality and grading assessment. In addition, we need prospective studies investigating steroid and non-steroid based management of IMH to determine the ideal treatment regimen and better delineate the threshold for appropriate treatment rechallenge after initial management.

Author contributions

ZL writes the original draft; YZ and HX review and edit the manuscript; ZZ is the instructors of this article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Efficacy and safety of concomitant use of proton pump inhibitors with aspirin-clopidogrel dual antiplatelet therapy in coronary heart disease: A systematic review and meta-analysis

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Background: Proton pump inhibitors (PPIs) are usually prescribed to prevent gastrointestinal (GI) complications in patients receiving dual antiplatelet therapy (DAPT). This systematic review and meta-analysis aimed to explore the efficacy and safety of the concomitant use of PPIs with aspirin-clopidogrel DAPT in patients with Coronary heart disease (CHD).

Method: The PubMed, Embase, Cochrane Library, and Web of Science databases were searched from inception to August 2022 for eligible studies. The adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to evaluate the clinical outcomes. Subgroup analysis was conducted according to different PPI subtypes, populations, follow-up times and study types. This study was registered on PROSPERO (CRD42022332195).

Results: A total of 173,508 patients from 18 studies [2 randomized controlled trials (RCTs), 3 *post hoc* analyses of RCTs, and 13 cohort studies] were included in this study. Pooled data revealed that coadministration of PPIs significantly increased the risk of major adverse cardiovascular events (MACEs) (HR = 1.15, 95% CI = 1.06–1.26, p = .001) and reduced the risk of gastrointestinal (GI) complications (HR = 0.44, 95% CI = 0.30–0.64, p < .0001). Subgroup analysis results showed that the esomeprazole users and patients with coronary stenting in the PPI group were associated with an increased risk of MACEs compared with the non-PPI group. The occurrence of MACEs in PPI users was more common than that in non-PPI users in long-term follow-up (≥12 months) studies and in the observational studies. There was no significant differences in the incidences of net clinical adverse events (NACEs), all-cause mortality, or cardiac death between the two groups.

Conclusion: In patients with CHD, the concomitant use of PPIs with aspirin and clopidogrel was associated with a reduced risk of GI complications but could increase the rates of MACEs (particularly in patients receiving esomeprazole or

with coronary stenting). There was no clear evidence of an association between PPI use and NACEs, all-cause mortality, or cardiac death. The results could have been affected by the follow-up time and study type. Further large-scale RCTs with long-term follow-up are needed.

KEYWORDS

proton pump inhibitors, aspirin, clopidogrel, coronary heart disease, medication interaction, meta-analysis

1 Introduction

Coronary heart disease (CHD) is one of the most common chronic illnesses and is the leading cause of death worldwide (Zhou et al., 2019; Voutilainen, et al., 2022). Dual antiplatelet therapy (DAPT) with aspirin plus clopidogrel is recommended for patients with CHD to reduce the risk of ischemic cardiovascular events while increasing the risk of bleeding compared with either of the regimens alone (Diener et al., 2004; Benavente et al., 2012; Valgimigli et al., 2018). Gastrointestinal (GI) bleeding accounts for a significant proportion of bleeding complications in DAPT, which can lead to DAPT cessation and has been identified as an independent risk factor for poor prognosis. (Capodanno et al., 2018). Because aspirin damages the gastric mucosa by suppressing the synthesis of prostaglandins (PGs) (Nishida et al., 2011), the antiangiogenic effects of clopidogrel could impair the healing of gastric erosions (Luo et al., 2016). These patients are frequently prescribed proton pump inhibitors (PPIs) to minimize GI complications (involving ulcers and bleeding) (Levine et al., 2016; Valgimigli et al., 2018). However, previous studies have indicated that coadministration of PPIs with aspirin-clopidogrel DAPT could be associated with adverse drug-drug interactions.

Aspirin is mainly absorbed in the acidic environment. PPIs inhibit gastric acid production and increase gastric pH, resulting in poor aspirin absorption (Gesheff et al., 2014; Vaduganathan and Bhatt, 2016). Clopidogrel is a prodrug that depends on cytochrome P450 isoenzyme (mainly CYP2C19) to metabolize into an active form. PPIs are also metabolized by CYP enzymes and thus could inhibit the conversion of clopidogrel into its active metabolite (Furuta et al., 2017). Furthermore, mounting clinical data have shown that long-term intake of PPIs increases the susceptibility of patients to serious adverse events, including cardiovascular events and damage to the lower GI tract (Lue and Lanas, 2016; Xie et al., 2019; Marlicz et al., 2022; Zhai et al., 2022). However, the existing clinical studies of the association between cardiovascular events and the concomitant use of PPIs with aspirin-clopidogrel DAPT in CHD have been conflicting (Ben Ghezala et al., 2022). Some meta-analyses were conducted to assess the clinical significance of this interaction and found that coadministration of PPIs could increase the rates of major adverse cardiovascular events (MACEs), stroke, revascularization, and stent thrombosis (ST) but not myocardial infarction (MI), all-cause mortality, or cardiac death (Guo et al., 2021; Hu et al., 2018; Li et al., 2021; Melloni et al., 2015; Sherwood et al., 2015). Interestingly, two recent clinical studies reported that the rates of MI, all-cause mortality and cardiac death were significantly increased in PPI users compared to non-users (Maret-Ouda et al., 2021; Mohammed et al., 2021). In these studies, hazard ratios (HRs) containing the status of event occurrence and the time when events happened were calculated by multivariable Cox proportional hazards regression models (Spruance et al., 2004). Moreover, net clinical adverse events (NACEs) are also an important clinical outcome in CHD (Chandrasekhar et al., 2017). However, to the best of our knowledge, no meta-analyses of previous studies have reported NACEs outcomes. In addition, the length of follow-up is vital for the clinical outcome evaluation of PPI coadministration, but it has rarely been considered in previous meta-analyses. Furthermore, the results of clinical studies have also been inconsistent regarding the protective effects of PPIs in the GI tract.

Therefore, we performed this meta-analysis to evaluate the efficacy and safety of the combination treatment of PPIs with aspirin-clopidogrel DAPT for CHD patients by extracting adjusted HRs to provide a theoretical basis for clinical, individualized practice. Furthermore, subgroup analysis was conducted according to different PPI subtypes, populations, follow-up times and study types to analyze the heterogeneity.

2 Methods

2.1 Search strategy

This study was conducted in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. The study was registered on PROSPERO (CRD42022332195). We searched the PubMed, Embase, the Cochrane Library, and Web of Science databases for relevant studies published in English from inception to August 2022. The following Medical Subject Headings (MeSH) and keywords were used for the literature retrieval: “proton pump inhibitors (PPIs),” “dual antiplatelet therapy (DAPT),” “aspirin,” “clopidogrel,” “acute coronary syndrome (ACS),” “myocardial infarction (MI),” “percutaneous coronary intervention (PCI),” and “coronary stenting.” We also searched

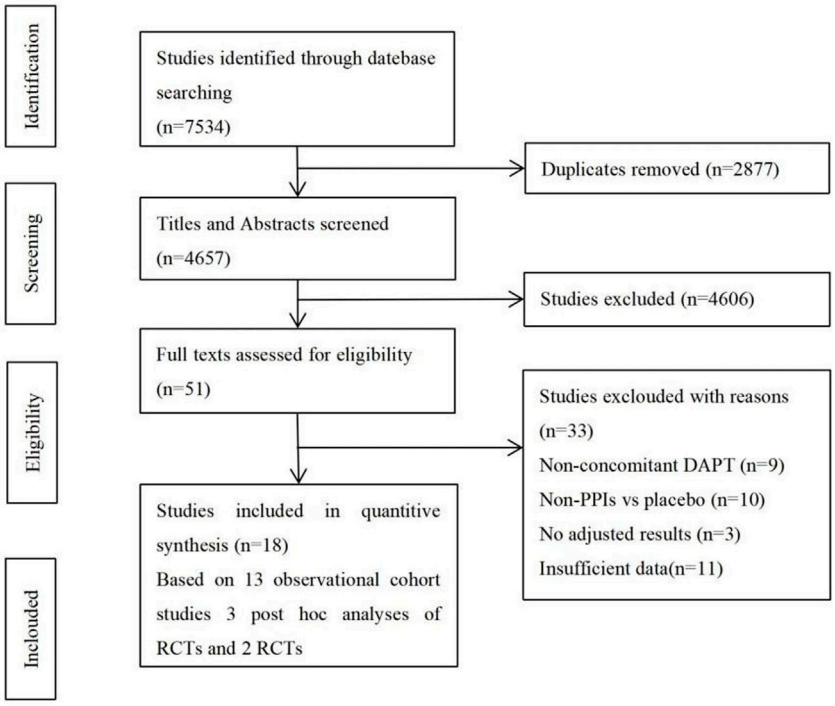

FIGURE 1
Flowchart of study selection.

conference abstracts and reviewed reference lists of relevant review articles to provide additional citations.

2.2 Study selection

Studies were selected based on the following inclusion criteria: 1) subjects: patients with ACS, PCI, or coronary stenting receiving aspirin-clopidogrel DAPT; 2) exposure intervention: the experimental group was treated with PPIs, whereas the control group was treated with a placebo or no PPIs; 3) outcome measures: the primary outcome was MACEs, and the secondary outcomes were NACEs, MI, stroke, revascularization, ST, all-cause mortality, cardiac death, and GI complications (involving ulcers and bleeding events); and 4) study design: randomized, controlled trials (RCTs) and observational studies.

Studies were excluded if they met the following exclusion criteria: 1) patients receiving aspirin or clopidogrel alone; 2) control group patients receiving H_2 receptor antagonists; 3) effect estimates of adjusted HRs and corresponding 95% confidence intervals (CIs) was not provided; 4) different reports of the same trial or duplicate data; and 5) case reports.

2.3 Data extraction and quality assessment

Two reviewers independently extracted data from eligible studies, including authors, publication year, region, population, type of PPI, follow-up time, study endpoints, study design, and sample size. The Jadad Scale and Newcastle-Ottawa Scale (NOS) scoring methods were used to assess the quality of RCTs and observational studies, respectively. Post hoc analyses of RCTs were regarded as observational studies to evaluate study quality. Discrepancies in data extraction and quality assessment, if necessary, were resolved by consultation with a third reviewer.

