REDUCING THE HARM OF MEDICATION - RECENT TRENDS IN PHARMACOVIGILANCE

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REDUCING THE HARM OF MEDICATION - RECENT TRENDS IN PHARMACOVIGILANCE

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EDITORIAL
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Editorial: Reducing the Harm of Medication–Recent Trends in Pharmacovigilance

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Keywords: adverse drug effect, medication without harm, Pharmacovigilance, Pharmacoepidemiology, causality assessment, adverse drug reaction

Editorial on the Research Topic

Reducing the Harm of Medication–Recent Trends in Pharmacovigilance

Two years ago, we launched this second Frontiers in Pharmacology Research Topic titled *Reducing the Harm of medication: Recent Trends in Pharmacovigilance*, following our first topic "Medication Harm: From Early Identification to Prevention". Adverse drug reactions (ADRs) are considered to be among the leading causes of morbidity and mortality with very high economic costs. An estimated 5–25% of hospital admissions are due to ADRs, and 6–15% of hospitalized patients experience serious ADRs, causing significant prolongation of hospital stay. Moreover, fatal ADRs are estimated to occur in 0.31% of hospitalized patients [*The Harm of Medication: From Early Identification to Prevention*/Frontiers Research Topic]. Randomized controlled trials are the main premarketing methods used to detect and quantify ADRs but these have numerous limitations, such as the exclusion of patients at higher risk of ADRs (e.g., children, elderly, comorbid, polymedicated), insufficient sample size for the detection of non-frequent ADRs, limited follow-up time for the detection of ADRs after long periods of exposure. In addition, the diagnosis of ADRs is complex and difficult in the Real World due to the non-specificity of ADR symptoms and signs, diagnostic tests are usually absent and a re-challenge is rarely ethically justified. At present, most countries participate in pharmacovigilance through spontaneous reporting systems of ADRs. However, spontaneous reporting systems have limitations such as under-reporting, of up to 90%, and reporting bias, of only ADRs easy to diagnose. Active pharmacovigilance programs may supplement such systems. In this Research Topic, we have had the opportunity to evaluate some of the latest strategies in pharmacovigilance programs to cover the much-needed timely and accurate identification of ADRs. Pharmacovigilance programs assessing ADRs occurring in routine clinical practice can contribute to a better knowledge of the risk profiles of a medication classes. A study using national health insurance data evaluated the association between antidepressant use dose/duration and cardiovascular disease (CVD) risk in patients without a history of CVD as a primary prevention measure Jang et al. The authors of this study found that even at low doses, the use of tricyclic antidepressants was associated with major adverse cardiovascular events. Further, the longer duration of tricyclic antidepressant use correlated significantly with a higher adjusted hazard ratio. In another national population-based retrospective cohort study the authors found that the use of "sleeping pills" was associated with an increased risk of chronic kidney disease. Liao et al.

The use of hospital databases is essential for evaluating the safety of medicines for hospital use and for making decisions for the prevention of serious ADRs. In a retrospective cohort from an Intensive Care Unit

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(ICU), the effect on survival and neurological outcomes of full-dose epinephrine when used in cardiopulmonary resuscitation was investigated Shi et al. The authors found an inverse association between total epinephrine dosage during resuscitation and worse neurological outcome 3 months after the cardiac event. This study concluded that the early benefit of a medication does not ensure good results in the medium and long term among these patients. The pharmacovigilance system could investigate the functional status and quality of life of patients as indicators of the impact of ADRs. In another retrospective cohort study Kunming et al., the authors aimed to characterize vancomycin-associated acute kidney injury (AKI) in Chinese hospitals. The authors found a higher frequency of concomitant nephrotoxic medication use compared to their use in the USA and that the incidence of vancomycin-associated AKI was higher in patients with a combined use of nephrotoxic medications. The study recommended improving serum creatinine measurement for the AKI diagnosis and the standardization of vancomycin therapeutic drug monitoring. In a multicenter retrospective study exploring the association between mean serum vancomycin trough concentration (VTC) and mortality in Intensive Care Units Hou et al. , the authors found that mean VTC was not associated with reduced ICU/hospital related mortality. These results indicate that further investigation is needed to address the issue of vancomycin dosing using mean VTC and support the recommendation of moving from trough levels to a 24-h area under the curve (AUC) to a minimum inhibitory concentration ratio. Another retrospective analysis was conducted on inpatients who received tigecycline treatment from January 2018 to January 2020 Shi et al. Based on the biochemical criteria of Drug-Induced Liver Injury (DILI) and the causality assessment by Roussel Uclaf Causality Assessment Method (RUCAM) using cases with a probable or highly probable causality grading. The author found that tigecycline was associated with liver injury, with a slightly higher incidence (5.7%) than the frequency of "frequent" (5%) defined by the Medical Dictionary for Regulatory Activities. In addition, tigecycline-associated DILI was related to a high maintenance dose, prolonged duration, and a number of concomitant medications with known hepatotoxicity.

It is well known that one of the major research challenges regarding drug safety is the identification of genetic variation of drug targets, drug-metabolizing enzymes, or drug transporters that can clarify the mechanisms of idiosyncratic ADRs. In the case-control study by Frogerini et al., the association between PTGS1 and NOS3 variant alleles and the risk of developing upper gastrointestinal bleeding (UGIB) secondary to complicated peptic ulcer disease was assessed. The authors have shown an increased risk of UGIB in the presence of some genetic variants, regardless of the medication exposure. On the other hand, these genetic variants are said to modify the magnitude of the risk of UGIB in nonsteroidal anti-inflammatory drugs and low-dose aspirin users, since variants rs10306114 and rs5788 are related to a COX-1 with less capacity to produce prostaglandins and reduced platelet aggregation, respectively. Therefore the need for personalized therapy for the prevention of these serious ADRs is emphasized.

Automated screening tools developed from quantitative methods in the large databases of suspected ADR reports allow signal detection to trigger further investigations. Improving pharmacovigilance disproportionality analysis to reduce methodological heterogeneity and substantial variability of results by pre-registering the protocols and presenting a set of secondary and sensitivity analyses rather than a single result may prevent selective reporting of results Khouri et al. In a study based on suspected ADRs from the World Health Organization (WHO) global database (VigiBase) Allouchery et al. the authors conducted a disproportionality analysis of ibrutininb, a Bruton tyrosine kinase inhibitor for the treatment of chronic lymphocytic leukemia, mantle cell lymphoma, Waldenström macroglobulinemia, with respect all other anticancer medications used as the reference group in order to identify of potential safety signals. The authors combined the use of two complementary disproportionality measures (proportional reporting ratio and information component) and found clinically relevant potential safety signals in patients exposed to ibrutinib, mainly ischemic heart diseases, pericarditis, uveitis, retinal disorders, and fractures. Immune checkpoint inhibitors (ICI) may be used to treat aggressive hematologic malignancies, either in refractory or relapsed lymphoma frequently before being treated by allogeneic hematopoietic stem cell transplantation (allo-HSCT) or in relapse after allo-HSCT. Nguyen et al. used the Bayesian estimate of disproportionality analysis of VigiBase to detect a signal of graft vs. host disease (GVHD), with subsequent mortality of 25.8%. This paper confirms previous results obtained from cohort studies on the association between GVHD and ICI. This manuscript is an example that the sum of evidence from different approaches in data analysis (experimental data, clinical trials, spontaneous notifications, case-control studies, cohort studies, and data mining) would allow definitive conclusions and decision-making in pharmacovigilance. Conducting a systematic review and meta-analysis of randomized controlled trials, Man et al. addressed the putative increase risk of malignancies and tuberculosis in patients with spondyloarthritis (SpA) treated with biologics. The authors showed an elevated risk of malignancy in patients with peripheral SpA treated with biologics, especially for IL-17 inhibitors, and an increased risk of tuberculosis in patients with axial SpA treated with anti-TNFα antibody. Since the sparse number of events of malignancy and tuberculosis, these results need to be confirmed by further studies with a larger population and longer follow-ups.

In this Research Topic, we have also evaluated the latest strategies to improve the safety of medication use. In this sense, an integrative systematic review of the practical considerations of Pro Re Nata medication management sought to summarize and integrate the practical considerations of healthcare professionals for the management of Pro Re Nata medications in different healthcare settings. Mardaniet al. On the other hand, high quality understandable, and accessible information helps patients to participate in decision-making about medicines prescribed for them by healthcare professionals. Despite a lot of information available in the media, patients considered patient information leaflets of medical products the most important source of information about medicines after medical prescription. Medina-Córdoba et al. evaluated patients' cognitive, behavioral, and emotional factors and characteristics of Patient Information Leaflets that can promote appropriate drug use practices. Another study tried to identify factors associated with a lack of awareness of the impact of improperly disposed of medications among the general population in Bandung, Indonesia, and to assess the associations of

awareness with medication disposal practices among this population. This study provides results that contribute to a better characterization of some aspects of patients' medication self-management. Alfian et al.

This topic also contains a case report with an objective to describe a simple, universal, and cost-effective method of microbiome analysis for clinical trials Zdziarski et al. This general method could raise the hypothesis of drug-associated dysbiosis. When testing the method in one of the patients treated with high doses of inhaled corticosteroids, the authors have come across the unexpected finding that severe dysbiosis was followed by seronegative Sjögren's syndrome.

Finally, a position paper from the ANSM (Agence Nationale de Sécurité du Médicament et des Produits de Santé) proposes some actions to be implemented to reduce the intentional unjustified use of medicines: 1) Early identification of situations in which inappropriate use can lead to a health risk comparing consumption in different countries. 2) Better collective engagement including educational programs and co-construction of action plans and improved communication with stakeholders Vignot et al.

We appreciate the good acceptance of this topic, and we will appreciate good reception among the authors to continue contributing effectively and share their interesting study findings in the second part of this topic (Volume II) [*Reducing the Harm of Medication - Recent Trends in Pharmacovigilance*, Volume II/Frontiers Research Topic].

Finally, we make some suggestions to encourage Pharmacovigilance activities going forward. The first is based on the fact that evidence suggests that individual case safety reports remain a very useful data source for detecting potential new safety issues. We suggest improving diagnostic tools, causality algorithms, and complementary drugs with other *in vitro* tests (pharmacogenetics, pharmacogenomics, pharmaco-immunology, pharmaco-proteomics), in the diagnosis of ADRs. In this sense, the implementation of expert medical specialties in the diagnosis of ADRs (e.g., Clinical Pharmacologists with specific training in the causality of ADRs) could give support and confidence to physicians in the generation of these new drug safety signals from primary and hospital care. On the other hand, electronic health records have proven to be more useful for evaluating problems already detected, allowing the implementation of prevention and early detection tools that minimize the risk of ADRs. Prospective cohort or retrospective observational database studies allow for longer follow-up periods of patients with a much broader range of characteristics, providing valuable means for the detection, quantification, and, where possible, reduction of ADRs, reducing health care costs in the process.

AUTHOR CONTRIBUTIONS

ER has been involved in drafting the manuscript and revising it critically for important intellectual content. MG-M, CK, and FA have been involved in revising the manuscript critically for important intellectual content.

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ORIGINAL RESEARCH
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Antidepressant Use and the Risk of Major Adverse Cardiovascular Events in Patients Without Known Cardiovascular Disease: A Retrospective Cohort Study

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Aims: Conflicting data exist on whether an association exists between antidepressants and the risk of major adverse cardiovascular events (MACEs) in patients with depression. This may be due to the use of various study designs and residual or unmeasured confounding. We aimed to assess the association between antidepressant use and the risk of MACEs while considering various covariates, including severity of depression and the cardiovascular disease (CVD) risk score.

Methods: Patients newly diagnosed with depression with no history of ischemic heart disease and stroke were followed-up from 2009 to 2015. We conducted Cox proportional hazard regression analysis to estimate hazard ratios (HRs) for each antidepressant for MACE risk.

Result: We followed-up (median, 4.4 years) 31,830 matched patients with depression (15,915 antidepressant users and 15,915 non-users). In most patients (98.7%), low-dose tricyclic antidepressants (TCAs) were related with a significantly increased risk of MACEs [adjusted HR = 1.20, 95% confidence interval (CI) = 1.03–1.40]. Duration response relationship showed a gradually increasing HR from 1.15 (95% CI = 0.98–1.33; <30 days of use) to 1.84 (95% CI = 1.35–2.51; ≥365 days of use) (p for trend <0.01). High Korean atherosclerotic CVD risk score (≥7.5%) or unfavorable lifestyle factors (smoking, alcohol intake, and exercise) were significantly associated with MACEs.

Conclusion: Even at low doses, TCA use was associated with MACEs during primary prevention. Longer duration of TCA use correlated with higher HR. Careful monitoring is needed with TCA use in patients with no known CVD history.

Keywords: antidepressive agents, serotonin uptake inhibitors, tricyclic antidepressive agents, serotonin and norepinephrine reuptake inhibitor, major adverse cardiovascular events, Korean atherosclerotic cardiovascular disease risk score

INTRODUCTION

Cardiovascular disease (CVD) and depression are currently the two most common causes of disability in high-income countries (World-Health-Organization, 2008). Many studies have reported that antidepressants can increase the CVD risk. Of these, some were conducted in patients with no CVD history (primary prevention) and some in those with underlying (secondary prevention) CVD. However, no randomized clinical trials (RCTs) exist on primary prevention, due to a low CVD incidence and long follow-up durations (Oh et al., 2014). Therefore, a well-designed real world study with a sufficiently large sample size would be more appropriate. Patients with depression have a known severity of depression and CVD risk. Therefore, controlling for these confounding factors is important for a valid study design.

To our knowledge, no available observational study in the primary prevention setting has adjusted for known severity of depression and CVD risk as confounding factors while using a sufficiently large sample size. Though the application of CVD risk score is highly recommended for the primary prevention of CVD in patients without CVD history (Goff et al., 2014), many studies had no access to physical examination data [blood pressure and serum cholesterol level (Oh et al., 2014)] due to the nature of their data sources. One study included each participants' CVD risk score (Framingham risk score) as a covariate; however, the results were based on the patients' ability to recall, which might be subject to recall bias (Rosenberg et al., 2010). Another study could not directly calculate CVD risk scores but used the number of cardiology visits instead (Huang et al., 2013). Many studies have evaluated depression severity using the depression severity code only (Coupland et al., 2011; Scherrer et al., 2011), depression index score (Rosenberg et al., 2010; Hamer et al., 2011), and number of depression diagnoses (Blanchette et al., 2008).

We aimed to examine whether antidepressant use was associated with the risk of CVD in patients with no known CVD by considering various covariates, including depression severity and CVD risk scores.

METHODS

Study Design and Data Sources

This study used a cohort study design and analyzed health insurance data officially provided by the Korean National Health Insurance Service (KNHIS) (Cheol Seong et al., 2017). The insurance data included the patients' demographic, diagnosis, procedure, and prescription data. Additionally, physical examination data were linked to the KNHIS data. Physical examination data included blood sample, anthropometric measurement (blood pressure), body mass index (BMI), smoking status, alcohol consumption, and exercise data. Causes of death information was also gathered using Korean National Statistics. The requirement for written informed consent from participants was waived because all participants were anonymized by a randomized identification number. This study was approved by the institutional review board of Seoul National University (IRB No. E1606/003-002).

Study Data

We performed analysis using KNHIS data from 2006 to 2015. Patients diagnosed with depression between 2006 and 2008 were identified using the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10). Patients who had been diagnosed with ischemic heart disease and stroke between 2006 and 2008 were excluded. Physician-diagnosed depression was defined by codes F06.3, F31.3, F31.4, F32, F33, F34.1, F38.1, and F41.2. Ischemic heart disease and stroke were identified by codes I20–I25 and I60-I64, respectively. We excluded patients with the following characteristics: age <40 or ≥80 years (the suitable age range for measuring Korean atherosclerotic cardiovascular disease [ASCVD] risk scores) at the time of enrollment (Goff et al., 2014; Jung et al., 2015); cancer diagnosis (C00-C97); no history of physical examination within 1 year before the index date; prescribed antidepressants before the depression diagnosis; and did not continue initial antidepressant treatment for at least 4 weeks.

Exposure Data

All patients' medication information (drug name, dosage, instruction, and period) was extracted. The medications were based on the anatomical therapeutic chemical (ATC) classification system. Antidepressants were grouped into three classes (tricyclic antidepressants [TCAs]; selective serotonin reuptake inhibitors [SSRIs]; and serotonin and norepinephrine reuptake inhibitors [SNRIs]). Individuals were classified to one of the three classes based on their first antidepressant and analyzed by the intention-to-treat method. Patients with no history of antidepressant use were included in the non-user group. We used the "proportion of days covered" method to evaluate each individuals' adherence and defined a patient as having discontinued antidepressants if the gap between prescription refills (permissible gap) was >7 days (Choudhry et al., 2009). Each daily dose was calculated by multiplying the number of tablets to be taken each day by the dose of each tablet, and this was converted to defined daily dose (DDD) which is assigned by the World Health Organization's Collaborating Center (WHOCC) for Drug Statistics Methodology (www.whocc.no/atc_ddd_index) (WHOCC, 2016), and weighted mean DDD was calculated. Individuals' DDD was categorized as low (<0.5 DDD), intermediate (0.5–1.0 DDD), and high (≥1.0 DDD).