2.4 Statistical analysis

Review Manager software, version 5.3, was used for the analysis of adjusted HRs. Between-study heterogeneity was calculated with Higgins's I^2 test: $I^2 > 50\%$ could represent substantial heterogeneity, and a random-effect model was applied; otherwise, a fixed-effect model was used. Subgroup analysis was conducted based on the PPI subclass, follow-up time, population, and study type. We estimated publication bias through a visual inspection of funnel plots.

TABLE 1 Main characteristics of included studies.

Author, year	Country	Population	Study design	Sample (PPIs/No PPIs)	Fellow-up (months)	PPIs
Mohammed et al. (2021)	Egypt	PCI	Cohort	375/175	18	NR
Maret-Ouda et al. (2021)	Swedish	PCI	Cohort	35772/64064	12	O, E, P
Zhu et al. (2017)	China	PCI	Cohort	2142/5725	24	NR
Chandrasekhar et al. (2017)	Fifteen centers from US and Europe	CS	Cohort	1062/3573	24	NR
Gargiulo et al. (2016)	Italy	CS	RCT	738/1232	24	O, E, P, R
Weisz et al. (2015)	US and Germany	CS	Cohort	2697/5885	24	NR
Zou et al. (2014)	China	CS	Post hoc	6188/1465	12	O, E, P
Hokimoto et al. (2014)	Japan	CS	Cohort	50/124	18	R
Goodman et al. (2012)	Europe,US,Asia	ACS	Post hoc	6539/12062	12	O, E, P, L, R
	Middle East, Africa, Australia					
Aihara et al. (2012)	Japan	CS	Cohort	1068/819	12	O, E, L
Simon et al. (2011)	France	MI	Post hoc	1453/900	12	O
Harjai et al. (2011)	United States of America	PCI	Cohort	751/1900	6	O
Burkard et al. (2012)	Switzerland	CS	Cohort	109/692	36	R
Bhatt et al. (2010)	393 sites in 15 countries	ACS or CS	RCT	1876/1885	15	O, P
Gaglia et al. (2010)	United States of America	CS	Cohort	318/502	12	O, E, P, L, R
Tentzeris et al. (2010)	Austria	CS	Cohort	691/519	12	O, E, P, L, R
Sarafoff et al. (2010)	Germany	CS	Cohort	698/2640	1	O, E, P, L, R
O'Donoghue et al. (2009)	United States of America and Europe	ACS with PCI	Post hoc	2257/4538	6	O, E, P, L

NR, not reported; *O*, omeprazole; *E*, esomeprazole; *P*, pantoprazole; *R*, rabeprazole; *L*, lansoprazole; *RCT*, randomized controlled trial; *CS*, coronary stenting; *US*, the Unite States.

3 Results

3.1 Search results and quality evaluation

The flowchart of study selection is shown in Figure 1. There were 7,534 studies identified in the preliminary electronic database search. After tiered screening, a total of 18 articles were selected for the quantitative analysis, including 2 RCTs (Bhatt et al., 2010; Gargiulo et al., 2016), 3 *post hoc* analyses of RCTs (Goodman, 2012; O'Donoghue, 2009; Simon, 2011), and 13 cohort studies (Aihara, 2012; Burkard, 2012; Chandrasekhar, 2017; Gaglia, 2010; Harjai, 2011; Hokimoto, 2014; Maret-Ouda, 2021; Mohammed, 2021; Sarafoff, 2010; Tentzeris, 2010; Weisz, 2015; Zhu, 2017; Zou, 2014). PPIs were used by 64,784 of the 173,508 patients (37.34%), and 108,700 patients did not use PPIs. Table 1 presents the main characteristics of the included studies, and the results of the quality evaluation are listed in Supplementary Tables S1, S2.

3.2 Quantitative synthesis

3.2.1 The primary outcome

Eighteen studies reported MACEs (Figure 2). The results indicated that PPIs significantly increased the occurrence of MACEs (HR = 1.15, 95% CI = 1.06–1.26; p = .001) with a random-effect model (P = .0007, I^2 = 59%).

Subgroup analyses of PPI subclasses, populations, follow-up times and study types were performed (Table 2).

With regard to the types of PPIs, the use of esomeprazole (HR = 1.23, 95% CI = 1.06–1.42; p = .006) was associated with a significant increase in the risk of MACEs but not omeprazole (HR = 1.02, 95% CI = 0.82–1.26; p = .87), pantoprazole (HR =

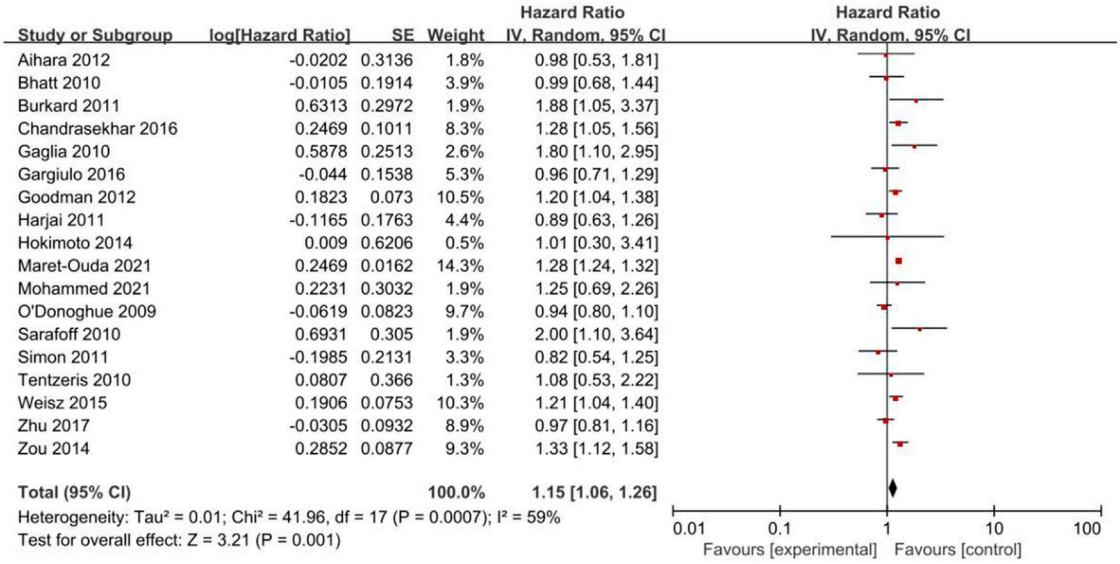

FIGURE 2
Forest plots of the risk of MACEs.

TABLE 2 Subgroup analysis of MACEs.

Outcome	Subgroup		Number of studies	Pooled HR (95%CI)	*P*-value	Heterogeneity		Analysis model
						P_h	I^2	
MACEs	Type of PPIs	Omeprazole	4	1.02 (0.82–1.26)	0.87	0.03	67	R
		Esomeprazole	3	1.23 (1.06–1.42)	0.006	0.54	0	F
		Pantoprazole	3	1.25 (0.90–1.74)	0.19	0.01	77	R
		Lansoprazole	2	0.96 (0.61–1.50)	0.85	0.40	0%	F
	Study design	RCTs	2	0.97 (0.77–1.23)	0.80	0.89	0%	F
		Observational studies	16	1.17 (1.07–1.28)	0.0005	0.001	60%	R
	Population	Coronary stenting	10	1.26 (1.16–1.38)	<0.00001	0.28	18%	F
		Mixed	8	1.05 (0.92–1.21)	0.46	<0.0001	77%	R
	Follow-up time	<12 months	5	0.98 (0.86–1.11)	0.73	0.19	34%	F
		≥12 months	13	1.26 (1.23–1.30)	<0.00001	0.04	45%	R

1.25, 95% CI = 0.90–1.74; p = .19), or lansoprazole (HR = 0.96, 95% CI = 0.61–1.50; p = .85). Only one study reported MACEs in patients treated with rabeprazole; therefore, subgroup analysis was not possible for rabeprazole users.

In the stratification analyses by population, we found that the occurrence of MACEs was higher in the coronary stenting group administered PPIs (HR = 1.26, 95% CI = 1.16–1.38; p < .00001) but not in the mixed group (HR = 1.05, 95% CI = 0.92–1.21; p = .46).

When stratified by length of follow-up, there was no significant difference in the incidences of MACEs between PPI users and non-PPI users in the short-term follow-up (<12 months) group (HR = 0.98, 95% CI = 0.86–1.11; p = .73). However, in the long-term follow-up (≥12 months) group, the occurrence of MACEs was higher in the patients administered PPIs (HR = 1.26, 95% CI = 1.23–1.30; p < .00001).

Subgroup analysis of observational studies (HR = 1.17, 95% CI = 1.07–1.28; p = .0005) showed that PPIs increased the

TABLE 3 Meta-analysis on outcomes.

Outcome	Number of studies	Pooled HR (95%CI)	*P*-value	Heterogeneity		Analysis model
				P_h	I^2 (%)	
MACEs	18	1.15 (1.06–1.26)	0.001	0.0007	59	R
NACEs	4	1.02 (0.93–1.13)	0.67	0.14	45	F
MI	13	1.18 (1.11–1.24)	<0.00001	0.26	18	F
Stroke	3	1.18 (1.03–1.35)	0.02	0.25	28	F
Revascularization	7	1.17 (1.06–1.30)	0.02	0.19	31	F
Stent thrombosis	11	1.21 (1.03–1.42)	0.02	0.65	0	F
All-cause mortality	13	1.15 (0.94–1.41)	0.18	<0.00001	78	R
Cardiac death	5	1.09 (0.80–1.48)	0.59	0.0006	80	R
GI complications	3	0.44 (0.30–0.64)	<0.0001	0.29	19	F

MACEs, major adverse cardiovascular events; *NACEs*, net clinical adverse events; *MI*, myocardial infarction; *GI*, gastrointestinal; *HR*, effect estimates of hazard ratio; *CI*, confidence interval; *R*, random effect model; *F*, fixed effect model.

occurrence of MACEs, while analysis of the RCTs (HR = 0.97, 95% CI = 0.77–1.23; p = .80) did not demonstrate statistical significance.

3.2.2 The secondary outcomes

All clinical outcomes are shown in Table 3. PPIs were associated with a significant increase in the risk of MI (HR = 1.18, 95% CI = 1.11–1.24; p < .00001, I^2 = 18%, Figure 3B), stroke (HR = 1.18, 95% CI = 1.03–1.35; p = .02, I^2 = 28%, Figure 3C), revascularization (HR = 1.17, 95% CI = 1.06–1.30; p = .02, I^2 = 31%, Figure 3D), and ST (HR = 1.21, 95% CI = 1.03–1.42; p = .02, I^2 = 0%, Figure 3E) but not NACEs (HR = 1.02, 95% CI = 0.93–1.13; p = .67, I^2 = 45%, Figure 3A), all-cause mortality (HR = 1.15, 95% CI = 0.94–1.41; p = .18, I^2 = 78%, Figure 4A) or cardiac death (HR = 1.09, 95% CI = 0.80–1.48; p = .59, I^2 = 80%, Figure 4B). However, PPIs significantly reduced the risk of GI complications (HR = 0.44, 95% CI = 0.30–0.64; p < .0001, I^2 = 19%, Figure 5).

3.3 Publication bias

A funnel plot was drawn for the primary outcome, and it showed symmetry on visual inspection, indicating that publication bias was not large (Supplementary Figure S1).

3.4 Sensitivity analysis

No single study markedly altered the overall effect in the sensitivity analysis, suggesting that the pooled HR of MACEs was stable.

4 Discussion

This systematic review and meta-analysis evaluated the efficacy and safety of the concomitant use of PPIs with aspirin-clopidogrel DAPT in CHD. The results showed that PPI coadministration decreased the risk of GI complications but could increase the rates of MACEs, stroke, revascularization and ST, in line with previous studies (Melloni et al., 2015; Hu et al., 2018). There were also no significant differences in the risks of all-cause mortality and cardiac death (Hu et al., 2018; Li et al., 2021; Melloni et al., 2015).

Interestingly, the incidence of MI in the PPI group was significantly increased in our study, inconsistent with previous meta-analysis results (Hu et al., 2018; Li et al., 2021). Several reasons account for this outcome. On the one hand, PPIs could decrease the effects of aspirin and clopidogrel on platelet aggregation (Zuern et al., 2010). On the other hand, it has been reported that PPIs could augment cardiovascular risk *via* platelet-independent biological pathways. One suggested mechanism is that PPIs inhibit the enzyme activity of dimethylarginine dimethylaminohydrolase (DDAH), thereby blocking the degradation of endothelial asymmetrical dimethylarginine (ADMA), an endogenous and competitive inhibitor of nitric oxide synthase. Excess ADMA in turn leads to impaired endothelial nitric oxide (NO) generation and reduced vascular function (Ghebremariam et al., 2013; Nolde et al., 2021; Zhai et al., 2022). In addition, a study investigating the long-term effect of PPIs on endothelial dysfunction found that chronic exposure to PPIs could expedite endothelial aging, which might explain the increased cardiovascular events (Yepuri et al., 2016). Therefore, the benefits for GI should be weighed against

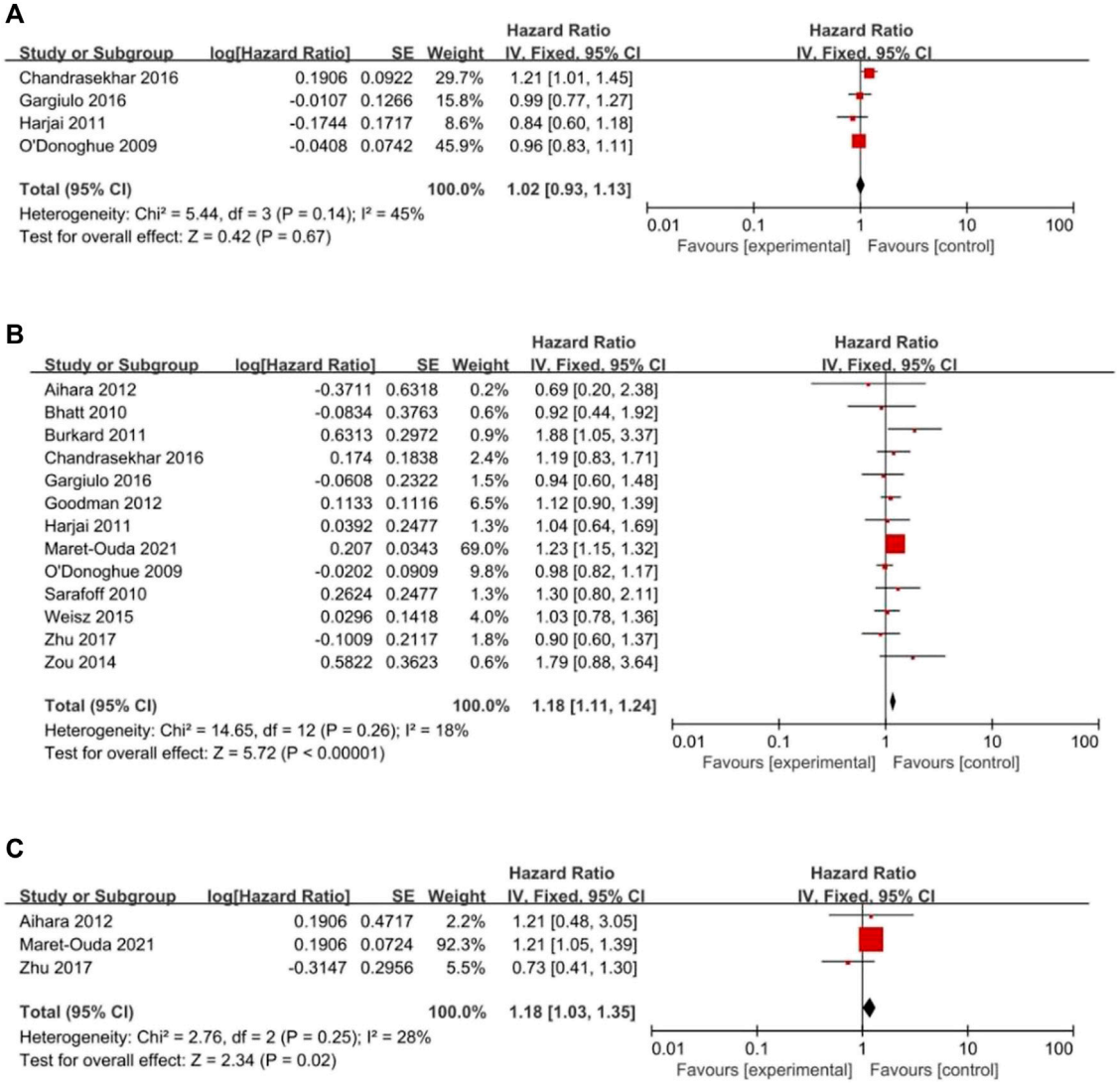

FIGURE 3
(Continued).

the recurrent ischemic cardiovascular events. Moreover, we found that there was no significant difference in the risk of NACEs between the two groups, although the risk was higher in the PPI group than in the non-PPI group.

Most interestingly, the elevated risk of MACEs for PPI users might be affected by the PPI subtype and population. *In vitro* studies suggested that different types of PPIs could affect CYP2C19 differently. Based on drug-drug interaction studies, the clinically relevant interaction tendency was the greatest for omeprazole and esomeprazole, with a moderate probability for lansoprazole and the lowest for pantoprazole and rabeprazole (Li et al., 2004; Norgard et al., 2009; Valgimigli et al., 2018). These results prompted our subgroup analyses of PPI subclasses. In agreement with the findings by Sherwood (Sherwood et al., 2015), the use of esomeprazole was associated with an increased risk of MACEs. Physicians should consider the potential risks with different PPIs when prescribing them for individual patients taking aspirin and clopidogrel. Because PCI with stent implantation is the most common interventional treatment for patients with coronary disease, we further performed stratification analyses of the population. In patients following coronary stenting, the occurrence of MACEs was higher in PPI users than in non-PPI users, which could have been driven by the significantly increased risk of ST (Zou et al., 2014).

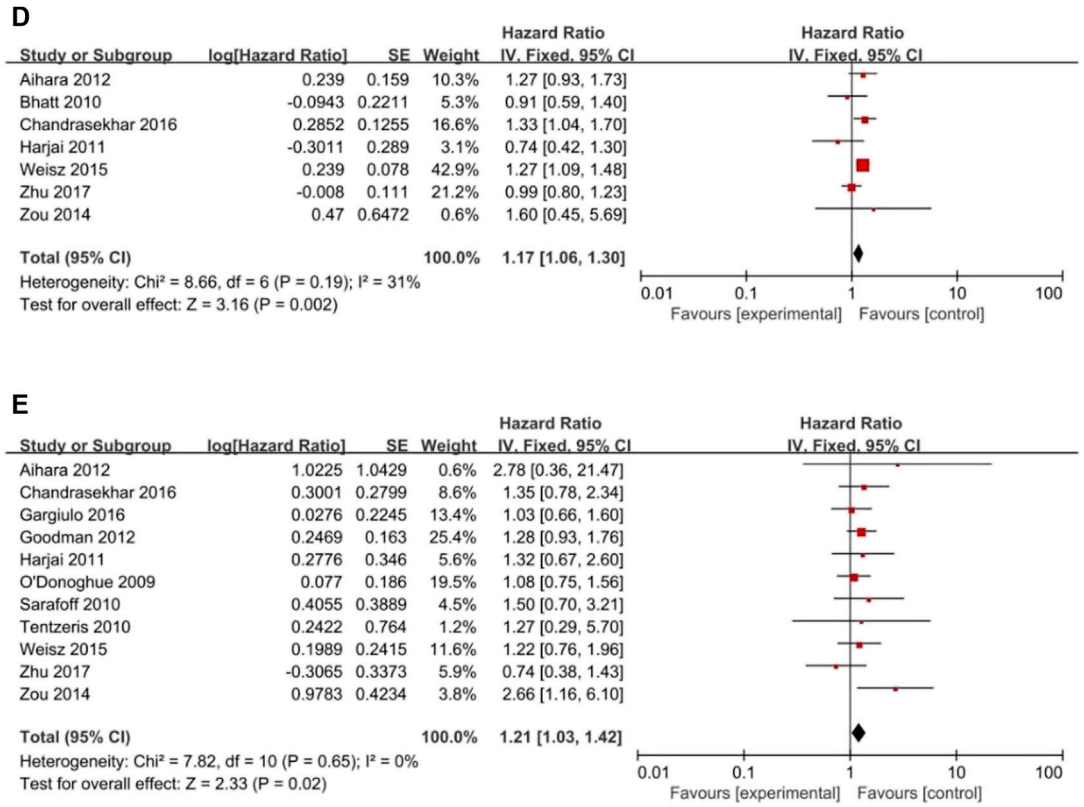

FIGURE 3
(Continued). Forest plots of **(A)** NACEs, **(B)** MI, **(C)** Stroke, **(D)** Revascularization and **(E)** Stent thrombosis.