Study Endpoints (Major Adverse Cardiovascular Events)

Individuals were followed-up until 2015, and outcomes were recorded between each individuals' index date and 2015. For CVD outcome, MACEs were used as the primary endpoints: myocardial infarction (MI) (I21), stroke (I60-I64), and CVD (I00-I99) related death. The use of invasive or surgical procedures during hospitalization for MI and stroke was additionally considered for validation (Yeom et al., 2015). Stroke was classified as hemorrhagic (I60-I62), ischemic (I63), or other (I64).

Confounding Variables

The following baseline characteristics, potentially influencing the study outcomes, were included: age (at enrollment); sex; economic status (assessed based on income-related insurance payment); comorbidities (dyslipidemia, diabetes, and hypertension); and concomitant medications (statins, antidiabetic, and antihypertensive) within one year of the index date. Furthermore, we collected information on exercise, smoking status, and alcohol consumption from questionnaire data and blood pressure, cholesterol level, and BMI from physical examination data. Each patient's Korean ASCVD risk scores were included as a covariate. Patients' age, sex, total cholesterol level (mg/dl), high density lipoprotein (mg/dl), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), high blood pressure treatment, diabetes diagnosis, and smoking status was used to calculate the Korean ASCVD risk score as described previously (Jung et al., 2015). For the information regarding depression severity, we used the number of outpatient visits during the first 6 months (180 days) as a proxy measure (Meunier et al., 2014).

Statistical Analysis

To address confounding due to treatment indications, the propensity-score matching method was applied. Matching was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, United States) Greedy 5→1 Digit Match macro (Parsons, 2001). The propensity score was obtained using logistic regression analysis to predict the class of antidepressants from age, sex, index year, economic status, comorbidities, co-medications, exercise, smoking status, alcohol consumption, the Korean ASCVD risk score, and the number of outpatient visits during the first 180 days after the index date. Distribution of patients' baseline covariates was evaluated with a standardized difference. Standardized difference of <0.1 was considered indicative of good balance (NCSS-statistical-Software, 2017).

To construct the outcome model, Cox proportional hazard regression was used to estimate the hazard ratio (HR) of each antidepressant for MACE risk, with 95% confidence interval (CI). Confounding factors were exercise, alcohol consumption, BMI, number of outpatient visits, and the Korean ASCVD risk score. The proportional hazards assumption was tested graphically and confirmed for each covariate using the log(-log) plot of hazard functions for each group and covariate. Subgroup analyses were performed to investigate the risk of MACEs by age, exercise status, smoking status, alcohol consumption, BMI, and the Korean ASCVD risk category (≥7.5%, high risk category; <7.5%, low risk category) (Goff et al., 2014).

To test the robustness of our model, sensitivity analyses were performed in three ways. First, we used cancer death as a negative control in our analysis. Second, we changed the predefined gap between prescription refills and checked whether the results were influenced by medication adherence. In our original study design, if the gap between prescription refills was >7 days, the patient was considered to have discontinued antidepressant use. To prevent the patients' medication adherence from affecting the main outcomes, we changed this gap to 14 days and 50% of each prescription period to test the effect on the main outcomes. Additionally, if the date of antidepressant initiation differed from the time of depression diagnosis, patients would have periods during which MACEs could not have been affected by treatment (immortal time) (Suissa, 2008). Therefore, we excluded patients meeting this criterion to minimize the immortal time bias. All analyses were performed with SAS software version 9.4 (SAS Institute Inc.)

RESULTS

Among 3,688,812 patients diagnosed with depression between 2009 and 2012, we excluded 1,523,433 patients owing to illness history (**Figure 1**). Patients not meeting the inclusion criteria or with wrong dosing information were excluded. The eligible study cohort included 70,524 patients (before propensity-score matching: 21,476 users and 49,048 non-users). Antidepressant users took more medications (statins, antidiabetics, and antihypertensive), had more comorbidities (dyslipidemia, diabetes mellitus, and hypertension), visited clinics more frequently, and had higher Korean ASCVD risk scores than non-users (**Supplementary Table S1**).

After propensity score matching, 15,915 antidepressant users were matched with 15,915 non-users. The above difference (comedications, comorbidities, number of clinic visit, Korean ASCVD risk scores) was reduced, and both groups were well balanced. Standardized differences were well below 0.1 for all covariates (**Table 1**). Median length of follow-up (4.4 years overall); median duration of antidepressants prescription during follow-up [94 (interquartile range 40–568) days]; and mean age of patients [56.4 years; men: 40.1% (n = 12,791)] were shown. Almost every patient (98.7%) had used low-dose TCAs (<0.5 DDD) while SSRIs were frequently used in both high (55.1%) and intermediate (43.1%) doses. Intermediate doses of SNRIs were the most frequently used (60.5%) (**Table 2**).

Association With the Major Adverse Cardiovascular Events Component

In composite MACEs endpoint, the average time to onset of the first MACEs was 410, 409 and 301 days for the TCAs, SSRIs, and SNRIs, respectively. Only the TCAs showed a significantly increased risk of MACEs [adjusted HR (aHR) = 1.20, 95% CI = 1.03–1.40] (**Table 3**). The SSRIs and SNRIs were not significantly associated with the composite MACEs. When examining each MACE component, TCAs significantly increased the HR of stroke (aHR = 1.21, 95% CI = 1.01–1.44). In subtypes of stroke, only ischemic stroke was significantly associated with TCAs (aHR = 1.23, 95% CI = 1.00–1.51). SSRIs showed no significant effect on MACE risk. SNRI was a significant risk factor for MI (aHR = 3.16, 95% CI = 1.49–6.69) and CVD-related death (aHR = 2.39, 95% CI = 1.20–4.80).

Risk of Major Adverse Cardiovascular Events According to Antidepressants Dose and Duration

Only low TCA doses (<0.5 DDD) were significantly associated with an increased MACE risk (aHR = 1.19, 95% CI = 1.02–1.40) (**Table 4**). In terms of duration of use, only the duration of TCA

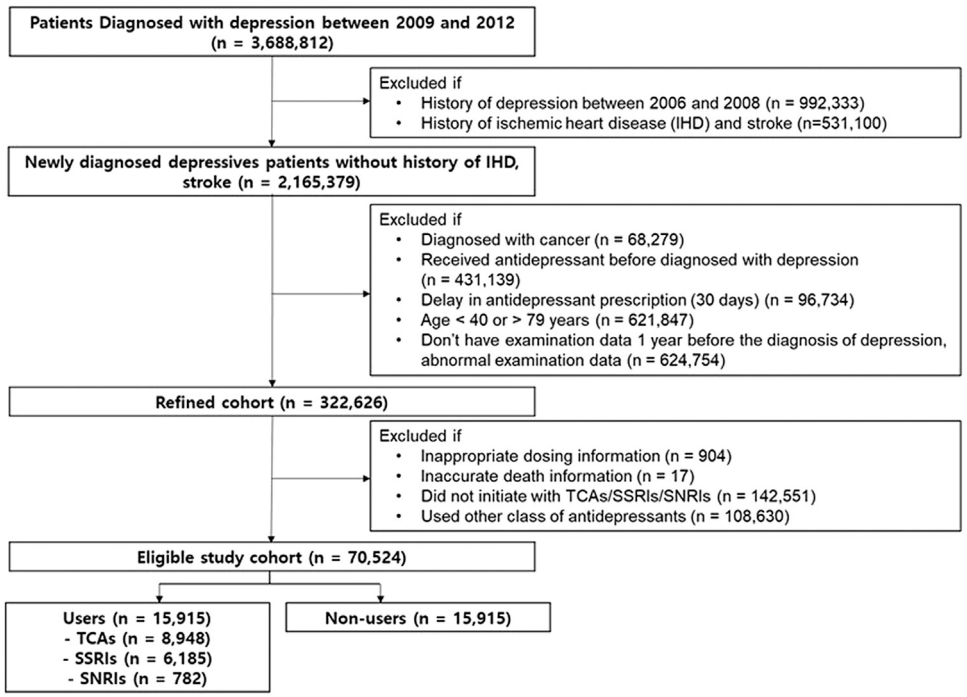

FIGURE 1 | Study flow chart. TCAs, tricyclic antidepressants; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin-norepinephrine reuptake inhibitors.

TABLE 1 | Characteristics of major adverse cardiovascular event-free patients diagnosed with depression after propensity-score matching.

Characteristics	Non-user	User				STD
	(*n* = 15,915)	TCAs (*n* = 8,948)	SSRIs (*n* = 6,185)	SNRIs (*n* = 782)	All (*n* = 15,915)	
Sex (male)	6,409 (40.3)	3,622 (40.5)	2,445 (39.5)	315 (40.3)	6,382 (40.1)	−0.01
Age (year)	56.6 ± 9.8	57.2 ± 9.5	54.7 ± 10.0	56.0 ± 9.9	56.2 ± 9.8	−0.05
Economic status[a]	3.3 ± 1.5	3.2 ± 1.5	3.3 ± 1.5	3.3 ± 1.5	3.2 ± 1.5	0.05
Medication						
Statins	3,127 (19.7)	1,681 (18.8)	920 (14.9)	142 (18.2)	2,743 (17.2)	0.06
Antidiabetics	2094 (13.2)	1,154 (12.9)	436 (7.1)	90 (11.5)	1,680 (10.6)	0.08
Antihypertensive	6,828 (42.9)	3,621 (40.5)	2,428 (39.3)	293 (37.5)	6,342 (39.9)	0.06
Comorbidities						
Dyslipidemia	5,211 (32.7)	3,052 (34.1)	1,661 (26.9)	259 (33.1)	4,972 (31.2)	0.03
Diabetes mellitus	3,176 (20.0)	1893 (21.2)	871 (14.1)	148 (18.9)	2,912 (18.3)	0.04
Hypertension	5,676 (35.7)	3,030 (33.9)	1713 (27.7)	236 (30.2)	4,979 (31.3)	0.09
History of smoke/Current smoker	2,719 (17.1)	1,485 (16.6)	1,048 (16.9)	154 (19.7)	2,687 (16.9)	−0.01
Number of outpatient visits[b]	1.1 ± 0.4	1.1 ± 0.5	1.3 ± 0.6	1.2 ± 0.6	1.2 ± 0.6	0.07
BMI (kg/m^2)	23.9 ± 3.1	24.0 ± 3.1	23.7 ± 3.1	23.6 ± 3.0	23.9 ± 3.1	0.01
Drink (time/week)	0.8 ± 1.5	0.8 ± 1.5	0.8 ± 1.5	0.9 ± 1.5	0.8 ± 1.5	0.005
Exercise (time/week)	1.0 ± 1.7	0.9 ± 1.7	1.0 ± 1.7	0.9 ± 1.6	1.0 ± 1.7	0.004
Korean ASCVD score (%)	7.4 ± 7.6	7.5 ± 7.6	6.2 ± 7.2	7.2 ± 8.0	7.0 ± 7.5	−0.05

Values are represented as mean ± standard deviation or number (%); TCAs, tricyclic antidepressants; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin-norepinephrine reuptake inhibitors; STD, standardized difference.

[a]*Economic status was assessed based on income-related insurance payment.*

[b]*The number of outpatient visits during the first six months was used as a proxy measure for severity of depression.*

use tended to be proportional to the MACE risk. TCA use <30 days showed no significant association with MACEs; however, with prolonged TCA use, the HR gradually increased from 1.15 (95% CI = 0.98–1.33) (<30 days of use) to 1.84 (95% CI = 1.35–2.51) (≥365 days) (p for trend <0.01). The HR was significantly increased in the group receiving SSRIs for 180–365 days (aHR = 1.40, 95% CI = 1.02–1.90), and there was a trend for an increasing HR in the SSRI users (0.91, 1.09,

TABLE 2 | Doses prescribed by antidepressants class in defined daily doses.

Defined daily dose (DDD)	Class		
	TCAs	SSRIs	SNRIs
<0.5 DDD	8,832 (98.7)	109 (1.8)	174 (22.3)
0.5–1.0 DDD	105 (1.2)	2,664 (43.1)	473 (60.5)
≥1.0 DDD	11 (0.1)	3,412 (55.1)	135 (17.2)
Total	8,948	6,185	782

Values are represented as number (%); TCAs, tricyclic antidepressants; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin-norepinephrine reuptake inhibitors.

TABLE 3 | Hazard ratios for major adverse cardiovascular events (Maces) components according to the classes of antidepressants in patients without cardiovascular disease diagnosed with depression.

Class	Events	Person-year	Hazard ratio (95% CI)	
			Unadjusted	Adjusted
Major adverse cardiovascular events				
Non-users	1,173	211,744	—	—
TCAs	780	116,899	1.21 (1.03–1.41)	1.20 (1.03–1.40)
SSRIs	426	79,795	0.96 (0.80–1.17)	1.07 (0.88–1.30)
SNRIs	81	9,993	1.47 (0.99–2.17)	1.37 (0.92–2.02)
Myocardial infarction				
Non-users	159	213,581	—	—
TCAs	129	118,255	1.47 (0.99–2.20)	1.45 (0.97–2.17)
SSRIs	51	80,605	0.86 (0.50–1.48)	0.95 (0.55–1.64)
SNRIs	24	10,089	3.23 (1.53–6.79)	3.16 (1.49–6.69)
Stroke				
Non-users	921	212,013	—	—
TCAs	618	117,155	1.22 (1.02–1.45)	1.21 (1.01–1.44)
SSRIs	342	79,845	0.99 (0.80–1.22)	1.09 (0.88–1.35)
SNRIs	45	10,005	1.04 (0.62–1.74)	0.96 (0.57–1.61)
Hemorrhagic stroke				
Non-users	270	210,460	—	—
TCAs	150	116,125	1.01 (0.71–1.42)	1.00 (0.71–1.42)
SSRIs	81	79,300	0.80 (0.52–1.22)	0.86 (0.56–1.33)
SNRIs	9	9,929	0.71 (0.22–2.23)	0.67 (0.21–2.11)
Ischemic stroke				
Non-users	666	211,438	—	—
TCAs	456	116,782	1.24 (1.01–1.52)	1.23 (1.00–1.51)
SSRIs	261	79,692	1.04 (0.81–1.33)	1.16 (0.90–1.48)
SNRIs	36	9,985	1.17 (0.64–2.05)	1.06 (0.59–1.90)
CVD-related death				
Non-users	222	213,929	—	—
TCAs	141	118,568	1.15 (0.80–1.66)	1.14 (0.79–1.64)
SSRIs	105	80,701	1.26 (0.84–1.89)	1.45 (0.97–2.18)
SNRIs	27	10,103	2.60 (1.3–5.19)	2.39 (1.20–4.80)

Hazard ratio was adjusted for exercise, alcohol consumption, body mass index, the number of outpatient visits, and the Korean atherosclerotic cardiovascular disease risk score. CI, confidence interval; TCAs, tricyclic antidepressants; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin-norepinephrine reuptake inhibitors.

TABLE 4 | Hazard ratios for major adverse cardiovascular events (MACEs) according to dose and duration of each antidepressant class in patients without cardiovascular disease diagnosed with depression.

	Events	Person-years	Hazard ratio (95% CI)	
			Unadjusted	Adjusted
Dose				
Non-users	1,173	211,744	—	—
TCAs (<0.5 DDD)	774	115,304	1.21 (1.03–1.42)	1.19 (1.02–1.40)
TCAs (0.5–1.0 DDD)	6	1,428	0.64 (0.16–2.56)	2.19 (0.54–8.81)
TCAs (≥1.0 DDD)	0	167	—	—
SSRIs (<0.5 DDD)	12	1,407	1.65 (0.62–4.43)	1.33 (0.49–3.58)
SSRIs (0.5–1.0 DDD)	195	33,956	1.09 (0.84–1.42)	0.89 (0.65–1.21)
SSRIs (≥1.0 DDD)	219	44,432	1.04 (0.81–1.34)	0.86 (0.64–1.15)
SNRIs (<0.5 DDD)	18	2,303	1.18 (0.53–2.64)	1.08 (0.40–2.90)
SNRIs (0.5–1.0 DDD)	51	6,145	1.47 (0.90–2.39)	0.93 (0.48–1.81)
SNRIs (≥1.0 DDD)	12	1,545	1.28 (0.48–3.44)	0.63 (0.20–1.98)
Duration				
Non-users	1,173	211,744	—	—
TCAs (<30 days)	204	33,639	1.10 (0.94–1.27)	1.15 (0.98–1.33)
TCAs (31–180 days)	492	75,955	1.17 (1.05–1.30)	1.18 (1.06–1.31)
TCAs (180–365 days)	42	4,445	1.71 (1.25–2.32)	1.37 (1.01–1.87)
TCAs (≥365 days)	42	2,860	2.65 (1.95–3.61)	1.84 (1.35–2.51)
SSRIs (<30 days)	93	20,213	0.83 (0.67–1.03)	0.91 (0.74–1.13)
SSRIs (31–180 days)	270	51,701	0.94 (0.83–1.08)	1.09 (0.95–1.25)
SSRIs (180–365 days)	42	5,145	1.47 (1.08–2.01)	1.40 (1.02–1.90)
SSRIs (≥365 days)	21	2,736	1.39 (0.90–2.13)	1.23 (0.80–1.90)
SNRIs (<30 days)	33	3,020	1.97 (1.39–2.79)	2.12 (1.50–3.00)
SNRIs (31–180 days)	45	5,963	1.37 (1.01–1.84)	1.27 (0.94–1.71)
SNRIs (180–365 days)	0	554	—	—
SNRIs (≥365 days)	3	456	1.18 (0.38–3.68)	0.77 (0.25–2.40)

Hazard ratio was adjusted for exercise, alcohol consumption, body mass index, the number of outpatient visits, and the Korean atherosclerotic cardiovascular disease risk score. CI, confidence interval; DDD, defined daily dose; TCAs, tricyclic antidepressants; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin-norepinephrine reuptake inhibitors.