In addition, we found that the length of follow-up time was quite different among our included studies, with a certain impact on the evaluation of MACEs (Harjai et al., 2011; Maret-Ouda et al., 2021). No previous meta-analyses were performed to evaluate such a difference. The incidence of MACEs varied according to different follow-up times in our study. When the follow-up periods were shorter than 12 months, there was no significant difference between the two groups, while the incidence of MACEs in the PPI group was significantly higher than that in the non-PPI group with longer follow-up (≥12 months). Consequently, long-term follow-up seems to be necessary for cardiovascular event investigations. Furthermore, the results were also inconsistent in different types of studies. The data from observational studies revealed that the use of PPIs increased the risk of MACEs, while the limited data from RCTs showed no significant difference.

There were several limitations to this study. First, most of our included articles were observational studies, and selection bias, along with unmeasured confounding, could account for these findings. Although we extracted the adjusted HRs, our results might still be biased by residual confounding. Second, a small number of RCTs (2 eligible for meta-analysis) were included, and the sample size of some subgroups might have been too small to indicate statistical significance and limit the representativeness of the results, again prompting more RCTs to assess the clinically relevant interactions. Third, we excluded many studies due to the inability to extract data, resulting in some bias. Fourth, subgroup analysis was conducted according to different PPI subtypes, populations, follow-up times and study types to analyze the heterogeneity in our study; however, clinical details, including the duration of DAPT and PPIs, type of stent, CYP2C19 genotypes, and concomitant diseases (such as diabetes), were insufficient in some articles, also potentially leading to heterogeneity among studies. Moreover, the included literature did not stratify the participants by the risk of cardiovascular events, and GI bleeding limited the evaluation of clinical outcomes. Thus, further studies regarding the efficacy and safety of concomitant use of PPIs with aspirin-clopidogrel DAPT should consider these limitations.

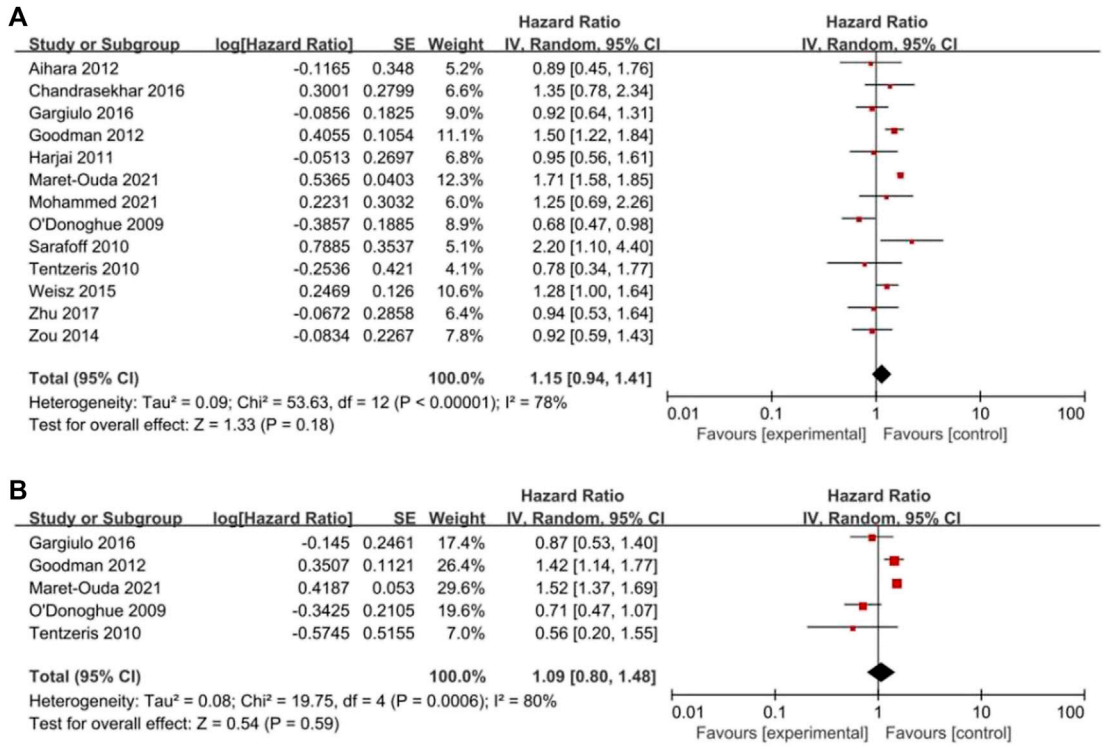

FIGURE 4
Forest plots of **(A)** all-cause mortality and **(B)** cardiac death.

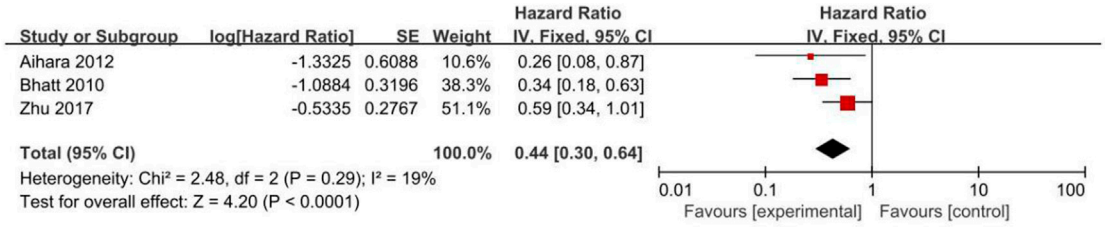

FIGURE 5
Forest plots of GI complications.

5 Conclusion

The concomitant use of PPIs was associated with a reduced risk of GI complications, while it could increase the rates of MACEs (particularly in patients receiving esomeprazole or with coronary stenting), MI, stroke, revascularization, and ST in CHD patients receiving aspirin-clopidogrel DAPT. There was no clear evidence of associations between PPI use and NACEs, all-cause mortality, or cardiac death. These results could have been affected by the follow-up time and study type. In light of the limitations of the current systematic review and meta-analysis, large-scale RCTs with longer-term follow-up are warranted to evaluate the safety and efficacy of PPIs with DAPT.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

XL, HX, XY and PZ contributed to the conception or design of the study. XL and SH contributed to the study selection, data extraction, quality assessment and statistical analysis. XY and YZ contributed to data acquisition and data interpretation. XL, MH and HX drafted the manuscript or substantively revised it. All the authors have final approval of the submitted manuscript and reached agreement to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Autoimmunity associates with severity of illness in elderly patients with drug-induced liver injury

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Background: Drug-induced liver injury (DILI) is a potentially serious adverse drug reaction. Due to the lack of definite etiology, specific clinical manifestations, and diagnostic methods, its prediction and diagnosis are challenging. Elderly individuals are deemed to be at high risk for DILI due to abnormal pharmacokinetics, aging tissue repair function, comorbidities, and taking multiple drugs. This study aimed to identify the clinical characteristics and explore the risk factors associated with the severity of illness in elderly patients with DILI.

Methods: In the present study, the clinical characteristics at the time of liver biopsy of consecutive patients with biopsy-proven DILI who presented at our hospital from June 2005 to September 2022 were evaluated. Hepatic inflammation and fibrosis were assessed according to the Scheuer scoring system. The presence of autoimmunity was considered if IgG level >1.1 × ULN (1826 mg/dL), or high titer (>1:80) of ANA, or SMA.

Results: In total, 441 patients were enrolled, and the median age was 63.3 years (IQR, 61.0–66.0); 122 (27.7%), 195 (44.2%), or 124 (28.1%) were classified as having minor, moderate, or severe hepatic inflammation, respectively; and 188 (42.6%), 210 (47.6%) or 43 (9.8%) patients presented minor, significant fibrosis or cirrhosis, respectively. Female sex (73.5%) and the cholestatic pattern (47.6%) were dominant in elderly DILI patients. Autoimmunity existed in 201 patients (45.6%). Comorbidities were not directly associated with the severity of DILI. PLT (OR: 0.994, 95% CI: 0.991–0.997; $p < 0.001$), AST (OR: 1.001, 95% CI: 1.000–1.003, $p = 0.012$), TBIL (OR: 1.006, 95% CI: 1.003–1.010, $p < 0.001$), and autoimmunity (OR: 1.831, 95% CI: 1.258–2.672, $p = 0.002$) were associated with the degree of hepatic

Abbreviations: ANA, anti-nuclear antibody; AMA, anti-mitochondrial antibody; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; BMI, body mass index; CI, confidence interval; DILI, drug-induced liver injury; EASL, uropean association for the study of the liver; HDS, herbal and dietary supplements; HIV, human immunodeficiency virus; HAI, histological activity index; IgG, immunoglobulin G; IQR, interquartile range; NSAID, non-steroidal anti-inflammatory drug; OR, odds ratio; PLT, platelet; PIM, potentially inappropriate medications; RUCAM, roussel uclaf causality assessment method; SMA, smooth muscle antibody; TBIL, total bilirubin; TCM, traditional Chinese medicine; ULN, upper limit of normal.

inflammation. Meanwhile, PLT (OR: 0.990, 95% CI: 0.986–0.993, $p < 0.001$), TBIL (OR: 1.004, 95% CI: 1.000–1.007, $p = 0.028$), age (OR: 1.123, 95% CI: 1.067–1.183, $p < 0.001$), and autoimmunity (OR: 1.760, 95% CI: 1.191–2.608, $p = 0.005$) were associated with the stage of hepatic fibrosis.

Conclusion: This study revealed that the presence of autoimmunity represents a more serious illness state of DILI, deserving more intensive monitoring and progressive treatment.

KEYWORDS

drug-induced liver injury (DILI), elderly, hepatic fibrosis, autoimmunity, liver biopsy

Introduction

In the case of a reasonable rule out of other causes, drug-induced liver injury (DILI), which remains one of the most challenging diseases faced by hepatologists, is a severe adverse drug reaction caused by hepatotoxic exogenous agents and their metabolites, such as prescriptions, over-the-counter drugs, herbs, and dietary supplements (Teschke et al., 2013; Kleiner et al., 2014; Navarro et al., 2014; EASL-ALEH Clinical Practice Guidelines;Asociacion Latinoamericana para el Estudio del Higado, 2015; Foureau et al., 2015; Bonkovsky et al., 2017; Kullak-Ublick et al., 2017; Andrade et al., 2019b). With the enhancement of people's health consciousness, herbal and dietary supplements (HDS) or traditional Chinese medicines (TCM) account for an increasing proportion of DILI events worldwide (Wai et al., 2007; Navarro et al., 2014; García-Cortés et al., 2016; Ji et al., 2018). This is especially applicable to body-building and fat-reducing supplements (Grewal and Ahmad, 2019; Santos et al., 2021), exposing people to more uncertain hepatotoxic drugs, which calls for clinicians to have a greater understanding of DILI.

Elderly individuals are at high risk for DILI (Danan and Benichou, 1993; Lucena et al., 2009; Andrade et al., 2019a). First, the distribution and release of drugs are affected by increased body fat in the aged (Lucena et al., 2009). Second, the decrease in liver and kidney function affects the metabolism and excretion of drugs, leading to abnormal pharmacokinetics (Tostmann et al., 2008; Klotz, 2009; Andrade et al., 2019a). At the same time, their tissue-repair capacity is reduced. In addition, the aged have many comorbidities and may require multiple medications, so DILI is complicated by drug-drug interactions and drug-host interactions (Chen et al., 2015; Andrade et al., 2019a). Marcum et al. (2012) reported that people older than 70 take an average of three to seven medications per day. A meta-analysis demonstrated that the prevalence of polypharmacy (using more than five drugs) and potentially inappropriate medications (PIM) in elderly Chinese patients was 48% and 39%, respectively (Tian et al., 2022). Similarly, Koçak et al. (2022) mentioned that the prevalence of PIM among nursing home residents aged ≥60 years was 47.6%.

DILI with features of autoimmunity represents an important category of hepatotoxicity due to medication exposure, which is a syndrome typically characterized by liver injury accompanied by hypergammaglobulinemia, circulating anti-nuclear antibody (ANA) and/or smooth muscle antibody (SMA) (deLemos et al., 2014). Circulating antibodies targeting intracellular components are also indicative of cellular damage. Furthermore, elderly individuals commonly present a low titer (<1:80) of serum autoantibodies (approximately 25%), making distinguishing between DILI and AIH more complicated (deLemos et al., 2014).

The exclusive diagnosis combined with causality assessment is an essential and arduous task to improve DILI diagnosis (Liu et al., 2021). There is considerable variability in the time to onset, severity, clinical manifestations, laboratory features, findings on liver biopsy, course, and outcome. Moreover, relatively few studies have involved elderly DILI patients with features of autoimmunity, making the database for this group somewhat limited. Thus, in the present study, we aimed to identify the clinical characteristics and explore the risk factors associated with the severity of illness in this special patient population to avoid progressing to poorer clinical outcomes.

Patients and methods

Study design and patients

The present study was a retrospective hospitalization-based cross-sectional study, which was approved by the Ethics Committees of Fifth Medical Center of Chinese PLA General Hospital (No. 2019024D). Written informed consent for liver biopsy was obtained from all patients, whereas patient consent for data collection was waived due to the study design.

The inclusion criteria included the following: 1) admitted from June 2005 to September 2022; 2) age ≥60 years old; 3) met the DILI definition (see definition section); 4) Roussel Uclaf Causality Assessment Method (RUCAM) score >6 points; and 5) the diagnosis of DILI was confirmed by liver biopsy. The exclusion criteria were as follows: 1) those with any other definite etiologies of liver disease (e.g., primary biliary cholangitis, primary sclerosing cholangitis, viral hepatitis, alcoholic or non-alcoholic liver disease, Gilbert syndrome, etc.) according to their relevant guidelines (Lindor et al., 2015; EASL–EASD–EASO Clinical Practice, European Association for the Study of Diabetes EASD, European Association for the Study of Obesity EASO, 2016; Hirschfield et al., 2017; Singal et al., 2018; Wagner et al., 2018; Te and Doucette, 2019); 2) those with severe systemic diseases affecting the liver (heart attack, stroke, kidney, or HIV infection); and 3) those with incomplete important data.

Procedures

The clinical data of the enrolled patients at the time of liver biopsy were retrieved through electronic medical records, such as age, sex, body mass index (BMI), implicated drugs, platelet (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST),

total bilirubin (TBIL), alkaline phosphatase (ALP), immunoglobulin G (IgG), anti-nuclear antibody (ANA), smooth muscle antibody (SMA), and anti-mitochondrial antibody (AMA).

Definition

DILI was defined as an adverse hepatic reaction that is unexpected based on the pharmacological action of the drug administered, including one of the following thresholds: 1) ≥5 × ULN elevation in ALT, 2) ≥2 × ULN elevation in ALP (particularly with accompanying elevations in concentrations of GGT in the absence of known bone pathology driving the rise in ALP level), or 3) ≥3 × ULN elevation in ALT and simultaneous elevation of TBIL concentration exceeding 2 × ULN, according to EASL DILI guideline 2019. Three patterns of DILI were categorized by using the R-value ([ALT/upper limit of the normal range (ULN)]/[ALP/ULN]): hepatocellular when ≥5, cholestatic when ≤2 and mixed when 2–5 (Andrade et al., 2019a).

The presence of autoimmunity was considered if IgG level >1.1 × ULN (1826 mg/dL), or high titer (>1:80) of ANA, or SMA (de Boer et al., 2017). The enrolled patients were divided into three groups by age: Group A, ≤65 years (n = 318 [72.1%]); Group B, 65–70 years (n = 92 [20.9%]); and Group C, >70 years (n = 31 [7.0%]).

Histological evaluations

Ultrasound-guided liver biopsy was performed on all patients, and hepatic inflammatory grades and fibrosis stages were evaluated according to the Scheuer scoring system (Scheuer, 1991). To ensure sufficient specimens with at least 12 portal vessels for histological evaluation, a minimum length of 15.0 mm was required for each liver specimen. Subsequently, two liver histopathologists independently evaluated tissue specimens, and when inconsistencies arose, both pathologists re-reviewed the specimens together. The grade of hepatic inflammation was defined as mild (G0-1), moderate (G2), and severe (G3-4). The stage of hepatic fibrosis was defined as mild (S0-1), significant (S2-3), and cirrhosis (S4).

Statistical analysis

Continuous variables were expressed as medians and interquartile ranges (IQR) and compared by using the Kruskal-Wallis test. Categorical variables were presented as numbers (percentage) and compared by Chi-square or Fisher's exact test. The trend was analyzed by the Cochran-Armitage trend test for the 2 × 3 tables (sex and autoimmunity) and the Jonckheere-Terpstra trend test for the 3 × 3 tables (age, liver damage pattern, medication). Multivariate ordinal polytomous logistic regression was performed to identify the independent risk factors associated with the severity of illness regarding hepatic inflammation or fibrosis. The odds ratio (OR) and 95% confidence interval (CI) were estimated simultaneously. The forest plot was established by using the ggplot2 package of R. A two-tailed *p-value* of <0.05 was considered statistically significant. All statistical analyses were performed using R software, version 4.2.1 (http://www.r-project.org/).

Results

Clinical characteristics

A total of 507 elderly patients with DILI who underwent liver biopsy were screened. Of these patients, 66 patients were excluded. Finally, 441 patients were enrolled in this study and were categorized into three groups according to hepatic inflammation grade or fibrosis stage (Figure 1).

The overall median age was 63.3 years (IQR, 61.0–66.0), and 324 patients (73.5%) were female. Regarding histological characteristics, 122 (27.7%), 195 (44.2%), or 124 (28.1%) patients were classified as having minor, moderate, or severe hepatic inflammation, respectively; 188 (42.6%), 210 (47.6%), or 43 (9.8%) patients presented minor, significant fibrosis, or cirrhosis, respectively. The clinical pattern of liver damage was given priority to cholestatic damage (47.6%), followed by hepatocellular damage (29.3%), and mixed damage (23.1%). The most common comorbidities were hypertension (28.1%), hyperlipidemia (27.7%), diabetes mellitus (11.8%), and coronary heart disease (4.8%). According to the results of autoimmune antibody detection, there were 143 patients (32.4%) with positive ANA, 30 patients (6.8%) with positive SMA, and 74 (16.8%) with ≥1.1 × ULN of IgG. Overall, 201 patients (45.6%) presented the feature of autoimmunity (Table 1).

Furthermore, we investigated the distribution of age stratified by sex. The number of cases was highest among those aged 60–62 years, and the percentage of females was significantly higher than that of males for every 1-year age group ($p < 0.05$, Figure 2A). The overall percentage of autoimmunity in females was 52.5%, which was significantly higher than that in males (25.6%, $p < 0.05$, Figure 2B).