1.40, 1.23), although the trend was not significant (p for trend = 0.40). There was an increased HR in patients receiving SNRIs for <30 days (aHR = 2.12, 95% CI = 1.50–3.00), and there was a trend (though not significant) for a reduction in HR (2.12, 1.27, 0.77) with an increasing duration of use (p for trend = 0.34).

Sensitivity Analyses

After the change from 7 to 14 days and 50% permissible gap, similar results were obtained (**Supplementary Table S2**). TCAs were still significantly associated with an increased MACE risk but SSRIs were not. An unstable result was observed for SNRIs, showing inconsistency in their level of significance for the results of each gap (7 days: aHR = 1.37, 95% CI = 0.92–2.02; 14 days: aHR = 1.49, 95% CI = 1.02–2.19; and 50% proportion: aHR = 1.25, 95% CI = 0.83–1.90). While analyzing cancer death as a negative control, all three classes showed neutral effects on MACEs (**Supplementary Table S3**). While testing for

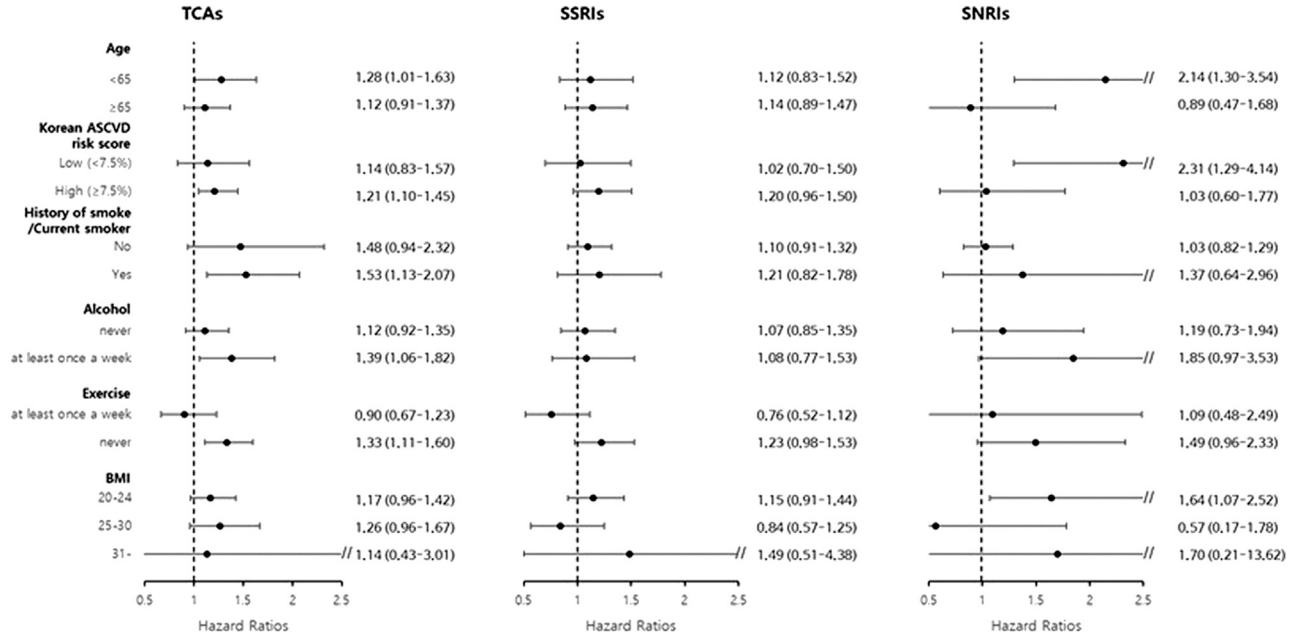

FIGURE 2 | Subgroup analysis of hazard ratio for major adverse cardiovascular events based on patients' age, exercise status, smoking status, alcohol consumption, BMI, and Korean atherosclerotic cardiovascular disease risk score category. TCAs, tricyclic antidepressants; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin-norepinephrine reuptake inhibitors; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index.

immortal time bias, we excluded 512 patients who had gaps between the index date and the first antidepressant exposure time. Similar results persisted for all three antidepressant classes (**Supplementary Table S4**).

Subgroup Analyses

In TCAs, while patients aged <65 years had significantly increased risk (aHR = 1.28, 95% CI = 1.01–1.63), no effect was seen on MACE risk in those aged >65 years (aHR = 1.12, 95% CI = 0.91–1.37) (**Figure 2**). However, patients in the high Korean ASCVD risk group (≥7.5%) showed significantly increased risk (aHR = 1.21, 95% CI = 1.10–1.45) unlike the low Korean ASCVD risk group (aHR = 1.14, 95% CI = 0.83–1.57). We also found that patients with unfavorable lifestyle factors (smoking, alcohol consumption, and exercise) had significantly increased risk (history of smoke/current smoker = 1.53, 95% CI = 1.13–2.07; drinking alcohol at least once a week = 1.39, 95% CI = 1.01–1.82; and no exercise = 1.33, 95% CI = 1.11–1.60).

DISCUSSION

Our study analyzed patients with no known CVDs that were diagnosed with depression through a long follow-up study. To our knowledge, this is the first study to consider the underlying disease condition/severity and physical examination data while using a sufficiently large sample size. In addition to the previous studies reporting on the harmful effect of TCAs, new information was provided by this study on the effects of the dose/duration of antidepressants. Our study results suggest that low doses of TCA use were associated with an increased risk of MACEs. A longer duration of TCA use correlated with a higher risk.

We found significant associations between antidepressant use, particularly TCA use, and MACEs in patients with depression. However, these associations were not found with SSRI or SNRI use. Yet, even in previous studies, there does not seem to be any definite association reported between each antidepressant and a specific MACE component. We found that TCAs had a significant adverse effect on MACEs and stroke (ischemic stroke reported in the stroke subtype analysis), consistent with previous findings. The harmful effect of TCAs on MACEs has been reported, along with an association with MI (Lapane et al., 1995; Penttinen and Valonen, 1996; Pratt et al., 1996; Cohen et al., 2000; Hippisley-Cox et al., 2001; Tata et al., 2005; Rosenberg et al., 2010). Other studies have shown partially significant results on MACE components: Smoller *et al.* showed that TCA use was only associated with an increased risk of all-cause mortality but not with MI or stroke (Smoller et al., 2009). TCA use may be associated with elevated CVD risk (MI and stroke) but not with coronary heart disease alone (excluding stroke from CVD) (Hamer et al., 2011).

Most studies have reported that SSRIs have a non-significant effect on MACE components (Cohen et al., 2000; Sauer et al., 2003; Monster et al., 2004; Smoller et al., 2009; Rosenberg et al., 2010; Hamer et al., 2011; Huang et al., 2013) or a protective effect against MI (Sauer et al., 2001). However, two studies have reported a significant increase in MACE risk with SSRIs (Blanchette et al., 2008; Coupland et al., 2011): Blanchette *et al.* reported an increased risk of acute MI (Blanchette et al., 2008), while another study reported an association with increased risks of both MI and stroke (Coupland et al., 2011).

TCAs have known potential cardio-toxic effects (reduced heart rate variability and QT interval prolongation) that could lead to fatal MI, stroke, and sudden death (Roose et al., 1998). Preclinical studies have shown that TCAs have cardiovascular ion-channel- (Na(+), Ca(2+), and K(+)) blocking activities (Pacher and Kecskemeti, 2004). SSRIs could partly share this mechanism; moreover, they have been known to block serotonin transporters (Hergovich et al., 2000; Zahradnik et al., 2008). Therefore, SSRIs have often been linked to bleeding complications like gastro-intestinal bleeding or hemorrhagic stroke (Dalton et al., 2003). Thus far, the pathogenesis appears to be multifactorial such that each MACE component cannot be said to be related to a specific drug class. Discrepancies between findings may be due to the differences in study samples, study designs, or statistical methods.

In our study, the analysis of the dose and duration of antidepressants use is noteworthy. Most TCA prescriptions (98.7%) in our study were <0.5 DDD. A study reported a proportion of 70.0% which is less than that in our study (Coupland et al., 2011). According to the WHOCC, the DDDs of TCAs are 75 mg/day for amitriptyline and nortriptyline and 100 mg/day for imipramine and clomipramine (WHOCC, 2016). Most of the participants in our study used <0.5 DDD of TCAs, meaning that they used <37.5 mg/day for amitriptyline and nortriptyline or <50 mg/day for imipramine and clomipramine. In other studies, a suitable low dose was suggested in the range of 75–100 mg/day as a way to reduce side effects (Furukawa et al., 2003; NICE-Guidance, 2010). The dose used by the participants in our study was less than 75–100 mg. It seems that psychiatrists might prescribe low doses of TCAs to minimize its known harmful effects on CVDs while preserving its clinical effect on depression. Kim *et al.* reported that it is common for psychiatrists in Korea to prescribe antidepressants in doses less than the minimum effective daily dose due to their side effects (Kim et al., 2019). Our results showed that TCA use was associated with an increased risk of MACEs even at its low doses, <0.5 DDD. In the duration analysis, a longer duration of TCA use correlated with a higher HR for the MACEs. The HR when TCAs were used for more than 365 days was 1.5 times the HR when they were used less than 30 days. In addition, the average time to onset of the first MACEs was 410 days for the TCAs. Considering that the long duration of time (>365 days) and the average occurrence time of MACEs (410 days) are similar in terms of time, it would be recommended that psychiatrists monitor the occurrence of MACEs when patients use TCAs for more than 1 year. Like our study, the cardiovascular side effects of a long-term therapy (≥53 weeks) with TCAs have also been reported (Rodstein and Oei, 1979). Nevertheless, another study reported that the duration of TCA use was not correlated with MI or stroke (Coupland et al., 2016). However, in the study design, the estimated risk was calculated by dividing the exposed and unexposed periods within each patient, meaning that it was not based on their continuous use of TCAs. Unlike the method in Coupland *et al*, we estimated the risk of MACEs based on the duration of the continuous TCA use and showed the elevated risk of long term use of TCAs. Therefore, careful monitoring is needed in patients using TCAs for a long period.

In the subgroup analysis, an unusual finding was found in the age analysis. TCAs showed a significantly higher risk in younger patients (<65 years) than in older patients (≥65 years). However, in the subgroup analysis by Korean ASCVD risk scores, patients with high Korean ASCVD risk scores (≥7.5%) showed a significantly increased risk. Although the age of patients is the most powerful factor in calculating the CVD risk (Karmali et al., 2014), age alone could not be used to estimate the CVD risk of each patient. Based on these results, it is recommended that physicians consider the CVD risk score, not age alone, of each patient when prescribing antidepressants (especially when TCAs are used). We also found that patients who were former- or current smokers, who drink alcohol, and do not exercise had an increased risk of MACEs compared to the others.

There are several limitations in our study. During applying an exclusion criteria, two criteria excluded quite many people (age <40 or age ≥80: 621,847 patients; did not take physical examinations within 1 year from the index date: 624,754 patients). One of the main purposes in this study was to calculated the Korean ASCVD risk scores of patients around each of their index dates. It was inevitable that we ended up excluding a large number of people who did not have a physical examination. SNRIs had only small proportion (n = 782) of the matched cohort, and unstable results were obtained in their sensitivity analysis. Interpretation of the clinical meaning of the SNRIs was difficult although they were observed as a significant risk factor for MI and CVD related death. In a real world setting, there would be a large number of switches between antidepressants drugs. We excluded patients if they did not stay with their initial antidepressant classes. Therefore, we could not consider every switch between the classes of antidepressants. Our study is a retrospective cohort design and not all information is included in the KNHIS data. Therefore, although we adjusted for all possible confounders, there still might be residual confounding factors present. This study may not have completely ruled out the effects of depression on MACEs because it used number of outpatient visits as an indirect measure of depression severity.

Our study results suggest that low doses of TCA use were associated with an increased risk of MACEs in primary prevention compared with other antidepressants. A longer duration of TCA use was correlated with a higher risk. The dose and duration of antidepressants use need to be considered when TCAs are used in patients with no known CVD. High Korean atherosclerotic CVD risk score and unfavorable lifestyle factors showed significant associations with MACEs. Because no RCT evidence is available, our findings could be used when physicians prescribe antidepressants. Further research is needed to elucidate the specific mechanism and clinical significance of our study results.

DATA AVAILABILITY STATEMENT

Data that can view all the records of a patient are difficult to share due to the policy of the National Health Insurance Service. It can only be viewed in anonymized form when analyzed. Therefore, if

there is a request for original data, the statistical data obtained after the desired statistical processing on the server will be shared.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of Seoul National University (IRB No. E1606/003-002). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HJ and JK contributed to conception and design of the study, data acquisition, analysis and interpretation of results, drafted, and revised the manuscript; Y-KS contributed to conception and design of the study, data acquisition, and revised the manuscript. J-YS contributed to design of the study, analysis of results, and revised the manuscript. H-YL and YA contributed to conception of the study, interpretation of results, and revised the manuscript. JO and I-WK contributed to conception and design of the study, analysis and interpretation of results, and revised the 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.2020.594474/full#supplementary-material.

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Taking Sleeping Pills and the Risk of Chronic Kidney Disease: A Nationwide Population-Based Retrospective Cohort Study

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Background: Sleeping disorder has been associated with chronic kidney disease (CKD); however, the correlation between sleeping pills use and CKD has not been investigated in-depth yet. This study elucidated the potential association of sleeping pill use with the risk of CKD and CKD progression to end-stage renal disease (ESRD) requiring dialysis.

Methods: This study was based on a population-based cohort that included 209,755 sleeping pill users among 989,753 individuals. After applying the exclusion criteria, 186,654 sleeping pill users and 373,308 nonusers were enrolled to monitor the occurrence of CKD. Using a cumulative daily dose, we analyzed the types of sleeping pills related to the risk of CKD and ESRD. Propensity score matching and analysis using Cox proportional hazards regression were performed with adjustments for sex, age, and comorbidities.

Results: Sleeping pill use was related to increased CKD risk after adjusting for underlying comorbidities (adjusted hazard ratio [aHR] = 1.806, 95% confidence interval [CI]: 1.617–2.105, $p < 0.001$). With the exception of hyperlipidemia, most comorbidities correlated with an increased risk of CKD. Persistent use of sleeping pills after CKD diagnosis increased the risk of concurrent ESRD (aHR = 7.542; 95% CI: 4.267–10.156; $p < 0.001$). After the subgroup analysis for sleeping pill use, brotizolam ($p = 0.046$), chlordiazepoxide ($p < 0.001$), clonazepam ($p < 0.001$), diazepam ($p < 0.001$), dormicum ($p < 0.001$), estazolam ($p < 0.001$), fludiazepam ($p < 0.001$), flunitrazepam ($p < 0.001$), nitrazepam ($p < 0.001$), trazodone ($p < 0.001$), zolpidem ($p < 0.001$), and zopiclone ($p < 0.001$) were found to have significant correlation with increased CKD risk.

Conclusion: Sleeping pill use was related to an increased risk of CKD and ESRD. Further studies are necessary to corroborate these findings.