Implicated drugs

In total, 202 (45.8%) patients had taken a combination of synthetic drugs and TCM or HDS, 129 (29.3%) had taken synthetic drugs, and 110 (24.9%) had taken TCM or HDS. The most commonly implicated drugs were cardiovascular drugs (17.0%), followed by herbal and dietary supplements (HDS) (12.9%), gastrointestinal drugs (6.3%), non-steroidal anti-inflammatory drugs (NSAIDs) (5.9%), etc. Meanwhile, there were 16.3% polypharmacy and 21.5% unspecified drugs (Supplementary Figure S1).

Distribution of clinical Parameters by histological assessment

All enrolled patients were categorized into three ordinal groups according to histological assessment and compared. The results showed that patterns of liver damage, PLT, ALT, AST, TBIL, and autoimmunity were significantly different in groups with increasing grades of hepatic inflammation ($p < 0.05$, Table 1) or in groups with

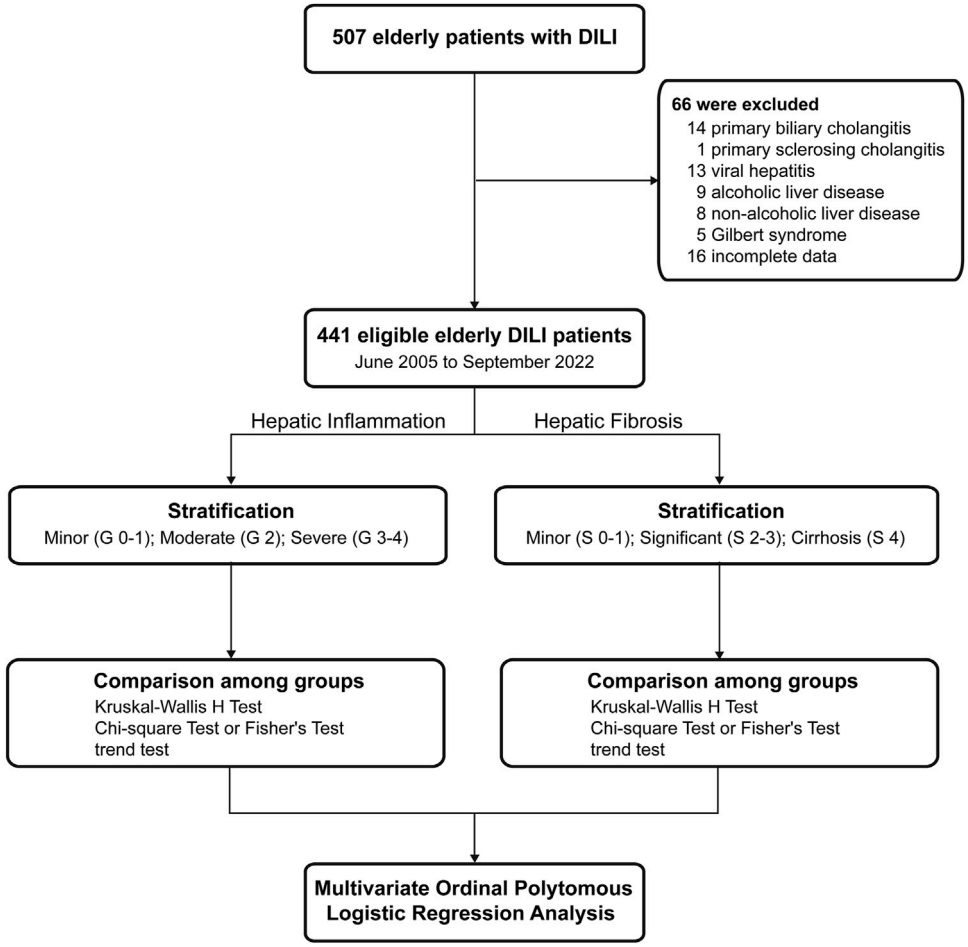

FIGURE 1
Flow chart of study population. DILI, drug-induced liver injury.

increasing stages of hepatic fibrosis ($p < 0.05$, Table 2). Furthermore, with the aggravation of hepatic inflammation grade, the proportion of hepatocellular pattern (13.9%, 31.8%, and 40.3%, p for trend = 0.037) and presence of autoimmunity (35.2%, 42.6%, and 60.5%, p for trend <0.001) increased significantly. Similarly, with the progression of hepatic fibrosis stage, the proportion of older age >70 years (2.7%, 9.5%, and 14.0%, p for trend = 0.003), cholestatic pattern (43.6%, 47.6%, and 65.1%, p for trend = 0.009), and presence of autoimmunity (36.7%, 51.0%, and 58.1%, p for trend = 0.002) significantly increased (Figure 3).

Multivariate ordinal polytomous logistic regression analysis

After screening by comparison among groups, collinearity analysis, and ordinal univariate analysis, variables with a p-value of <0.1 were included in the multivariate ordinal polytomous logistic regression analysis (Table 3). Ultimately, PLT (OR: 0.994, 95% CI: 0.991–0.997; $p < 0.001$), AST (OR: 1.001, 95% CI: 1.000–1.003, $p = 0.012$), TBIL (OR: 1.006, 95% CI: 1.003–1.010, $p < 0.001$), and autoimmunity (OR: 1.831, 95% CI: 1.258–2.672, $p = 0.002$) were associated with the degree of hepatic inflammation. Meanwhile, PLT (OR: 0.990, 95% CI: 0.986–0.993, $p < 0.001$), TBIL (OR: 1.004, 95% CI: 1.000–1.007, $p = 0.028$), age (OR: 1.123, 95% CI: 1.067–1.183, $p < 0.001$), and autoimmunity (OR: 1.760, 95% CI: 1.191–2.608, $p = 0.005$) were associated with the stage of hepatic fibrosis.

Taking the cholestatic damage pattern as a reference, hepatocellular (OR: 1.735, 95% CI: 1.027–2.932, $p = 0.038$) and mixed (OR: 1.981, 95% CI: 1.249–2.932, $p = 0.004$) damage were independent risk factors for hepatic inflammation. However, cholestasis (OR: 2.686, 95% CI: 1.563–4.673, $p < 0.001$) and mixed (OR: 2.738, 95% CI: 1.527–4.959, $p = 0.001$) were independent risk factors for hepatic fibrosis when referring to the hepatocellular damage pattern. In addition, sex was not associated with either aspect (Figure 4).

Discussion

With the aging of the population, the incidence of elderly DILI is expected to increase (Andrade et al., 2019a). Meanwhile, DILI causes the progression of underlying diseases due to drug withdrawal, a worse quality of life, and an immense economic burden (Stevens and

TABLE 1 Clinical characteristics stratified by hepatic inflammation.

	Overall (*N* = 441)	Minor (*N* = 122)	Moderate (*N* = 195)	Severe (N = 124)	*p*-value
Age (years)	63.3 (61.0–66.0)	62.0 (61.0–65.0)	64.0 (61.1–66.0)	63.3 (61.0–66.0)	0.191
Age groups					0.916
≤65 years	318 (72.1)	92 (75.4)	138 (70.8)	88 (71.0)	
65–70 years	92 (20.9)	22 (18.0)	43 (22.1)	27 (21.8)	
>70 years	31 (7.0)	8 (6.6)	14 (7.2)	9 (7.3)	
Female sex	324 (73.5)	85 (69.7)	140 (71.8)	99 (79.8)	0.152
BMI (kg/m^2)	23.8 (22.2–25.6)	23.5 (22.4–25.0)	24.0 (22.5–25.6)	23.8 (21.8–26.1)	0.276
Hypertension	124 (28.1)	34 (27.9)	58 (29.7)	32 (25.8)	0.746
Hyperlipidemia	122 (27.7)	39 (32.0)	49 (25.1)	34 (27.4)	0.415
Diabetes mellitus	52 (11.8)	11 (9.0)	27 (13.8)	14 (11.3)	0.422
Coronary heart disease	21 (4.8)	5 (4.1)	8 (4.1)	8 (6.5)	0.581
Class of Implicated drugs					0.790
TCM or HDS	110 (24.9)	27 (22.1)	47 (24.1)	36 (29.0)	
Synthetic drugs	129 (29.3)	37 (30.3)	58 (29.7)	34 (27.4)	
Combined	202 (45.8)	58 (47.5)	90 (46.2)	54 (43.5)	
Pattern of liver damage					<0.001
Hepatocellular	129 (29.3)	17 (13.9)	62 (31.8)	50 (40.3)	
Cholestatic	210 (47.6)	81 (66.4)	88 (45.1)	41 (33.1)	
Mixed	102 (23.1)	24 (19.7)	45 (23.1)	33 (26.6)	
PLT (×10^9/L)	170.0 (127.0–209.0)	185.0 (136.5–232.5)	170.0 (122.5–206.0)	154.0 (122.8–190.8)	0.009
ALT (U/L)	76.0 (35.0–203.0)	36.0 (22.0–96.2)	89.0 (42.5–246.5)	124.5 (53.0–333.5)	<0.001
AST (U/L)	72.0 (38.0–183.0)	32.5 (25.2–55.5)	80.0 (44.5–181.0)	132.5 (80.5–324.0)	<0.001
TBIL (μmol/L)	18.2 (11.0–37.3)	11.5 (8.7–17.3)	18.6 (11.6–35.9)	35.4 (17.1–80.5)	<0.001
ALP (U/L)	130.0 (97.0–179.0)	114.0 (79.2–156.0)	134.0 (101.5–181.5)	138.5 (103.0–180.5)	0.001
IgG ≥1.1 × ULN	74 (16.8)	8 (6.6)	32 (16.4)	34 (27.4)	<0.001
ANA positive	143 (32.4)	33 (27.0)	60 (30.8)	50 (40.3)	0.068
SMA positive	30 (6.8)	4 (3.3)	12 (6.2)	14 (11.3)	0.040
Autoimmunity	201 (45.6)	43 (35.2)	83 (42.6)	75 (60.5)	<0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ANA, anti-nuclear antibody; AMA, anti-mitochondrial antibody; BMI, body mass index; HDS, herbal and dietary supplements; IgG, immunoglobulin G; PLT, platelet; SMA, smooth muscle antibody; TBIL, total bilirubin; TCM, traditional Chinese medicine; ULN, upper limits of normal.

Baker, 2009; Kullak-Ublick et al., 2017). We revealed that the presence of autoimmunity was associated with a higher level of illness severity concerning hepatic inflammation and fibrosis in elderly patients with DILI, providing a reference for the strategy of intensive monitoring and aggressive treatment.

DILI is an invisible killer, and although most cases are improved after drug withdrawal (Aithal et al., 2011; Chen et al., 2011), serious clinical outcomes such as chronic hepatitis, liver failure, cirrhosis, liver transplantation, or even death may occur if long-term drug use is combined with irregular liver function reexamination (Raschi and De Ponti, 2017; Weaver et al., 2020; Wang et al., 2021). In the USA and Europe, DILI accounts for the most cases of acute liver failure (Ostapowicz, 2002; Kullak-Ublick et al., 2017). A prospective study showed that patients aged over 60 years with comorbidities had a higher mortality rate (Chalasani et al., 2015). A retrospective analysis including 595 patients demonstrated that the incidence of chronic hepatitis, liver failure, cirrhosis, and death was 13.4%, 7.9%, 7.6%, and 4.5% in China, respectively (Zhu et al., 2016). The present study showed that 9.8% of patients presented cirrhosis, implying that the adverse outcome of DILI, especially in elderly individuals, might be underestimated and need more attention.

Our study found that the most commonly-used implicated drug in the elderly was cardiovascular drugs, which was different from the ordinary patient population. Previous studies demonstrated antibiotics to be the most commonly implicated agents (Andrade et al., 2019b). This might lie in the dominance of cardiovascular disease as an underlying disease in elderly individuals. Additionally, the elderly population was also found to be exposed to greater usage of over-the-counter drugs and herbal supplements due to the increased consciousness about health in the present study. Moreover, some features in the medication of the elderly should be noted: 1) higher level of polypharmacy usage, 2) more cases of unclear medication duration, 3) longer duration of drug intake

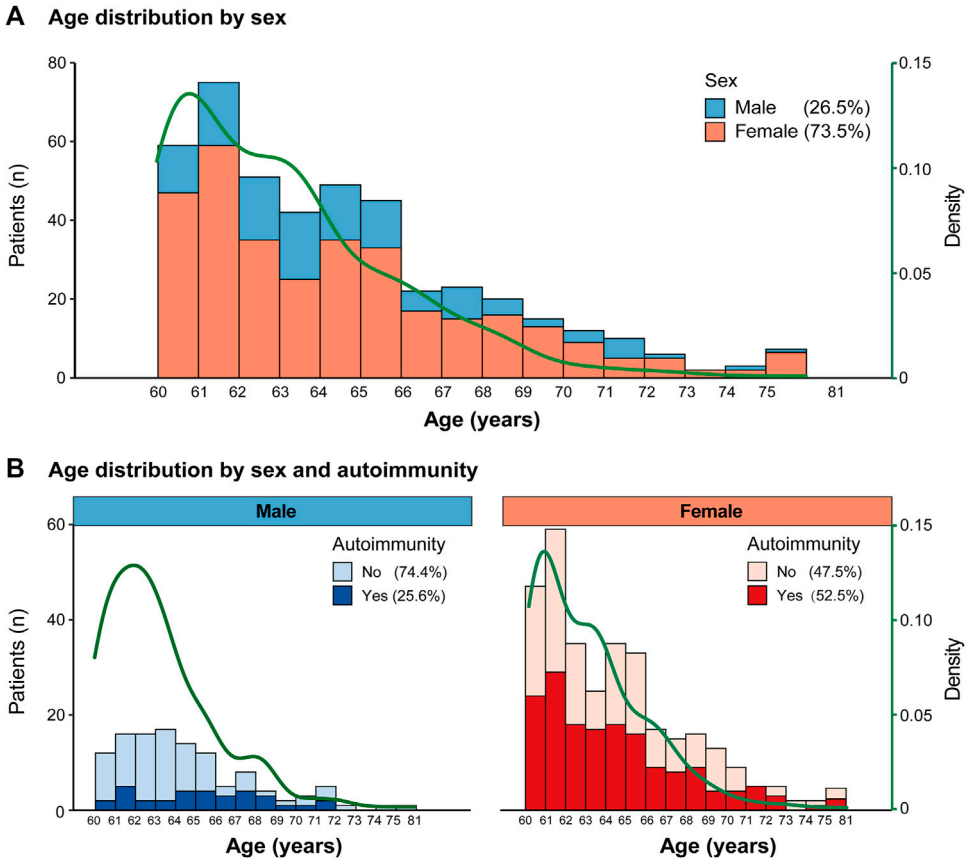

FIGURE 2
Age distribution **(A)**. Age distribution by sex; **(B)**. Age distribution by sex and autoimmunity.

until the onset of DILI, and 4) more cases of the cholestatic pattern of liver damage, which should not be neglected before the prescription.

Regarding clinical characteristics, 73.5% of participants were female, and the presence of autoimmunity was found to be more common in specimens from female patients, which is consistent with previous studies (Amacher, 2014; Chalasani et al., 2015) suggesting that females are more susceptible to DILI and autoimmunity. Levels of immunoglobulins and autoantibodies are higher in female, especially, postmenopausal women with DILI and abnormal IgG levels are more likely to progress autoimmune hepatitis (Sakiani et al., 2013; He et al., 2022). This may be attributed to immune function impacted by periodicity of estrogen and progesterone levels (Moulton, 2018). In our study, the elderly female patients were all postmenopausal women with a great potential for autoimmunity. Moreover, sex differences in percentage of body fat, cytochrome P450 isozymes and drug-binding proteins may also explain this phenomenon (Amacher, 2014). The cholestatic (47.6%) pattern was the main pattern of liver damage. Likewise, Lucena et al. (2009) proposed that agedness is positively associated with cholestatic liver injury. This may be explained by the high utilization rate of hyperlipophilic drugs and decreased biliary function (Hunt et al., 2014). Additionally, prolonged tubular excretion and cholangiocyte exposure may be the reason for activating immune responses (Weersink et al., 2021).

There was no statistically significant difference in hepatic inflammation or fibrosis for comorbidities, such as hypertension, hyperlipidemia, diabetes mellitus, or coronary heart disease. A Spanish DILI registry study suggested that hypertension and diabetes may be detrimental to the repair of liver injury, contributing to chronic outcomes (Medina-Caliz et al., 2016). Weersink et al. (2021) showed increasing comorbidity burden ($p < 0.001$) and polypharmacy ($p < 0.001$) in elderly patients with DILI, which may explain the increased non-liver-related mortality ($p = 0.030$). It may be that comorbidities are not direct factors contributing to the illness progression of DILI but make patients forced to take multiple drugs or actively seek informal medical methods such as folk remedies or dietary supplements.

Furthermore, according to the multivariate ordinal polytomous logistic regression analysis, autoimmunity was an independent risk factor for hepatic inflammation (OR: 1.831; 95% CI: 1.258–2.672) or fibrosis (OR: 1.760; 95% CI: 1.191–2.608), promoting illness progression in elderly DILI patients. Moreover, DILI with autoimmunity was not rare, accounting for 45.6% of cases, in the present study. An analysis of the autoimmune features of DILI from the DILI Network prospective study found that the majority of patients (60%–70%) were positive for ANA and SMA, and approximately 40% had elevated IgG serum levels (de Boer et al., 2017). Multidrug compatibility in the elderly can alter the immune and inflammatory response environment (Chen et al., 2015). Damage-associated molecular patterns (DAMPs) released by drug-injured hepatocytes activate innate immunity and lead to sterile inflammation, which further amplifies tissue damage (Mosedale and Watkins, 2017; Gerussi et al., 2021). Once more, inappropriate maturation of dendritic cells due to tissue injury in the elderly may

TABLE 2 Clinical characteristics stratified by hepatic fibrosis.

	Minor (*N* = 188)	Significant (*N* = 210)	Cirrhosis (*N* = 43)	*p*-value
Age (years)	62.2 (61.0–65.0)	64.0 (61.1–67.0)	63.4 (61.6–67.9)	0.002
Age group				0.002
≤65 years	152 (80.9)	139 (66.2)	27 (62.8)	
65–70 years	31 (16.5)	51 (24.3)	10 (23.3)	
>70 years	5 (2.7)	20 (9.5)	6 (14.0)	
Female sex	135 (71.8)	159 (75.7)	30 (69.8)	0.574
BMI (kg/m^2)	23.5 (22.0–25.1)	24.0 (22.3–26.0)	24.2 (22.6–25.2)	0.206
Hyperlipidemia	19 (10.1)	25 (11.9)	8 (18.6)	0.296
Hypertension	43 (22.9)	73 (34.8)	8 (18.6)	0.011
Diabetes mellitus	7 (3.7)	12 (5.7)	2 (4.7)	0.648
Coronary heart disease	55 (29.3)	57 (27.1)	10 (23.3)	0.710
Class of Implicated drugs				0.570
TCM or HDS	49 (26.1)	51 (24.3)	10 (23.3)	
Synthetic drugs	61 (32.4)	55 (26.2)	13 (30.2)	
Combined	78 (41.5)	104 (49.5)	20 (46.5)	
Pattern of liver damage				0.001
Hepatocellular	66 (35.1)	62 (29.5)	1 (2.3)	
Cholestatic	82 (43.6)	100 (47.6)	28 (65.1)	
Mixed	40 (21.3)	48 (22.9)	14 (32.6)	
PLT (×10^9/L)	184.0 (149.5–234.2)	157.5 (118.2–196.8)	117.0 (93.5–162.0)	<0.001
ALT (U/L)	99.5 (34.5–253.8)	76.5 (38.0–209.5)	52.0 (31.0–66.5)	0.006
AST (U/L)	61.0 (31.8–176.2)	91.5 (45.5–224.8)	63.0 (43.5–95.0)	0.005
TBIL (μmol/L)	15.1 (9.8–32.3)	22.0 (12.1–42.9)	15.6 (11.3–24.4)	0.002
ALP (U/L)	123.5 (91.0–178.0)	138.0 (103.0–182.0)	120.0 (93.5–171.5)	0.086
IgG ≥ 1.1 × ULN	16 (10.0)	45 (25.0)	13 (41.9)	<0.001
ANA positive	46 (24.5)	79 (37.6)	18 (41.9)	0.008
SMA positive	9 (4.8)	16 (7.6)	5 (11.6)	0.223
Autoimmunity	69 (36.7)	107 (51.0)	25 (58.1)	0.004

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ANA, anti-nuclear antibody; BMI, body mass index; HDS, herbal and dietary supplements; IgG, immunoglobulin G; PLT, platelet; SMA, smooth muscle antibody; TBIL, total bilirubin; TCM, traditional Chinese medicine; ULN, upper limits of normal.

alter the balance between immune function and tolerance, triggering the propensity of autoimmunity (Agrawal et al., 2012; Tajiri and Shimizu, 2013). Despite the application of high-dose glucocorticoids in the follow-up, drug-mediated autoimmune hepatitis mostly occurrs in elderly patients (75% aged >60 years) predisposed to late relapse (Yeong et al., 2016). Thus, our conclusion that autoimmunity promotes the illness progression of elderly DILI is of significant clinical manfulness.