Keywords: peritoneal dialysis, hemodialysis, end-stage renal disease, chronic kidney disease, sleeping pills

INTRODUCTION

Chronic kidney disease (CKD) is characterized by abnormalities of kidney function or structure presenting for >3 months and associated with health problems depending on the causes, glomerular filtration rate category, and albuminuria category. CKD is known to contribute to the risk of cardiovascular events, cardiovascular mortality, and all-cause mortality. Many drugs have been associated with CKD occurrence. The complex and dynamic relationship between sleeping pills and CKD remains relatively poorly understood. Furthermore, the burdens of sleeping pills on CKD and progression to end-stage renal disease (ESRD) are rarely discussed (Turek et al., 2012). Taiwan is well known for its high prevalence of CKD (11.9%) and ESRD among adults, with a higher prevalence among the elderly (37.2%), which makes them important study subjects. According to the report, the prevalence of sleep disturbances is estimated to be 80% among patients with CKD; thus, the use of sleeping pills may have a significant impact on this group of people (Turek et al., 2012).

Sleeping pills, primarily benzodiazepines, nonbenzodiazepine omega-receptor agonists (zolpidem), tricyclic antidepressants, selective gamma-aminobutyric acid (GABA) agents, and antihistamines, have been widely introduced in an era where sleeping and anxiety disorders are common. Approximately 2–5% of sleeping pill users are young adults, while most are older adults and women. Sleeping pills are well known for their undesired side-effects, including daytime sedation, confusion, cognitive deficits, dependency, withdrawal, rebound symptoms, ataxia, dysarthria, diplopia, and vertigo (Wang et al., 2001). Benzodiazepine can cause various adverse events such as falls, major fractures, and motor vehicle accidents. The elderly are at a particular risk of such adverse events because of poor elimination of the metabolized drugs (Tamblyn et al., 2005). Furthermore, the use of benzodiazepine and zolpidem was associated with a 15% increase in mortality rate. Winkelmayer et al. previously concluded that frequent use of benzodiazepines and zolpidem may lead to higher mortality rates in patients undergoing dialysis (Winkelmayer et al., 2007). Various studies have reported sleep disorders in patients with CKD and those receiving dialysis (Winkelmayer et al., 2007; Natale et al., 2019). Sleep disorders may increase the use of sleeping pills. In patients with CKD and ESRD, the elimination of these drugs may be impaired or shortened, thus leading to a higher risk of adverse events. As the relationship between the cumulative use of sleeping pills and the subsequent risk of CKD and ESRD is still poorly understood, the purpose of this study was to assess the impact of sleeping pill exposure on CKD and further ESRD.

MATERIALS AND METHODS

Data Source

The present study was designed as a longitudinal data study and time-to-event analysis and was conducted over a 13-year period, using data acquired from the National Health Insurance Research Database (NHIRD), which is maintained by the National Health Research Institutes. The NHIRD consists of broad population-based datasets covering 99.9% of the Taiwanese inhabitants. We applied inpatient and outpatient datasets that had diagnoses clarified using the International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM) codes. All data were anonymized to ensure the privacy of all the participants.

Inclusion and Exclusion Criteria

The details of the inclusion and exclusion criteria are shown in **Figure 1**. Those patients who were using sleeping pills were matched with those not using sleeping pills (control group) by propensity score matching (two controls for each sleeping pill user) according to age, sex, and index date, using the same exclusion criteria. The index dates of sleeping pill users and sleeping pill nonusers were matched in the same month. CKD was represented with ICD-9-DM code 585. The patients with ESRD requiring dialysis (ESRDd) were represented with the ICD-9-DM codes for peritoneal dialysis and hemodialysis. With the criteria of absence of evidence of CKD and ESRDd over the previous 3 years (1997–1999), the study subjects were enrolled from January 1, 2000, until December 31, 2013, and defined as newly diagnosed with CKD/ESRDd (**Figure 1**).

Outcome and Comorbidities (Potential Confounders)

With the exception of individuals who had withdrawn from the insurance program or were lost to follow-up until the end of 2013, the person-years of follow-up for the patients newly diagnosed with CKD/ESRDd were calculated. Between the index date and January 1, 2013, CKD risk factors were identified as potential confounders, including comorbidities such as diabetes mellitus (ICD-9-CM code 250), hypertension (codes 401–405), chronic liver disease and cirrhosis (LC) (code 571), chronic obstructive pulmonary disease and allied conditions (COPDa; codes 490–496), hyperlipidemia (code 272), congestive heart failure (CHF; code 428), coronary artery disease (CAD; codes 410–414), and stroke (codes 430–438). Nephrotoxic drugs such as NSAIDs (nonsteroidal anti-inflammatory drugs), antibacterial agents (such as aminoglycosides), antifungal or antiviral agents, immunosuppressants, and ACEI (angiotensin-converting enzyme inhibitors)/ARB (angiotensin II receptor blocker) were identified as potential confounders.

Evaluation of Sleeping Pill Exposure

Those who were not exposed to sleeping pills for >3 years prior to the study period (1997–1999) were included as nonusers of sleeping pills. Regular sleeping pill use was defined as constant sleeping pill use for >90 days after the index date. Sleeping pill use periods with intervals between successive prescriptions exceeding 90 days were abandoned. To judge the association between sleeping pill exposure and further risk of ESRDd, we analyzed the incidence of ESRDd after CKD in those using sleeping pills compared with those not using sleeping pills.

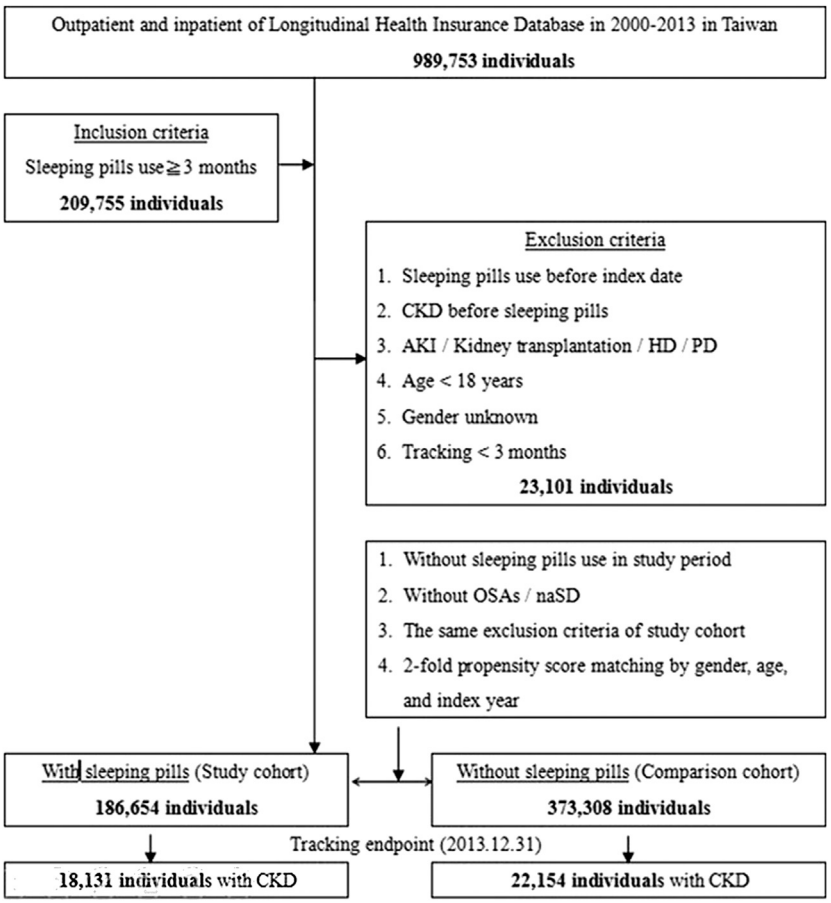

FIGURE 1 | The flowchart of study sample selection from the National Health Insurance Research Database in Taiwan.

Statistical Analysis

The incidence rate of CKD (per 10^5 person-years) was calculated for both cohorts. The chi-square test was used for categorical variables and *t*-test for continuous variables in our study. Multivariate-adjusted Cox proportional hazard regression models were used to analyze associations between sleeping pill use and CKD incidence. The models were adjusted for age, sex, hypertension, diabetes mellitus, stroke, LC, COPDa, CHF, CAD, hyperlipidemia, Charlson comorbidity index (CCI) score, city location, urbanization, level of care, and season. A large number of confounding variables with p values of <0.05 after 13 years of follow-up were included in the adjusted model in **Table 1**. The Kaplan–Meier analysis was applied to assess the cumulative incidence curves of CKD for both cohorts. The log-rank test was applied to assess the differences between the two cohorts. Results are presented as hazard ratios (HR) with 95% confidence intervals (CIs). The SAS statistical software (Version 9.3 for Windows; (SAS Institute, Inc., Cary, NC, United States) was used for all the statistical analyses.

Study Approval

This study was approved by the Institutional Review Board of the Tri-Service General Hospital (study approval code: TSGHIRB No. 2-106-05-029). The NHIRD encrypted the personal information of the patients and provided researchers with anonymous identification numbers associated with relevant claim information and prescriptions, medical services received, sex, and date of birth; therefore, patient consent was not required for this study.

RESULTS

In total, 209,755 regular sleeping pill users (i.e., use for >3 months) were enrolled between 2000 and 2013. CKD was observed in 18,131 of 186,654 sleeping pill users and in 22,154 of 373,308 nonusers. **Figure 1** shows the research design of this study.

Table 2 shows the comorbidities and demographic features of the sleeping pill users (186,654) and nonusers (373,308) from 2000 to 2013. People >60 years of age comprised 40% of both cohorts with similar age and sex distributions. Men accounted for 58% of the population in both groups. Sleeping pill use occasionally occurred concurrently in the patients with comorbidities such as hypertension, diabetes mellitus, stroke, LC, COPDa, CHF, and CAD (all $p < 0.05$), with a similar

TABLE 1 | Factors of CKD at the end of the follow-up period stratified by Cox regression.

Variables	With sleeping pills			Without sleeping pills (reference)			Ratio	Adjusted HR (95%CI)	*p* value
Stratified	Events	PYs	Rate	Events	PYs	Rate			
Overall	18,131	1,852,718.01	978.62	22,154	3,841,800,12	576.66	1.697	1.806 (1.617–2.105)	<0.001*
Gender									
Male	10,201	1,033,370.53	987.16	10,865	2,199,862.43	493.89	1.999	2.127 (1.904–2.479)	<0.001*
Female	7,930	819,347.49	967.84	11,289	1,641,937.69	687.54	1.408	1.498 (1.341–1.746)	<0.001*
Age, year									
18–29	301	170,506.59	176.53	128	113,031.10	113.24	1.559	1.659 (1.485–1.934)	<0.001*
30–39	664	238,047.58	278.94	571	383,830.78	148.76	1.875	1.993 (1.784–2.326)	<0.001*
40–49	1,398	295,634.89	472.88	1,099	433,670.36	253.43	1.866	1.985 (1.779–2.139)	<0.001*
50–59	1,736	330,859.05	524.69	1,635	604,582.89	270.43	1.940	2.065 (1.843–2.404)	<0.001*
≧60	14,032	817,669.90	1,716.10	18,721	2,306,684.89	811.60	2.114	2.224 (2.001–2.638)	<0.001*
Comorbidity									
HTN	3,612	38,631,915	934.98	3,598	841,576.62	427.53	2.187	2.327 (2.084–2.715)	<0.001*
DM	5,651	315,775.954	1,789.56	6,200	696,874.46	889.69	2.011	2.143 (1.918–2.499)	<0.001*
CHF	1,586	77,023.53	2,059.11	1,567	179,608.20	872.45	2.360	2.511 (2.240–2.938)	<0.001*
Stroke	1,533	144,467.46	1,061.14	1,786	326,806.34	546.50	1.942	2.066 (1.835–2.421)	<0.001*
COPDa	2,341	175,313.84	1,335.32	1,913	303,463.12	630.39	2.118	2.254 (2.011–2.623)	<0.001*
LC	1,835	104,988.14	1,747.82	1,667	209,419.66	796.01	2.196	2.337 (2.095–2.731)	<0.001*
CAD	2,582	153,669.22	1,680.23	2,612	361,016.51	723.51	2.322	2.474 (2.217–2.890)	<0.001*
Hyperlipidemia	297	58,928.35	504.00	633	123,502.54	512.54	0.983	1.046 (0.937–1.222)	0.108
Without hyperlipidemia	17,834	1,793,789.66	994.21	21,521	3,718,297.58	578.79	1.718	1.828 (1.637–2.131)	<0.001*
Insured premium									
<18,000	16,121	1,820,315.56	885.62	16,252	3,773,394.06	430.70	2.056	2.188 (1.859–2.651)	<0.001*
18,000–34,999	567	26,915.50	2,106.59	1,339	56,153.61	2,384.53	0.883	1.340 (1.010–1.596)	0.039*
≧35,000	1,443	5,486.96	26,298.71	4,563	12,252.45	37,241.53	0.706	0.852 (0.673–1.207)	0.246
Medications									
NSAIDs	6,756	438,592.56	1,540.38	8,382	676,197.76	1,239.58	1.243	1.322 (1.182–1.569)	<0.001*
ACEI and ARB	3,230	231,055.30	1,397.93	4,052	342,282.57	1,183.82	1.181	1.257 (1.126–1.463)	<0.001*
Antibacterial agents	573	45,644.48	1,255.35	897	81,151.43	1,105.34	1.136	1.209 (1.083–1.409)	<0.001*
Antifungal agents	444	33,318.88	1,332.58	765	62,036.12	1,233.15	1.081	1.150 (1.028–1.347)	0.018*
Antiviral agents	599	60,266.22	993.92	1,125	128,154.63	877.85	1.132	1.205 (1.079–1.401)	<0.001*
Immunosuppressive agents	402	30,157.96	1,332.98	711	56,738.13	1,253.13	1.064	1.132 (1.015–1.322)	0.036*

*PYs, person-years; rate, per 10^5 PYs; ratio = rate in cases ÷ rate in controls; adjusted HR, adjusted for all the variables of age, gender, and comorbidities, including the following: HTN, hypertension; DM, diabetes; CHF, congestive heart failure; stroke, COPDa, chronic obstructive pulmonary disease and allied conditions; LC, chronic liver disease and cirrhosis; CAD, coronary artery disease; hyperlipidemia. *p value <0.05.*

prevalence ($p = 0.485$). No cooccurrence was observed in all the patients with hyperlipidemia. We also observed a higher prevalence of sleeping pill use in northern Taiwan, areas of higher urbanization, and our local hospital.

The incidence rate of progression to CKD was 978 per 100,000 person-years for the sleeping pill users compared with 576 per 100,000 for the nonusers. The incidence rate of CKD was 1.69-fold higher in those who used sleeping pills than in the nonusers. We further evaluated the risk of ESRDd after CKD diagnosis in those who continuously used sleeping pills using the Cox regression model, which revealed an increased risk of CKD progression (adjusted HR [aHR] = 7.542; 95% CI: 4.267–10.156; $p < 0.001$; **Table 3**).

Sleeping pill users were shown to have an increased risk of CKD compared with the nonusers after univariate and multivariate analyses were performed with adjustments for age, sex, and comorbidities (aHR = 1.806; 95% CI: 1.617–2.105; $p < 0.001$).

The aHR for CKD was much greater for elderly individuals (age: 60 years; aHR = 4.678: 95% CI: 4.351–5.209; $p < 0.001$) than for those aged 18–29 years. Patients with comorbidities such as hypertension, diabetes mellitus, stroke, LC, COPDa, CHF, CAD, and hyperlipidemia exhibited an increased risk for developing CKD. Higher insurance premiums, lower urbanization, and central facilities were all associated with an increased risk of CKD. Medications such as NSAIDs, ACEI, and ARB were all associated with an increased risk of CKD. Furthermore, each increase in CCI score led to a 1.025% increase in CKD risk (table not shown). After stratification by sleeping pill use, a risk increase was apparent according to sex (both), age, and comorbidities such as hypertension, diabetes mellitus, CHF, stroke, COPDa, LC, CAD, and nephrotoxic agents. Hyperlipidemia (aHR = 1.046; 95% CI: 0.937–1.222; $p = 0.108$) and higher insurance premium of 35,000 Taiwanese dollars (aHR = 0.852; 95% CI: 0.673–1.207; $p = 0.246$) were not significant contributors to the risk after stratification (**Table 1**).

After stratification of the sleeping pills by generic drug classification, most sleeping pills were associated with an increased risk of CKD, except for alprazolam (aHR = 1.487; 95% CI: 0.931–1.772; $p = 0.068$), amitriptyline (aHR = 1.358; 95% CI: 0.930–1.628; $p = 0.070$), doxepin (aHR = 1.595; 95% CI: 0.885–2.702; $p = 0.129$), flurazepam (aHR = 1.804; 95% CI: 0.841–2.490; $p = 0.158$), lorazepam (aHR = 1.702; 95%

TABLE 2 | Demographic characteristics and comorbidities in cohorts with and without sleeping pills use.

Variables	Total	Sleeping pills use		*p* value
		Yes	No	
N (%)	559,962	186,654 (33.33)	373,308 (66.67)	
Age, year				0.999
18–29	72,924 (13.02)	24,308 (13.02)	48,616 (13.02)	
30–39	79,017 (14.11)	26,339 (14.11)	52,678 (14.11)	
40–49	95,658 (17.08)	31,886 (17.08)	63,772 (17.08)	
50–59	91,041 (16.26)	30,347 (16.26)	60,694 (16.26)	
≧60	221,322 (39.52)	73,774 (39.52)	147,548 (39.52)	
Sex				0.999
Female	233,547 (41.71)	77,849 (41.71)	155,698 (41.71)	
Male	326,415 (58.29)	108,805 (58.29)	217,610 (58.29)	
Comorbidity				
Hypertension	79,325 (14.17)	33,112 (17.74)	46,213 (12.38)	<0.001
DM	59,735 (10.67)	21,194 (11.35)	38,541 (10.32)	<0.001
CHF	11,073 (1.98)	4,112 (2.20)	6,961 (1.86)	<0.001
Stroke	39,517 (7.06)	14,534 (7.79)	24,983 (6.69)	<0.001
COPDa	39,346 (7.03)	15,624 (8.37)	23,722 (6.35)	<0.001
LC	29,967 (5.35)	8,957 (4.80)	21,010 (5.63)	<0.001
CAD	38,602 (6.89)	12,616 (6.76)	25,986 (6.96)	<0.001
Hyperlipidemia	16,327 (2.92)	5,314 (2.85)	11,013 (2.95)	0.485
CCI_R	0.49 ± 1.88	0.76 ± 2.52	0.36 ± 1.44	<0.001
Season				<0.001
Spring (March–May)	148,649 (26.55)	48,130 (25.79)	100,519 (26.93)	
Summer (June–August)	139,393 (24.95)	47,206 (25.29)	92,487 (24.77)	
Autumn (September–November)	130,474 (23.30)	46,851 (25.10)	83,623 (22.40)	
Winter (December–February)	141,146 (25.21)	44,467 (23.82)	96,679 (25.90)	
Location				<0.001
Northern Taiwan	209,137 (37.35)	60,208 (32.26)	148,929 (39.89)	
Middle Taiwan	163,183 (29.14)	62,875 (33.69)	100,308 (26.87)	
Southern Taiwan	147,442 (26.33)	48,121 (25.78)	99,321 (26.61)	
Eastern Taiwan	36.888 (6.59)	14,341 (7.68)	22,547 (6.04)	
Outlets Islands	3,312 (0.59)	1,109 (0.59)	2,203 (0.59)	<0.001
Urbanization level				0.701
1 (the highest)	180,200 (32.18)	51,949 (27.83)	128,251 (34.36)	<0.001
2	231,075 (41.27)	76,190 (40.84)	154,885 (41.49)	<0.001
3	49,432 (8.83)	19,899 (10.66)	29,533 (7.91)	<0.001
4 (the lowest)	99,255 (17.73)	38,616 (20.69)	60,639 (16.24)	<0.001
Level of care				<0.001
Medical center	159,653 (28.51)	50,524 (27.07)	109,129 (29.23)	
Region hospital	186,615 (33.33)	78,429 (42.02)	108,186 (28.98)	
Local hospital	213,694 (38.16)	57,701 (30.91)	155,993 (41.79)	
Insured premium				<0.001
<18,000	483,656 (86.37)	161,245 (86.26)	327,095 (87.62)	
18,000–34,999	54,313 (9.70)	18,010 (9.65)	36,303 (9.72)	
≧35,000	21,993 (3.93)	7,399 (3.96)	14,594 (3.91)	
Medications				
NSAIDs	110,876 (19.80)	44,124 (23.64)	66,752 (17.88)	<0.001
ACEI and ARB	57,034 (10.19)	23,245 (12.45)	33,789 (9.05)	<0.001
Antibacterial agents	12,603 (2.25)	4,592 (2.46)	8,011 (2.15)	<0.001
Antifungal agents	9,476 (1.69)	3,352 (1.80)	6,124 (1.64)	<0.001
Antiviral agents	18,714 (3.34)	6,063 (3.25)	12,651 (3.39)	0.006
Immunosuppressive agents	8,635 (1.54)	3,034 (1.63)	5,601 (1.50)	<0.001

Chi-square/Fisher's exact test; continuous variable: t-*test.* *p *value <0.05. CKD, chronic kidney disease; DM, diabetes mellitus; CHF, congestive heart failure; COPDa, chronic obstructive pulmonary disease and allied conditions; LC, chronic liver disease and cirrhosis; CAD, coronary artery disease; CCI_R, Charlson comorbidity index with DM, CHF, stroke, COPDa, LC, CAD, and hyperlipidemia removed. NSAIDs, nonsteroidal anti-inflammatory drugs; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker.*

CI: 0.866–2.192; $p = 0.137$), quetiapine (aHR = 1.196; 95% CI: 0.715–1.783; $p = 0.286$), and triazolam (aHR = 1.886; 95% CI: 0.848–2.519; $p = 0.153$) (**Table 4** and **Figure 2**). Compared with the nonusers, the sleeping pill users demonstrated a significantly higher cumulative risk of CKD based on the Kaplan–Meier analyses ($p < 0.001$); (**Figure 3**).

DISCUSSION

After adjusting for potential confounders, sleeping pill use was correlated with a significant (80%) increase in the risk of CKD (**Table 1**). In addition, this study revealed that all the sleeping pills except alprazolam, amitriptyline, doxepin, flurazepam,

TABLE 3 | Factors of ESRD among CKD patients by using Cox regression.

Variables[a]	Population	Events	PYs	Rate	Crude HR	95% CI	*p* value	Adjusted HR	95% CI	*p* value
Sleeping pills[a]	18,131	7,862	412,986.55	1,903.69	7.688	4.832–10.996	<0.001*	7.542	4.267–10.156	<0.001*

*PYs, person-years; rate, per 10^5 PYs; HR, hazard ratio; CI, confidence interval; adjusted HR: adjusted for all the variables of age, gender, and comorbidities, including HTN, hypertension; DM, diabetes; CHF, congestive heart failure; stroke; COPDa, chronic obstructive pulmonary disease and allied conditions; LC, chronic liver disease; cirrhosis; CAD, coronary artery disease; and hyperlipidemia. *p value <0.05.*

TABLE 4 | Crude and adjusted odds ratios of CKD associated with various sleeping pills administration during the follow-up period in the study cohort.

Variables[a]	Crude HR	95% CI	*p* value	Adjusted HR	95% CI	*p* value
Sleeping pills[a]	1.818	1.789–1.902	<0.001	1.806	1.617–2.105	<0.001*
Alprazolam	1.594	1.030–1.895	0.018	1.487	0.931–1.772	0.068
Amitriptyline	1.498	1.002–1.706	0.048	1.358	0.930–1.628	0.070
Brotizolam	1.398	1.111–1.564	0.003	1.276	1.003–1.513	0.046*
Chlordiazepoxide	1.506	1.2244–1.765	<0.001	1.501	1.110–1.673	<0.001*
Clonazepam	1.449	1.398–1.501	<0.001	1.368	1.282–1.501	<0.001*
Diazepam	1.799	1.642–1.836	<0.001	1.627	1.527–1.736	<0.001*
Dormicum	1.813	1.299–2.012	0.001	1.705	1.179–1.970	<0.001*
Doxepin	1.701	0.972–2.982	0.134	1.595	0.885–2.702	0.129
Estazolam	1.906	1.807–2.072	<0.001	1.736	1.591–1.907	<0.001*
Fludiazepam	1.535	1.384–1.803	0.007	1.426	1.244–1.624	<0.001*
Flunitrazepam	1.678	1.271–1.895	<0.001	1.555	1.172–1.770	<0.001*
Flurazepam	2.174	0.973–2.997	0.070	1.804	0.841–2.490	0.158
Lorazepam	1.911	1.012–2.551	0.038	1.702	0.866–2.192	0.137
Nitrazepam	1.924	1.568–2.497	<0.001	1.775	1.328–2.147	<0.001*
Quetiapine	1.355	0.864–2.012	0.246	1.196	0.715–1.783	0.286
Trazodone	1.801	1.245–2.131	0.001	1.627	1.162–1.927	<0.001*
Triazolam	1.996	0.931–2.724	0.070	1.886	0.848–2.519	0.153
Zolpidem	2.019	1.672–2.486	<0.001	1.872	1.519–2.091	<0.001*
Zopiclone	1.905	1.539–2.11	<0.001	1.897	1.638–2.213	<0.001*

*HR, hazard ratio; CI, confidence interval; adjusted HR, adjusted for all the variables of age, gender, and comorbidities, including HTN, hypertension; DM, diabetes; CHF, congestive heart failure; stroke; COPDa, chronic obstructive pulmonary disease and allied conditions; LC, chronic liver disease and cirrhosis; CAD, coronary artery disease; hyperlipidemia. *p value <0.05.*
[a]Without the disease or medication as reference.

lorazepam, quetiapine, and triazolam were associated with an increased risk of CKD. No study has previously rigorously examined the adverse effects of sleeping pill use (including benzodiazepines). The prevalence of sleeping pill use in a previous Brazilian population study was 7.6% and was closely associated with female sex, age ≥60 years, and smoking (Kodaira and Silva, 2017).

A significantly higher prevalence of sleeping pill use (5.5%) was observed among morbidly obese (body mass index (BMI) ≥ 35 kg/m^2) men and underweight (BMI < 18.5 kg/m^2) women in a Canadian study (Vozoris and Leung, 2011). In this study, the prevalence of sleeping pill use generally increased with age. A previous study reported that 2–5% of young adults used benzodiazepines or nonbenzodiazepine omega-receptor agonists and the use was particularly high in older adults, women, Caucasians, and current smokers. In the study by Winkelmayer et al., hypertension and diabetes mellitus were not associated with benzodiazepine use, while several other comorbid conditions such as CAD, CHF, peripheral vascular disease, COPD, and malignancy were significantly associated with the use of benzodiazepines (Winkelmayer et al., 2007). These comorbidity results are compatible with the findings in our study regarding CAD, CHF, and COPDa. Conversely, our study demonstrated that sleeping pill use was more frequently observed in patients with hypertension, diabetes mellitus, stroke, liver cirrhosis, and hyperlipidemia. The percentages of patients with CKD and ESRD using sleeping pills are seemingly rising, owing to the significant psychiatric burden of physical illness (Pham et al., 2017).

According to Winkelmayer et al., dialysis patients using benzodiazepines were predominantly Caucasian (77% vs. 60%, $p = 0.001$) and female (53% vs. 45%, $p = 0.002$), had lung disease (odds ratio [OR] = 1.43; 95% CI: 1.03–1.98), and seldom had cerebrovascular diseases (Winkelmayer et al., 2007). Benzodiazepines such as temazepam, lorazepam, alprazolam, and clonazepam have reportedly been prescribed in dialysis populations for the treatment of anxiety, sleep disorders, restless leg syndrome, and depression (Fukuhara et al., 2006). Sedative use was previously associated with increased mortality; however, the reasons were unknown (Hausken et al., 2007). Although our study confirmed an association between sleeping pill use and CKD risk, research discussing this relationship is limited. Mechanisms underlying the association between sleeping pill use and the deterioration of kidney function are still unclear.

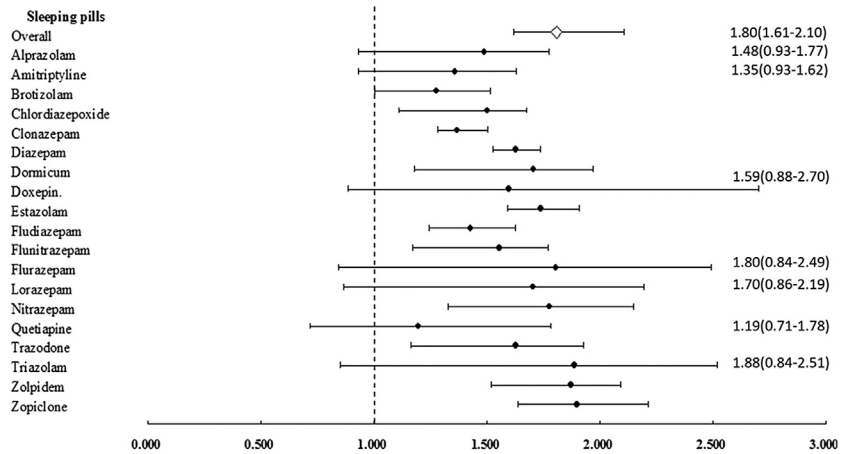

FIGURE 2 | Adjusted hazard ratio of chronic kidney disease (CKD) in individuals with affected sleeping pills. Error bars indicate 95% confidence interval (CI).

According to our study, those using sleeping pills usually had underlying comorbidities (e.g., concomitant hepatic disease or CHF), which may contribute to the risk of nephrotoxicity in patients with advancing age, volume depletion, and selected high-risk therapies such as NSAIDs, aminoglycosides, ACE inhibitors, and radiographic contrast media (Ponticelli et al., 2015). In relation to the previous aspect, it should be taken into account that when a patient uses a high number of drugs concomitantly, generally, it is considered polypharmacy from 5. This is an important aspect when the outcome is renal damage. Benzodiazepines are the most common class drugs used as sleep aids. Their action is based on an inhibitory neurotransmitter called GABA, which specifically acts in a transmembrane structure on GABA receptors (GABA agonists) known as the GABAA receptor protein complex. This results in the opening of chloride channels with the consequent influx of ions into neurons, causing hyperpolarization. Undesirable side-effects are due to the prolonged action caused by renal or liver failure, as the liver and kidney are the primary organs for benzodiazepine metabolism and excretion. Benzodiazepines that include active metabolites such as clorazepate, chlordiazepoxide, diazepam, and flurazepam should be prohibited in patients with renal insufficiency and those with ESRD (Wyne et al., 2010). Renal and nonrenal drug clearance mechanisms, such as the CYP3A4 clearance of benzodiazepines, may be compromised in CKD and ESRD conditions, leading to drug side-effects and possible cumulative effects on the kidneys (Velenosi et al., 2012; Thomson et al., 2015). Both the dibenzodiazepine derivative quetiapine (an atypical or second-generation antipsychotic) and olanzapine have been reported to induce chronic interstitial nephritis (He et al., 2013). Furthermore, propylene glycol is contained in parenteral formulations of benzodiazepines (e.g., lorazepam, chlordiazepoxide, and diazepam), which may cause acute kidney injury, proximal tubule injury, hyperosmolarity, and sepsis-like syndrome (Cawley, 2001). Worsening renal failure and metabolic acidosis have both been correlated with prolonged infusion of solutions containing propylene glycol. Withholding these agents has been recommended in critically ill patients with a creatinine clearance of ≤30 ml/min (Cawley, 2001). Some benzodiazepines and antidepressants/mood stabilizers, such as amitriptyline and doxepin, have been correlated with rhabdomyolysis (Coco and Klasner, 2004).

However, in our study, the use of alprazolam, amitriptyline, doxepin, flurazepam, lorazepam, quetiapine, or triazolam was not significantly associated with CKD. It seems that the lack of statistical significance for these drugs is due to a problem of imprecision. It is not appropriate to state that these drugs are risk-free. According to the studies of Kim et al. and Choi et al., CKD was strongly suggested to be significantly associated with long sleep duration (≥9 h/day). However, these studies failed to report on the effect of benzodiazepine use (Choi et al., 2017; Kim et al., 2017).

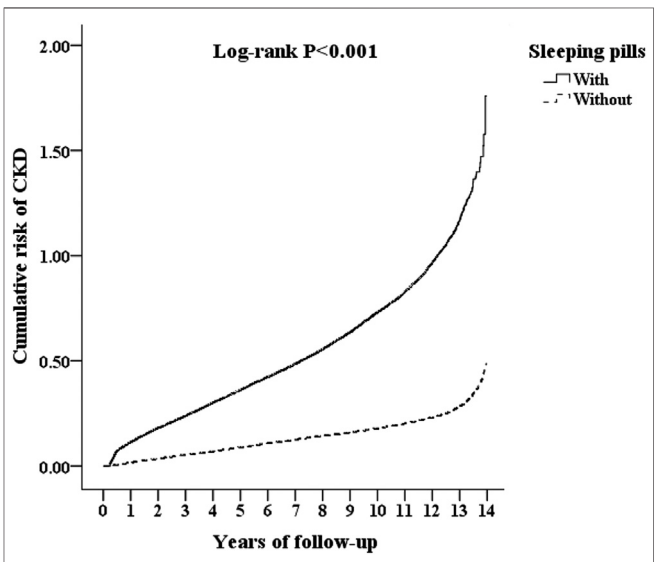

FIGURE 3 | Kaplan–Meier analysis for cumulative risk of CKD among those aged 18 and over, stratified by sleeping pills with the log-rank test.