Therefore, elderly DILI patients with autoimmunity need more frequent follow-up and aggressive treatment. Over the years, several models (Ashby et al., 2021; Li et al., 2021) have been proposed to predict the outcome of DILI. Our previous study found that significant hepatic inflammation (HAI ≥10) was an independent risk factor for biochemical resolution (OR: 21.278, 95% CI: 14.780, 30.632) (Wang et al., 2022), which was consistent with the present result.

The primary treatment for DILI is the discontinuation of suspected drugs, and corticosteroids can improve the condition of DILI with autoimmune features (Chalasani et al., 2021; Björnsson et al., 2022). Nevertheless, clinicians must weigh the risks of infection, osteoporosis, cognitive decline, etc., against the progression of DILI when treating older adults with corticosteroids (Lee et al., 2021).

The strengths of the present study included that: 1) a multilevel ordinal logistic regression analysis was performed to obtain a reliable estimate and standard error, 2) a large sample size of elderly patients was enrolled, which had adequate power to detect the true effect of the independent variables, and 3) a liver biopsy was required to ensure the accurate assessment for hepatic inflammation and fibrosis and exclusion of patients with other etiologies, which is scarce in the current literature.

Although the findings have important clinical implications, several limitations should be noted. First, the cross-sectional study design cannot establish the causal relationship between the severity of DILI (hepatic inflammation or fibrosis) and identified independent variables

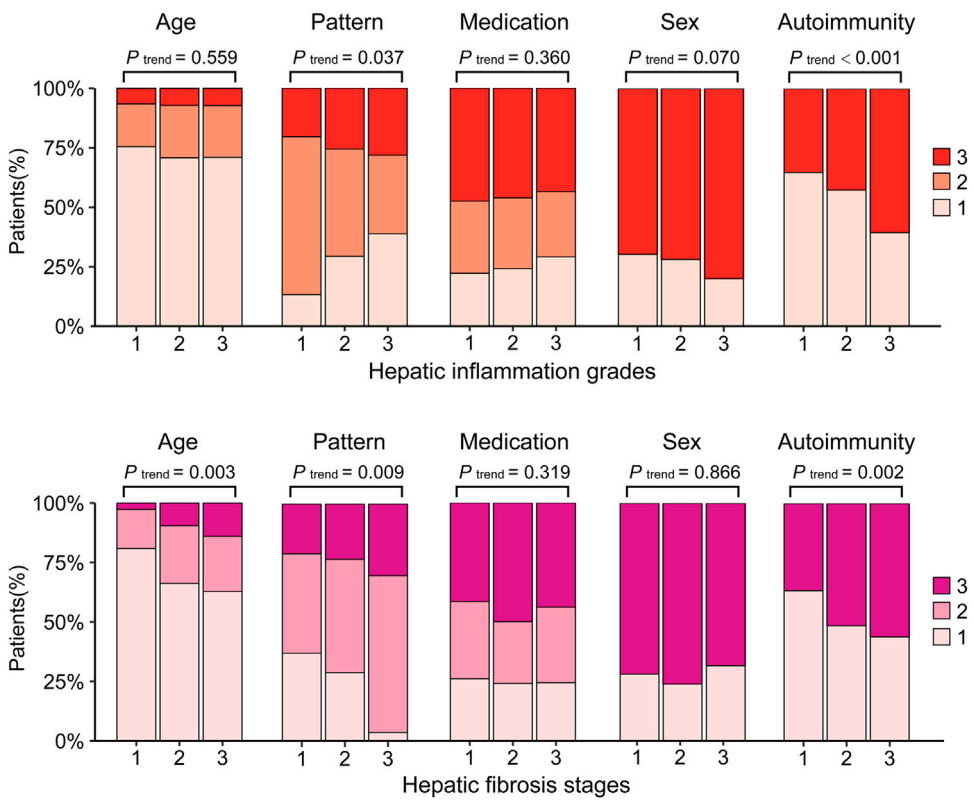

FIGURE 3
The clinical characteristics by histological evaluation.Hepatic inflammation grade: 1: G0-1; 2: G2; 3: G3-4. Hepatic fibrosis stage: 1: S0-1, 2: S2-3, 3: S4. Age, 1: ≤65 years, 2: 65–70 years, 3: >70 years. Pattern, 1: hepatocellular; 2: Cholestatic; 3: Mixed. Medication, 1: Synthetic drugs; 2: Traditional Chinese Medicine or herbal and dietary supplements; 3: Combined. Sex, 1: Male; 3: Female. Autoimmunity: 1: No; 3: Yes.

TABLE 3 Univariate ordinal polytomous logistic regression analysis.

	Hepatic inflammation		Hepatic fibrosis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age (years)	1.017 (0.970–1.067)	0.489	1.111 (1.057–1.168)	<0.001
Female sex	1.432 (0.969–2.122)	0.072	1.086 (0.722–1.637)	0.691
Hypertension	0.931 (0.634–1.367)	0.717	1.300 (0.878–1.928)	0.190
Pattern of liver damage				
Hepatocellular	3.110 (2.050–4.749)	<0.001	Reference	
Cholestatic	Reference		1.932 (1.268–2.959)	0.002
Mixed	2.066 (1.320–3.247)	0.002	1.939 (1.175–3.215)	0.01
PLT ($\times10^9$/L)	0.996 (0.994–0.999)	0.009	0.990 (0.987–0.993)	<0.00
AST (U/L)	1.003 (1.002–1.004)	<0.001	1.000 (0.999–1.001)	0.82
TBIL (μmol/L)	1.009 (1.006–1.012)	<0.001	1.001 (0.998–1.003)	0.62
Autoimmunity	2.058 (1.444–2.942)	<0.001	1.856 (1.291–2.678)	0.001

PLT, platelet; AST, aspartate aminotransferase; TBIL, total bilirubin.

(e.g., autoimmunity, patterns of injuries, etc.). Second, implicated agents might be misclassified due to the multiple underlying diseases and complexity of drug use. Finally, as this study is a retrospective single-center study, there might be some biases, such as admission rate bias. There were no patients of other races, which might limit the generalizability of this conclusion to broader populations. Further multicenter prospective studies with a larger sample size are warranted to verify the results.

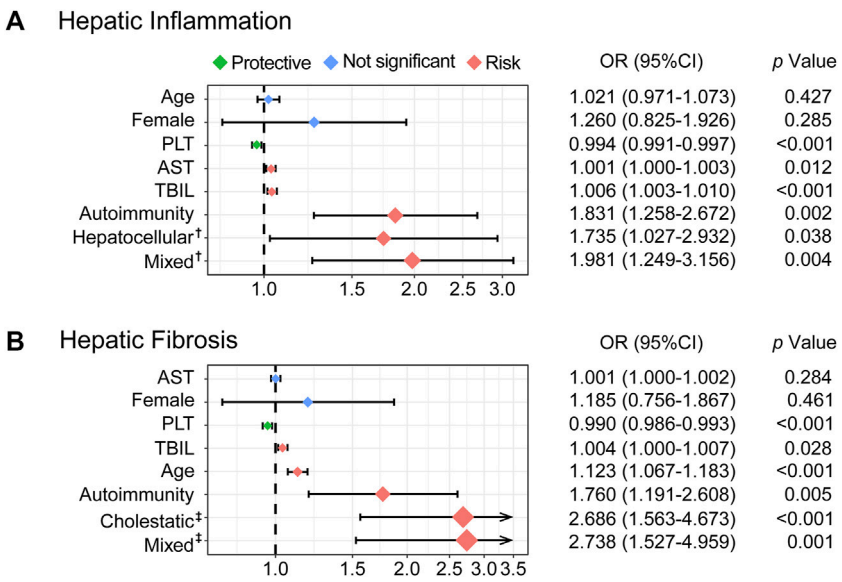

FIGURE 4
Forest plots based on multivariable ordinal polytomous logistic regression analysis. The *X*-axis was transformed as a log scale **(A)**. The risk factors associated with hepatic inflammation **(B)**. The risk factors associated with hepatic fibrosis. The colored solid diamonds represent the OR and the black lines show 95% CI.OR, odds ratio; CI, confidence interval; AST, aspartate transaminase; TBIL, total bilirubin; PLT, platelet.

Conclusion

Female sex and the cholestatic liver damage pattern are dominant in the elderly patients with DILI, and comorbidities are not directly associated with the severity of the illness. The presence of autoimmunity represents a more serious illness state of DILI, deserving more intensive monitoring and progressive treatment.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Ethics Committees of Fifth Medical Center of Chinese PLA General Hospital (No. 2019024D). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

DJ was responsible for the study design and supervision. Y-TX, J-FW, and X-XN were responsible for manuscript writing and data analysis. DJ, JZ, and Y-MF were responsible for critical revision of the manuscript. All authors were responsible for data acquisition and approval of the final version of the manuscript for submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1071709/full#supplementary-material

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