We propose that benzodiazepine overuse may simultaneously prolong sleep duration and lead to CKD. In our study, hyperlipidemia and higher insurance premium were not associated with CKD risk associated with sleeping pill use. Associations between hyperlipidemia and sleeping pill use are therefore still not clear. The collinearity of hypertension and hyperlipidemia may explain this phenomenon. In addition, the use of lipid-lowering drugs may have renal protective effects that alleviate the renal toxicity associated with sleeping pill use (McWilliam et al., 2018).

Individuals with low insurance premiums are often equal to the group with a low socioeconomic status in Taiwan who were at risk of CKD events in our study. A study by Bello et al. previously reported that people of lower socioeconomic status have a higher risk of ischemic heart disease events than those of higher socioeconomic status (Bello et al., 2008). In adults with CKD, death risk can be attributed to nonrestorative sleep, short sleep duration, and restless leg syndrome (Ricardo et al., 2017). Thus, a balance between adequate sleep quality and evaluating the impact of sleeping pills in this population remains to be determined. The strengths and limitations of our study may be similar to those in most retrospective studies. The greatest strengths of this study were its long follow-up duration of 13 years and large-scale population-based data source. Patients who died within 90 days after the index date were excluded to reduce survival bias. To reduce off-label use of sleeping pills, patients with obstructive sleep apnea and nonapnea sleep disorders were excluded from our study. Our study had some pertinent limitations. First, data from insurance claims did not include measurements of microalbuminuria and serum creatinine; therefore, we may not have included patients with early CKD in our study. The frequency of sleeping pill use may have been increasing owing to the mental burden of CKD and ESRD. Although we excluded patients who had CKD and ESRD before entering our study, analysis of antecedents and consequences may have been confused or misinterpreted. Second, the results of our study are inappropriate for interpretation in patients with CKD of different ethnicities, as our study cohort was composed mostly of Taiwanese people. Third, we failed to adjust several possible unmeasured confounding variables for CKD that were unavailable in the NHIRD dataset (e.g., individual blood pressure control status, diet preference, and smoking status), although we adjusted for potential confounders during the statistical analysis. Finally, the study method defined sleeping pills use after 2000, which could result in underestimation of the drug effects on CKD if the drug was administered before 2000.

CONCLUSION

In brief, sleeping pill use and the risk of CKD significantly were correlated (101%). The risk and impact on public health should be reevaluated because sleeping pills are a commonly prescribed medication. However, the use of benzodiazepines such as alprazolam, flurazepam, lorazepam, and triazolam, and other agents such as amitriptyline, doxepin, and quetiapine may be warranted in susceptible CKD cases. Additional studies, particularly prospective randomized trials with longer prescription durations, are required to confirm our findings.

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 Institutional Review Board of the Tri-Service General Hospital (study approval code: TSGHIRB No. 2-106-05-029). Patient consent was not required for this study as the NHIRD had encrypted patient personal information and provided researchers with anonymous identification numbers associated with relevant claim information, including sex, date of birth, medical services received, and prescriptions.

AUTHOR CONTRIBUTIONS

C-YL contributed to manuscript writing. C-HC contributed to data collection and analysis. K-CL contributed to data interpretation. C-YC contributed to data interpretation. S-SY contributed to data interpretation. W-CC contributed to data collection and analysis. C-CW contributed to the idea of the manuscript and manuscript editing.

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BRIEF RESEARCH REPORT
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Graft Versus Host Disease Associated with Immune Checkpoint Inhibitors: A Pharmacovigilance Study and Systematic Literature Review

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Background: In patients with allogenic hematopoietic stem cell transplantation (allo-HSCT), immune-checkpoint inhibitors (ICI) are used to treat malignancy recurrence. However, ICI are also associated with graft vs. host disease (GVHD). In this pharmacovigilance analysis, we aimed to characterize cases of GVHD associated with ICI, drawn from the World Health Organization pharmacovigilance database, VigiBase®, and from literature.

Methods: We performed VigiBase® query of cases of GVHD associated with ICI. These cases were combined with those of literature, not reported in VigiBase®. The Bayesian estimate of disproportionality analysis, the information component, was considered significant if its 95% credibility interval lower bound was positive; denoting a significant association between GVHD and the suspected ICI. Time to onset between ICI and GVHD onset and subsequent mortality were assessed.

Results: Disproportionality analysis yielded 93 cases of GVHD associated with ICI (61.8% men, median age 38 [interquartile range = 27; 50] years). Cases were mostly associated with nivolumab (53/93, 57.0%), pembrolizumab (23/93, 24.7%) and ipilimumab (12/93, 12.9%) monotherapies. GVHD events occurred after 1 [1; 5.5] injection of ICI, with a time to onset of 35 [IQR = 14; 176] days. Immediate subsequent mortality after GVHD was 24/93, 25.8%. There was no significant difference in mortality depending on the molecule (p = 0.41) or the combination regimen (combined vs. monotherapy, p = 0.60). Previous history of GVHD was present in 11/18, 61.1% in cases reported in literature.

Conclusion: In this worldwide pharmacovigilance study, disproportionality yielded significant association between GVHD and ICI, with subsequent mortality of 25.8%. Previous history of GVHD was reported in more than half of cases.

Clinicaltrials.gov identifier: NCT03492242

Keywords: immunotherapy, pharmacovigilance, vigibase®, graft-versus-host disease, adverse (side) effects

BACKGROUND

Immune checkpoint inhibitors (ICI) may be used to treat aggressive hematologic malignancies, either in refractory or relapsed lymphoma frequently before being treated by allogeneic hematopoietic stem cell transplantation (allo-HSCT) or in relapse after allo-HSCT. Indeed, in both situations, relapse mechanisms include immune escape by the tumor, T-cell anergy, down-regulation of regulatory T cells and activation of immune checkpoints (Barrett and Battiwalla, 2010). They include drugs targeting programmed death-1 receptor (anti-PD-1), its ligand (anti-PD-L1) and cytotoxic T lymphocyte antigen-4 (anti-CTLA-4).

Like other therapies used in these indications (i.e., donor lymphocytes infusion, chemotherapy, immunotherapy, CAR T cell therapy), the aim of ICI is to enhance the immune system so that it may be effective against malignancy, by restoring T-cell function, activating lymphocytes and inducing a sustainable graft-versus-tumor (GVT) effect.

However, ICI are associated with immune-related adverse events, secondary to the over-activation of the immune system, which may, in turn, cause auto-immune-like complications, including graft-versus-host disease (GVHD). GVHD is serious adverse event in patients with allo-HSCT, initially described as an aggravated manifestation of regular inflammation, in which, donor lymphocytes interact with recipient antigens which may cause multi-organ dysfunction (Ramachandran et al., 2019).

Previously, cohort studies showed that ICI used before allo-HSCT were associated with an incidence of GVHD varying from 41 to 56% (Merryman et al., 2017; Ijaz et al., 2019). When used after allo-HSCT, the incidence depended whether there was a history of previous GVHD (55%) or if it was the first episode (30%) (Haverkos et al., 2017; Herbaux et al., 2018).

To comfort these results yielded from cohort studies; in the present work, we used disproportionality methodology to present characteristics of GVHD following ICI administration. This method is based on a large pharmacovigilance database, VigiBase®, which collects worldwide reports of drug-related adverse events in the World Health Organization network (Lindquist 2008). In addition, whenever possible, we combined the individual reports yielded from VigiBase® to de-duplicated cases reported in the literature to expand the cohort.

METHODS

Study Design

This work combines a worldwide pharmacovigilance observational case-control cross-sectional study focusing on GVHD related to the usage of ICI, and a systematic literature case report analysis. The pharmacovigilance part relies on VigiBase®, a database encompassing 22 million individual case safety reports (ICSR) received worldwide (Lindquist 2008). It is freely accessible upon request, increasing possibilities for external validation ("VigiAccess." from http://www.vigiaccess.org/). ICSRs include administrative information (country, type of report, qualification of reporter), patient data (age, sex), date of onset of reaction(s) and nature of the outcome using the latest version of MedDRA (Medical Dictionary for Regulatory Activities) terms (currently v22.1). Drug(s) involved (name, drug start and stop dates, indication, dose) are also indicated.

We searched for cases flagged with MedDRA preferred terms (PT) reflecting GVHD (see below), and associated with ICI molecules (nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, ipilimumab, tremelimumab and cemiplimab) from VigiBase® creation through January 05, 2020.

This work is ancillary to the Immune CHeckpoint Inhibitors Monitoring of Adverse Drug ReAction (CHIMeRA) registry (clinicaltrials.gov registry number NCT03492242).

Disproportionality Analyses

To assess whether a GVHD adverse event was associated with an ICI molecule in VigiBase®, we used disproportionality analysis (also known as case–non-case analysis) methodology. Briefly, the estimate of disproportionality analysis can be calculated by the information component (IC) for which IC_{025} is the lower end of its 95% credibility interval. A positive IC_{025} is statistically significant (Bate et al., 1998; Norén et al., 2013). The IC calculation was based on the number of GVHD reported with each ICI molecule, vs. all ADR with all medicines reported in VigiBase®. Like others, our research group previously used this method and database to describe the spectrum of cardiovascular diseases and other immune-related adverse events associated with ICI (Salem et al., 2018; Nguyen et al., 2020).

Calculation of the IC using a Bayesian confidence propagation neural network was developed and validated by the Uppsala Monitoring Center as a flexible, automated indicator value for disproportionate reporting that compares observed and expected drug–ADR associations to find new drug–ADR signals with identification of probability difference from the background data (full database) (Bate et al., 1998). Probabilistic reasoning in intelligent systems (information theory) has proved to be effective for the management of large datasets, is robust in handling incomplete data, and can be used with complex variables. The information theory tool is ideal for finding drug–ADR combinations with other variables that are highly associated compared with the generality of the stored data (Bate, Lindquist et al., 1998). Several examples of validation with the IC exist, showing the power of the technique to find signals sooner after drug approval than by a regulatory agency (e.g., an association between captopril and coughing), and to avoid false positives, whereby an association between a common drug and a common ADR occurs in the database only because the drug is widely used and the ADR is frequently reported (Bate, Lindquist et al., 1998; Norén, Hopstadius et al., 2013). Furthermore, our group recently published several studies using VigiBase® and disproportional reporting calculation to characterize and identify new drug-ADR associated signals, which were subsequently corroborated by preclinical mechanistic studies or prospective cohorts (Salem et al., 2018; Salem et al., 2019a; Salem et al., 2019b; Salem et al., 2019c).

The statistical formula is as follows:

$$IC = \log 2\left[\frac{(N_{observed} + 0.5)}{(N_{expected} + 0.5)}\right]$$

where

$$N_{expected} = \left[\frac{(N_{drug} \times N_{effect})}{N_{total}}\right]$$

$N_{expected}$ is the number of case reports expected for the drug–ADR combination.

$N_{observed}$ is the actual number of case reports for the drug–ADR combination.

N_{drug} is the number of case reports for the drug, regardless of ADR.

N_{effect} is the number of case reports for the ADR, regardless of drug.

N_{total} is the total number of case reports in the database.

IC_{025} is the lower end of a 95% credibility interval for the IC.

A positive IC_{025} value (>0) is the traditional threshold deemed statistically significant. IC_{025} values have only been validated for comparison of drug-specific ADR vs. the full database and cannot be used to compare disproportionate reporting among different ICI regimens. All patients were included in these analyses.

List of all MedDRA preferred terms (PT) related to GVHD, used in VigiBase® query.

Acute graft vs. host disease (PT), Acute graft vs. host disease in intestine (PT), Acute graft vs. host disease in liver (PT), Acute graft vs. host disease in skin (PT), Chronic graft vs. host disease (PT), Chronic graft vs. host disease in intestine (PT), Chronic graft vs. host disease in liver (PT), Chronic graft vs. host disease in skin (PT), Graft vs. host disease (PT), Graft vs. host disease in eye (PT), Graft vs. host disease in gastrointestinal tract (PT), Graft vs. host disease in liver (PT), Graft vs. host disease in lung (PT), Graft vs. host disease in skin (PT).

Literature Review

A systematic literature review of all published cases of GVHD associated with ICI was performed, spanning from January 1st, 2010 to February 1st, 2020, on MEDLINE using PubMed® search engine. The methodological search strategy included keywords associated with Medical Subject Headings (MeSH) terms related to ICI and GVHD: "immune checkpoints inhibitors" OR ("immune" AND "checkpoints") OR ("checkpoint" AND "inhibitor") OR "nivolumab" OR "pembrolizumab" OR "ipilimumab" OR "cemiplimab" OR "avelumab" OR "durvalumab" OR "atezolizumab" OR "PD-1 blockade") AND ("graft vs. host disease" OR ("graft vs. host" AND "disease") AND ("Allogeneic stem cell transplantation" OR ("allogeneic" AND "stem cell transplant"). From this search, 715 articles were found which contained any of these terms, but the combination of these terms in the format of case reports or case series after adequate filtering yielded 22 cases.

Statistics

Continuous variables are presented as median [interquartile-range] and categorical variables as number (percentage), with relevant comparison tests, accounting for data distribution. GraphPad Prism v6.0 (GraphPad Software, California, United States) was used for statistics and figures, except **Figure 2** drawn using R software (R project, worldwide community project).

RESULTS

Overall, we included 93 deduplicated cases (91 reported in VigiBase® and 22 in literature, among which 20 were reported

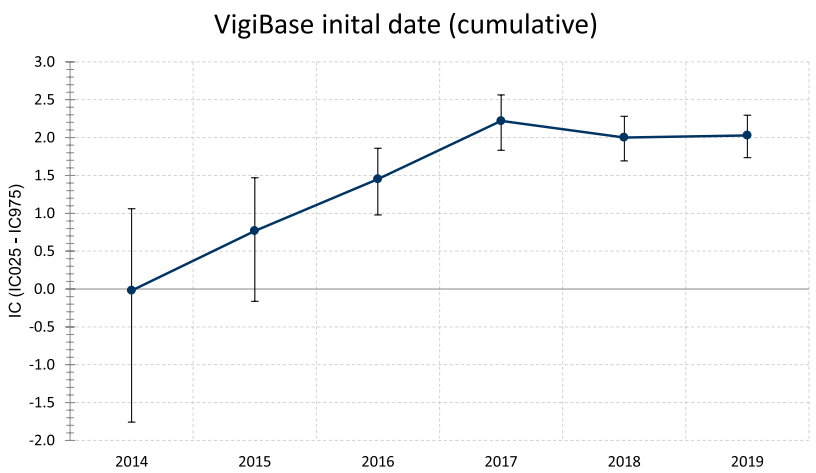

FIGURE 1 | Evolution of information component (IC) over time of ICI associated GVHD (thru December 2019). Whiskers represent the 95% credibility interval lower and upper bounds (respectively IC_{025} and IC_{975}). IC is considered significant when IC_{025} is >0.Statistics: $IC = \log 2\ [(N_{observed} + 0.5)/(N_{expected} + 0.5)]$, where $N_{expected} = (N_{drug} \times N_{effect})/N_{total}$, with $N_{expected}$ being the number of ICSRs expected for the drug-ADR combination; $N_{observed}$ being the actual number of ICSRs observed for the drug-ADR combination; N_{drug} being the number of ICSRs for the drug, regardless of ADR; N_{effect} being the number of ICSRs for the ADR, regardless of the drug; and N_{total} being the total number of ICSRs in the database. ADR = adverse drug reaction; ICSR = individual case safety report.

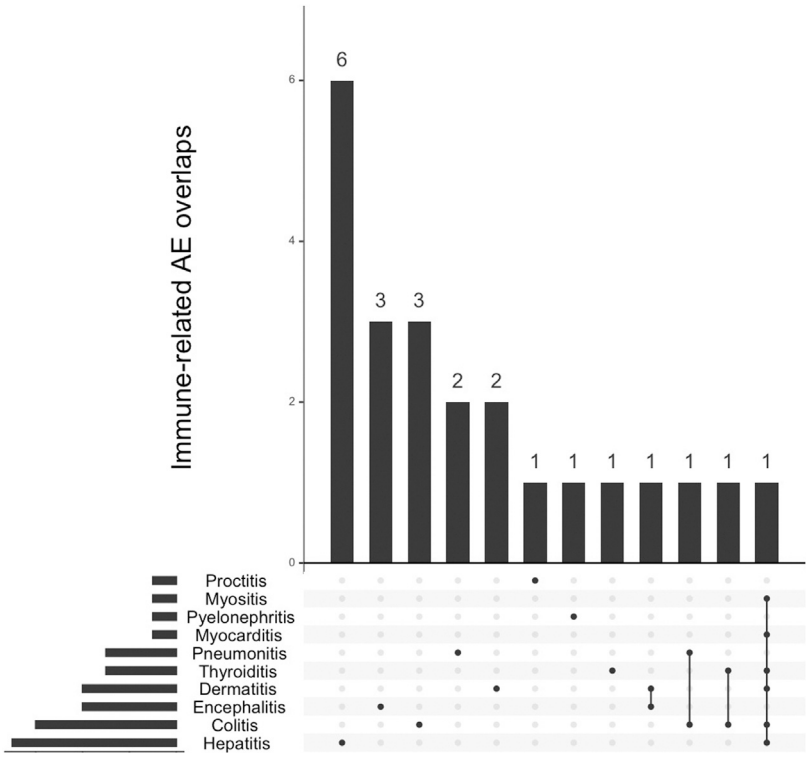

FIGURE 2 | Overlap between concurrent immune-related adverse events (IrAE) with graft vs. host disease events associated with immune-checkpoint inhibitors (n = 23).

in VigiBase® that we accounted for). In VigiBase®, disproportionality analysis yielded significant association between GVHD events and ICI molecules, with an IC = 2.0 and IC_{025} = 1.7 (see **Figure 1**). The first reported case was with ipilimumab in 2014. The most represented drug-ADR association was nivolumab with GVHD (n = 33, IC = 2.4, IC_{025} = 1.9). The most common ICI indications reported were Hodgkin disease in 47/81, 58.0% and non-Hodgkin lymphoma in 14/81, 17.3%. ICI were prescribed in monotherapy, in 88/93, 94.6% (as compared to combination therapy involving several ICI). There was no relevant information regarding GVHD grade in VigiBase®.

In the aggregated dataset of 93 cases, men represented 42/68, 61.8% of cases (**Table 1**). Median age was 38 [interquartile range (IQR) = 27; 50] years. Events occurred after a median of 1 [IQR = 1; 5.5] ICI injection. Median time to onset of GVHD after first ICI injection was 35 [IQR = 14; 176] days (data available in 19/93, 20.4%).

Overall mortality associated with ICI-associated GVHD was 24/93, 25.8%. This mortality was that reported in VigiBase® and implies a very short follow-up after the episode (less than a month). Therapy regimen (combination vs. monotherapy) did not affect subsequent mortality (respectively, 22/88, 25.0% vs. 2/5, 40.0%, p = 0.60). Mortality was not affected by the type of molecule (p = 0.41). Immune-related adverse events were co-reported in 23/93, 24.7%, mostly with hepatitis in 6/23, 26.1%.

In case reports extracted from literature (Singh et al., 2016; Boekstegers et al., 2017; Braun et al., 2017; Haverkos et al., 2017; Klobuch et al., 2017; Onizuka et al., 2017; Herbaux et al., 2018; Charles et al., 2019; Kim et al., 2019; Minson et al., 2019), when mentioned, ICI was indicated for disease relapse after allo-HSCT in 17/22, 77.3%. In one case of nivolumab-associated GVHD, biopsy showed PD-L1 expression in skin, liver and muscular tissues (those with active GVHD) (Charles et al., 2019).

Finally, we observed 11/18, 61.1% of patients had prior history of GVHD before ICI administration, although this information was lacking in 75/93, 80.6% of reports.

DISCUSSION

In this work, we analyzed GVHD related to ICI, by combining a systematic disproportionality analysis relying on the WHO pharmacovigilance database, VigiBase®; and case-reports drawn from literature, amounting a total of 93 cases to further describe risk factors of GVHD and prognosis.

In the cases we reviewed, ICI were mostly indicated for disease relapse after allo-HSCT, aiming at restauration of T-cell function and appropriate graft-versus-tumor effect (Godfrey et al., 2017). Whether previous GVHD is an additive or synergistic risk factor of GVHD remains to be explored, however better characterization of GVHD events may benefit from adding any prior history of GVHD, which remains the risk factor most associated with this event. Previous reports confirmed that history of GVHD was a risk factor, even for GVHD

TABLE 1 | Reports of graft vs. host disease (GVHD) associated with immune checkpoint inhibitors (ICI) aggregated from VigiBase® (thru January 05, 2020) and systematic literature search (thru February 2020).

	n/N (%)	Data available, n (%)
Reporting region		All
United States	50/93 (53.8)	
Europe	23/93 (24.7)	
Australia	7/93 (7.5)	
Asia	13/93 (14.0)	
Reporting year		All
2019	32/93 (34.4)	
2018	19/93 (20.4)	
2017	29/93 (31.2)	
2016	9/93 (9.7)	
2015	3/93 (3.2)	
2014	1/93 (1.1)	
Reporters		89/93 (95.7)
Healthcare professional	82/89 (92.1)	
Non-healthcare professional	7/89 (7.9)	
Reports in the course of clinical studies	11/93 (11.8%)	All
Sex		68/93 (73.1)
Men	42/68 (61.8)	
Women	26/68 (38.2)	
Age at onset, mean ± SD, years	39.03 ± 16.25	61/93 (65.6)
(min-max)	(3–74)	
Death	24/93 (25.8%)	All
Suspected drugs		All
Only ICI	69/93 (74.2)	
ICI +1 other drug	11/93 (11.8)	
ICI + ≥2 other drugs	13/93 (14.0)	
Drugs		All
Monotherapy with anti PD-1/PD-L1		
Nivolumab	53/93 (57.0)	
Pembrolizumab	23/93 (24.7)	
Monotherapy with anti CTLA-4		
Ipilimumab	12/93 (12.9)	
Combination therapy		
Nivolumab + Ipilimumab	5/93 (5.4)	
Indications for ICI		81/93 (87.1)
Hodgkin disease	47/81 (58.0%)	
Non-hodgkin lymphoma	14/81 (17.3%)	
Other	20/81 (24.7%)	
Timing of ICI administration		22/93 (23.7)
Before allo-HCST	5/22 (22.7)	
After allo-HCST	17/22 (77.3)	
Time between first dose of ICI and GVHD onset, days:		19/93 (20.4)
Median; [IQR]	35 [14–176]	
(min-max)	(3–240)	
Type of graft		17/93 (18.3)
Matched related donor	12/17 (70.6)	
Matched unrelated donor	4/17 (23.5)	
Cord blood	1/17 (5.9)	
Previous GVHD		18/93 (19.4)
Yes	11/18 (61.1)	
No	7/18 (38.9)	

allo-HSCT: allogenic hematopoietic cell transplantation; IQR: interquartile range; PD-1: programmed cell death protein 1; PD-L1: Programmed death-ligand 1; CTLA4: cytotoxic T-lymphocyte-associated protein 4.

related to ICI after allo-HSCT (12/17, 70.6% vs. 5/17, 29.4%) (Haverkos et al., 2017).

While VigiBase® cannot be used to compute true incidence of adverse serious events, in this worldwide pharmacovigilance database analysis, we confirmed a significant association between GVHD and ICI administration. Time to onset was 35 days after first treatment, however, residual effects of ICI, due to a long half-life and extended pharmacodynamic effects, even substantially beyond its administration prior to allo-HSCT (Geraud et al., 2021). Indeed, expression of PD-1 on T cells was found significantly decreased up to 6 months after allo-HSCT, despite a last dose of ICI more than a month prior (Merryman

et al., 2017). In patients not treated by ICI, PD-1 expression after allo-HSCT was also associated with increased risk of mortality (Schade et al., 2016).

Literature search yielded in one case of nivolumab-associated GVHD, a PD-L1 expression in skin, liver and muscular tissues (those with active GVHD) (Charles et al., 2019). As of yet, PD-L1 expression has not been described in regular GVHD reports (Ramachandran et al., 2019). Crosstalk between PD-1/PD-L1 and CTLA4 may play an important role in GVHD mechanisms. Indeed, while ICI may induce GVHD, reversion by abatacept was described (Nahas et al., 2018).

Interestingly, we found similar results as those found in the largest dataset to date, of GVHD due to ICI, aggregating 283 cases from several studies albeit with a reported overall mortality after GVHD of 11%, lower than that observed in our study. There were 107 cases occurring prior to allo-HSCT, and 176 after, akin to the results we observed in our dataset (Ijaz et al., 2019).

Our findings support the fact that ICI use in patients with allo-HSCT needs to be carefully monitored, as these patients are at high risk of developing GVHD. Regarding event prediction, risk factors of developing immune-related adverse events are currently under investigation (Shankar et al., 2020), and biomarkers involved in the mTOR pathway have been suggested (Esfahani et al., 2019).

We acknowledge several limitations to the present work. First, retrospective pharmacovigilance analyses present inherent intrinsic limitations; while disproportionality reporting used here (IC_{025}) have been demonstrated to accurately to identify signals of cardiovascular adverse events associated with anti-cancer drugs in various settings (Salem et al., 2018; Salem et al., 2019b), risk of false signals remain possible. Reporting and publication bias need to be addressed: in most cases, details on patients such as previous history of GVHD were not mentioned in VigiBase® making it hard to assess its weight into the added risk of developing GVHD after ICI administration. Similarly, indications for ICI treatment or previous HSCT were not always clearly stated, making it hard to categorize these events. Cotreatments used to treat lymphoma relapse were not always stated, which makes the assertion of cumulative risk of GVHD difficult, as previous other treatments used in these indications may also be associated with GVHD, such as donor lymphocytes infusions. Finally, these analyses do not allow the computation of true incidence, as by essence, disproportionality analyses report relative incidences. True incidence would require a denominator which would include all worldwide prescriptions of ICI in the study period. Yet, our observations confirm several cohort studies and we hope these findings may help oncologists, hematologists and pharmacovigilance specialists to better interact in order to improve reporting and management of GVHD related to ICI intake.

CONCLUSION

This worldwide pharmacovigilance database analysis confirmed a significant association between GVHD and ICI.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: http://www.vigiaccess.org.

AUTHOR CONTRIBUTIONS

LN and LR equally contributed to the manuscript. They wrote the draft, performed analyses, reviewed literature. BL-V provided VigiBase data and provided critical review to the manuscript. J-ES supervised the project, performed analyses and provided critical review to the manuscript.

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Disclaimer: The supplied data from VigiBase 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 WHO. The authors have nothing to disclose related to this study. The data, analytic methods, and study materials are available to other researchers for purposes of reproducing the results or replicating the procedure at http://www.vigiaccess.org/.

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frontiers in Pharmacology

ORIGINAL RESEARCH
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Vancomycin Associated Acute Kidney Injury: A Longitudinal Study in China

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Background: Vancomycin-associated acute kidney injury (VA-AKI) is a recognizable condition with known risk factors. However, the use of vancomycin in clinical practices in China is distinct from other countries. We conducted this longitudinal study to show the characteristics of VA-AKI and how to manage it in clinical practice.

Patients and Methods: We included patients admitted to hospital, who received vancomycin therapy between January 1, 2016 and June 2019. VA-AKI was defined as a patient having developed AKI during vancomycin therapy or within 48 h following the withdrawal of vancomycin therapy.

Results: A total of 3719 patients from 7058 possible participants were included in the study. 998 patients were excluded because of lacking of serum creatinine measurement. The incidence of VA-AKI was 14.3%. Only 32.3% (963/2990) of recommended patients performed therapeutic drug monitoring of vancomycin. Patients with VA-AKI were more likely to concomitant administration of cephalosporin (OR 1.55, 95% CI 1.08–2.21, p = 0.017), carbapenems (OR 1.46, 95% CI 1.11–1.91, p = 0.006) and piperacillin-tazobactam (OR 3.12, 95% CI 1.50–6.49, p = 0.002). Full renal recovery (OR 0.208, p = 0.005) was independent protective factors for mortality. Compared with acute kidney injury stage 1, AKI stage 2 (OR 2.174, p = 0.005) and AKI stage 3 (OR 2.210, p = 0.005) were independent risk factors for fail to full renal recovery.

Conclusion: Lack of a serum creatinine measurement for the diagnosis of AKI and lack of standardization of vancomycin therapeutic drug monitoring should be improved. Patient concomitant with piperacillin-tazobactam are at higher risk. Full renal recovery was associated with a significantly reduced morality.

Keywords: vancomycin, acute kidney injury, risk factors, renal recovery, morality

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INTRODUCTION

Currently, vancomycin is the first-line treatment for methicillin-resistant *Staphylococcus aureus* (MRSA) infections. However, it has also been associated with significant acute kidney injury (AKI) (Chen et al., 2011; Liu et al., 2011), which is a common disorder with a high risk of mortality, the development of chronic kidney disease, and substantial medical expense (Yang et al., 2015). There is considerable variation in the incidence of reported vancomycin-associated AKI (VA-AKI), which ranges from 5 to 43% (van Hal et al., 2013). There are numerous potential risk factors for VA-AKI including race, obesity, vancomycin exposure, pre-existing kidney disease, severity of illness,

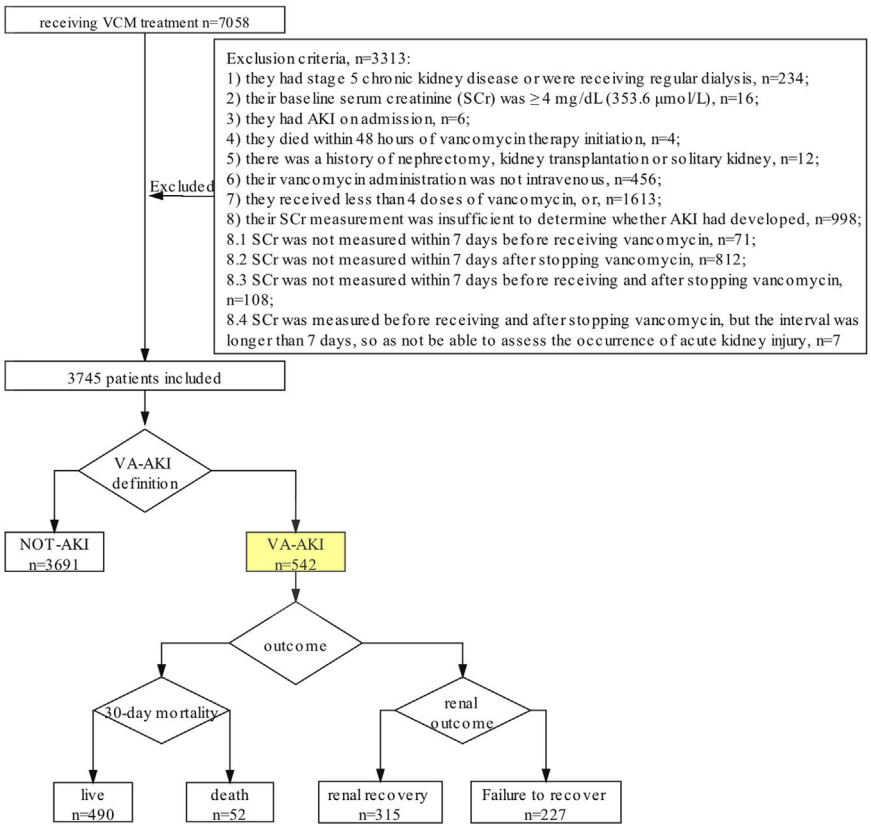

FIGURE 1 | Study Design.

concurrent nephrotoxin exposure, concurrent piperacillin-tazobactam use, etc. (Filippone et al., 2017). However, due to variations in study populations and sample sizes, different studies have identified conflicting risk factors. Several studies have shown that specific races (e.g., African-Americans) have a higher risk for VA-AKI (Bosso et al., 2011; Womble et al., 2019); although, studies specifically investigating Asian populations are lacking. For patients developed VA-AKI, how to reduce mortality and improve renal recovery is still a difficult problem to be explored.

We previously have reported that current literature on VA-AKI mainly came from American hospitals (Pan et al., 2019). However, the clinical use of vancomycin in China is distinct from other countries. For instance, vancomycin and piperacillin-tazobactam are among the most commonly prescribed antibiotics in American hospitals, which are associated with significant increases in the incidence of AKI compared to vancomycin monotherapy or other empirical combinations (Balci et al., 2018; Carreno et al., 2018; Ide et al., 2019; Avedissian et al., 2020; Ciarambino et al., 2020). In contrast, previous studies have shown that, in China, the most common antibiotic combinations with vancomycin are carbapenems (Pan et al., 2017; Pan et al., 2018). Liang et al. found that vancomycin nephrotoxicity was significantly correlated with the trough concentration and reported the first cut-point as 13 mg/L for the Chinese population (Liang et al., 2018). This was in contrast to trough concentrations exceeding 15 mg/L cited in American guidelines (Rybak et al., 2009; Ye et al., 2016). (Yang et al., 2015). found that, in China, a higher proportion of nephrotoxic drug exposure (71.6%) occurred before or while AKI develops as opposed to what has been reported by developed countries (20–50%) (Yang et al., 2015). Therefore, we designed this cohort study to include large sample patients, who are widely distributed and included a comprehensive number of risk factors. We believe that data from China, the most populous country in Asia, and the world's largest developing nation, will provide valuable information for assessing the burden of VA-AKI in this population, as well as describe its clinical characteristics, show how to recognize and manage VA-AKI in clinical practice.

METHODS

Study Design and Patient Population

This was a retrospective observational cohort study performed at Zhongshan Hospital Fudan University, a comprehensive, 2005-bed teaching hospital. The survey of VA-AKI was designed to include three steps (**Figure 1**). First, all adult inpatients treated with vancomycin from January 2016 to June 2019 were evaluated for study inclusion. Patients were excluded if 1) they had stage 5 chronic kidney disease or were receiving regular dialysis; 2) their

baseline serum creatinine (SCr) was ≥4 mg/dL (353.6 μmol/L); 3) they had AKI on admission; 4) they died within 48 h of vancomycin therapy initiation; 5) there was a history of nephrectomy, kidney transplantation or solitary kidney; 6) their vancomycin administration was not intravenous; 7) they received less than four doses of vancomycin, or; 8) their SCr measurement was insufficient to determine whether AKI had developed.

Second, we recorded the SCr of the included patients and separated the patients into two groups: the NOT-AKI group and the VA-AKI group. We used the 2012 Kidney Disease:Improving Global Outcomes (KDIGO) definition of AKI as the primary screening criterion, e.g., an increase in SCr by ≥ 0.3 mg/dL (≥26.5 μmol/L) within 48 h or an increase in SCr to ≥1.5 times baseline, which was known or presumed to have occurred within the prior 7 days (KDIGO Acute Kidney Injury Work Group, 2012). VA-AKI was defined as a patient having developed AKI during vancomycin therapy or within 48 h following the withdrawal of vancomycin therapy.

Third, for patients who developed AKI, we further analyzed the severity and outcome of the condition. Severity was assessed based on the highest AKI stage (1, 2, or 3) according to the KDIGO criterion. VA-AKI outcomes for the study included length of hospital stay (LOS), renal recovery, and 30-day mortality rates. Renal recovery was categorized into three levels: full recovery, partial recovery and failure to recover. We defined renal recovery at discharge as full recovery with SCr decreased to the baseline. We defined partial recovery as SCr decreased by 25% or more from peak concentration but remaining higher than baseline. We defined failure to recover as patient still dependent on dialysis or SCr decreased by less than 25% from peak concentration until discharged.

Data Collection

Data was extracted from the hospital's electronic database. A researcher uninvolved in the study anonymized patient information. The following variables were collected: demographic information, concomitant underlying diseases, severity of disease, vancomycin exposure, vancomycin variety (Wenkexin vs. Laikexin; trade name: Wenkexin, generic name: Vancomycin Hydrochloride for Injection, manufacturer: VIANEX S.A. (PLANT C), Greece, specification: 500 mg/bottle; and, trade name: Laikexin, generic name: Vancomycin Hydrochloride for Injection, manufacturers: Zhejiang Medicine Co., Ltd. Xinchang Pharmaceutical Factory, China, specification: 500 mg/bottle), therapeutic drug monitoring (TDM) rates, and concomitant nephrotoxic drugs. We also collected data on economic factors and patient outcomes including renal recovery, LOS, and 30- and 90-day mortality rates (**Supplementary Table S1**).

Data Analysis

Variables were assessed for normality using the Kolmogorov-Smirnov test. Based on these tests, quantitative variables are presented as means and standard deviations (SDs) or medians and interquartile ranges (IQRs). Variables were then compared between groups using independent t-tests or rank-sum tests. Qualitative variables are presented as frequencies and corresponding percentages and were compared using chi-squared or Fisher's exact tests.

A multivariate logistic regression analysis was used to assess independent risk factors for VA-AKI occurrence, full renal recovery and mortality. All potential risk factors with a p value ≤ 0.05 in the univariate analysis were used in the multiple logistic regression analysis (**Supplementary Table S2**). A backwards conditional approach was used to enter new terms into the logistic regression. The good of fit was evaluated by the analysis of Hosmer and Lemeshow. All p values were two-sided, and a p value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using SPSS statistics version 26.0 (IBM Inc., Armonk, NY, United States).

RESULTS

There were 7058 patients evaluated for study inclusion. After applying the exclusion criteria, 3339 (47.3%) patients were omitted from the study. Of those excluded, 998 patients lacked a SCr measurement, typically within 7 days after receiving vancomycin therapy (**Figure 1**). A total of 3719 patients were included for analysis. Of these, 66.3% were male and 33.7% were female. The median age was 60 years (IQR, 48.0–68.0).

The incidence of VA-AKI was 14.3% (532/3719) and occurred after 3.0 (IQR, 1.0–7.0) days of treatment. During vancomycin therapy, 86.2% of the patients received at least one nephrotoxic drug. The percentage of patients who received nephrotoxic drugs in combination with vancomycin was 36.8% for one drug, 32.8% for two drugs and 12.5% for three drugs (**Supplementary Table S3**). The ROC curve analysis indicated that a limit of 1.5 combined nephrotoxic agents was the optimal cut-off value for defining VA-AKI high-risk individuals (**Supplementary Figure S1**). The most common antibiotic used in combination therapy was carbapenem (58.7%, 2186/3719), while the rate of piperacillin-tazobactam use was 1.6% (62/3719).

Regional Distribution of the Patients Included

Patients included in the study came from 219 (65.6%, 219/334) municipal boroughs in 30 (88.2%, 30/34) provincial-level administrative regions in China. There are 34 provincial-level administrative regions includes 23 provinces, five autonomous regions, four municipalities and two special administrative regions in China (China Government Network, 2005) (See **Supplementary Table S4** for details).

Therapeutic Drug Monitoring of Patients

According to the vancomycin TDM guidelines issued by the Chinese Pharmacological Society (Ye et al., 2016), 3524 patients were recommended to receive TDM. However, only 1051 (29.8%) patients received it. Monitoring was initiated before the fourth or fifth vancomycin administration in 21.5% patients. A steady state valley concentration between 10 and 20 μmol/L occurred in 42.6% patients, while 27.6% had a concentration greater than

TABLE 1 | Demographic information and clinical characteristics of patients with and without VA-AKI.

Demographic information	Patients without VA-AKI N = 3187	Patients with VA-AKI N = 532	*p* value
Gender (male)	2094 (65.7)	372 (69.9)	0.057
Age			0.713
< 60 (years)	1579 (49.6)	259 (48.7)	
≥60 (years)	1608 (50.5)	273 (51.3)	
Body mass index			0.034
< 30 (kg/m^2)	2617 (82.1)	417 (78.4)	
BMI ≥ 30 (kg/m^2)	92 (2.9)	24 (4.5)	
Concomitant underlying diseases			
Chronic kidney diseases	34 (1.1)	34 (6.4)	<0.001
Chronic hepatic insufficiency	98 (3.1)	43 (8.1)	<0.001
Hypertension	645 (20.2)	127 (23.9)	0.056
Coronary heart disease	322 (10.1)	60 (11.3)	0.409
Heart failure	29 (0.9)	14 (2.6)	0.001
Atrial fibrillation	302 (9.5)	60 (11.3)	0.194
Valvular heart disease	1160 (36.4)	222 (41.7)	0.018
Chronic obstructive pulmonary disease	52 (1.6)	5 (0.9)	0.229
Diabetes	315 (9.9)	53 (10.0)	0.955
Cancer	767 (24.1)	83 (15.6)	<0.001
Anaemia	111 (3.5)	14 (2.6)	0.313
Severity of illness			
Admission to the ICU	1194 (37.5)	282 (53.0)	<0.001
Shock or concomitant vasopressors	345 (10.8)	149 (28.0)	<0.001
Trauma	7 (0.2)	1 (0.2)	1.000[a]
Cardiac surgery	1339 (42.0)	276 (51.9)	<0.001
Major non-cardiac surgery	279 (8.8)	49 (9.2)	0.731
Sepsis	1135 (35.6)	208 (39.1)	0.121

[a]*The calibration of the chi-square test. F refers to Fisher's exact test.*

Data are described as mean (SD), n (%), or median (IQR); VA-AKI, vancomycin-associated kidney injury; ICU, intensive care unit.

20 μmol/L. The highest monitoring rates occurred in patients with hepatic insufficiency (48.2%) and renal insufficiency (45.5%) (**Supplementary Table S5**).

Comparison of Risk Factors Between Patients with and Without VA-AKI

Table 1 displays patient demographic information, concomitant underlying diseases, and severity of illness. **Table 2** lists patient vancomycin exposure and concomitant nephrotoxic drugs. The multivariable logistic regression of factors for development of VA-AKI can be seen in **Table 3**. Patients with VA-AKI were more likely to concomitant with BMI ≥ 30 kg/m^2 (OR 1.64, 95% CI 1.00–2.69, $p = 0.05$) than those without VA-AKI. There was no significant difference in age or sex between the two groups.

More patients in VA-AKI group had concomitant chronic kidney disease (OR 5.49, 95% CI 2.82–10.68, $p < 0.001$) or chronic hepatic insufficiency (OR 2.42, 95% CI 1.45–4.04, $p = 0.001$) and were more likely to have concomitant heart failure (2.6 vs. 0.9%, $p = 0.001$) and valvular heart disease (41.7% vs. 36.4%, $p = 0.018$), but less likely to have cancer (15.6 vs. 24.1%, $p < 0.001$). Patients in VA-AKI group were also more likely to be admitted to the ICU (OR 1.44, 95% CI 1.15–1.80, $p = 0.001$), to experience shock or be given concomitant vasopressors (OR 2.35, 95% CI 1.80–3.05, $p < 0.001$) and undergo cardiac surgery (OR 1.43, 95% CI 1.11–1.84, $p = 0.005$).

Patients with VA-AKI received more Wen Kexin (vs. Lai Kexin) (OR 1.76, 95% CI 1.37–2.25, $p < 0.001$), compared with those without VA-AKI. In addition, patients in the VA-AKI group underwent a longer therapy course. Exposure to loop diuretics (5.5 vs. 2.2%, $p < 0.001$), tacrolimus (OR 2.92, 95% CI 1.63–5.22, $p < 0.001$), and radio-contrast agents (OR 2.51, 95% CI 1.55–4.07, $p < 0.001$) were also more frequent in the VA-AKI group. Furthermore, patients with VA-AKI were more likely to concomitant with concomitant administration of cephalosporin (OR 1.55, 95% CI 1.08–2.21, $p = 0.017$), carbapenems (OR 1.46, 95% CI 1.11–1.91, $p = 0.006$) and piperacillin-tazobactam (OR 3.12, 95% CI 1.50–6.49, $p = 0.002$).

Comparison of Medical Costs and Outcomes for Patients with and Without VA-AKI

Patients with VA-AKI were more likely to have higher medication costs (6.1 vs. 3.6 thousand US dollars, $p < 0.001$), treatment costs (0.7 vs. 0.4 thousand US dollars, $p < 0.001$) and total costs (19.2 vs. 12.7 thousand US dollars, $p < 0.001$). Patients in the VA-AKI group also had longer hospital stays (23 vs. 20 days, $p < 0.001$) and a higher 30-days mortality rate (8.8% vs. 1.5%, $p < 0.001$) (**Table 4**).

Severity and Outcomes of VA-AKI Patients

There were 343 VA-AKI patients (64.5%) with KDIGO stage 1 AKI. Thirty-eight patients (7.1%) received dialysis, and those with stage 3 VA-AKI experienced the highest dialysis rate (29.2%).

The 30-day mortality rate of the VA-AKI patients was 8.8%, and 29.8% (14/47) of patients had SCr within the normal range

TABLE 2 | Vancomycin exposure and concomitant nephrotoxic drugs of patients with and without VA-AKI.

	Patients without VA-AKI N = 3187	Patients with VA-AKI N = 532	*p* value
Vancomycin exposure			
Vancomycin varieties			<0.001
Wen Kexin	1937 (60.8)	409 (76.9)	
Lai Kexin	1250 (39.2)	123 (23.1)	
Length of vancomycin therapy			<0.001
<7 days	1732 (54.3)	224 (42.1)	
≥7 days and <14 days	1038 (32.6)	182 (34.2)	
≥14 days	417 (13.1)	126 (23.7)	
Dose			0.055[a]
<4 g/d	3186 (100.0)	530 (99.6)	
≥4 g/d	1 (0.03)	2 (0.4)	
Concomitant nephrotoxic drugs			
Aminoglycoside antibiotics	19 (0.6)	2 (0.4)	0.530
Antiviral drugs	103 (3.2)	22 (4.1)	0.284
Rifampin	39 (1.2)	14 (2.6)	0.011
Quinolone antibiotics	62 (2.0)	11 (2.1)	0.851
Sulfonamides	42 (1.3)	11 (2.1)	0.177
β- Lactam antibiotics			<0.001
Vancomycin monotherapy	1001 (31.4)	108 (20.3)	
Cephalosporin	462 (14.5)	98 (18.4)	
Carbapenems	1678 (52.7)	310 (58.3)	
Piperacillin-tazobactam	46 (1.4)	16 (3.0)	
Loop diuretic	1400 (43.9)	294 (55.3)	<0.001
Cyclosporine A	15 (0.5)	3 (0.6)	1.000[b]
Tacrolimus	35 (1.1)	34 (6.4)	<0.001
Chemotherapy	9 (0.3)	1 (0.2)	1.000[b]
Radiocontrast agents	70 (2.2)	29 (5.5)	<0.001
Reninangiotensin system blockers	495 (15.5)	37 (7.0)	0.443
NSAIDs	121 (3.8)	17 (3.2)	0.497

[a]*Fisher's exact test.*

[b]*The calibration of the chi-square test.*

Data are described as mean (SD), n (%), or median (IQR). NSAIDs = Non-steroidal anti-inflammatory drugs. The number of concomitant amphotericin B or traditional Chinese medicine was zero. ICU, intensive care unit; VA-AKI, vancomycin-associated kidney injury.

(44–115 μmol L^{-1}) at the time of death. For patients with stage 3 AKI the mortality was 16.9%.

58.6% (312/542) of VA-AKI patients have a renal recovery (full recovery or partial recovery), of which 40.2% (218/542) patients fully recovered. The median time to renal recovery is 4.1 (IQR = 5.0) days after VA-AKI occur. Patients with stage 1 AKI had the highest renal recovery rate (46.9%) (**Table 5**).

Risk Factors for Mortality of VA-AKI Patients

Multiple logistic regression analysis revealed that gender (male) (OR 3.053, $p = 0.035$) and age (≥60 years) (OR 3.13, $p = 0.007$) were independent risk factors for mortality. Compared with AKI stage 1, AKI stage 3 (OR 3.352, $p = 0.007$) was an independent risk factor for mortality. Full renal recovery (OR 0.208, $p = 0.005$) and admission to the ICU (OR 0.414, $p = 0.034$) were independent protective factors for mortality (**Table 6**).

Risk Factors for Fail to Full Renal Recovery of VA-AKI Patients

Multiple logistic regression analysis revealed that cancer (OR 2.447, $p = 0.004$) was an independent risk factor for fail to full renal recovery. Compared with AKI stage 1, AKI stage 2 (OR 2.174, $p = 0.005$) and AKI stage 3(OR 2.210, $p = 0.005$) were independent risk factors for fail to full renal recovery. Admission to the ICU (OR 0.626, $p = 0.023$) and shock or concomitant vasopressors (OR 0.526, $p = 0.003$) were independent protective factors for fail to full renal recovery (**Table 7**).

DISCUSSION

This single-center cohort study, including 3719 patients from 30 of 34 provincial-level administrative regions in China, have investigated the burden and characteristics of VA-AKI in China. Our survey, with to our knowledge, the largest sample size so far and covering patients from different areas in China, further uncovered the risk factors for prognosis of VA-AKI patients.

Our results showed that the incidence of VA-AKI was 14.3%, however, this could be an underestimate as 998 patients were excluded due to insufficient SCr measurements, which is consistent with our previous research (Pan et al., 2018). Therefore, our results may have missed a number of VA-AKI patients.

Currently, TDM is an effective measure used to reduce VA-AKI. However, we found several issues with its use, including an

TABLE 3 | Multivariable logistic regression of factors for development of VA-AKI.

	B	S.E.	OR	95% CI. for OR		*p* value
				Lower	Upper	
Body mass index (≥30 kg/m^2)	0.50	0.25	1.64	1.00	2.69	0.05
Chronic kidney diseases	1.70	0.34	5.49	2.82	10.68	< 0.001
Chronic hepatic insufficiency	0.89	0.26	2.42	1.45	4.04	0.001
Admission to the ICU	0.37	0.11	1.44	1.15	1.80	0.001
Circulatory shock or vasopressors	0.85	0.13	2.35	1.80	3.05	< 0.001
Cardiac surgery	0.36	0.13	1.43	1.11	1.84	0.005
Vancomycin varieties (Wen Kexin)	0.56	0.13	1.76	1.37	2.25	< 0.001
LOT < 7 days						<0.001
LOT ≥ 7 days and < 14 days	0.26	0.13	1.30	1.01	1.67	0.043
LOT ≥14 days	0.79	0.15	2.20	1.64	2.94	< 0.001
β-Lactam antibiotics (none)						0.003
β-Lactam antibiotics (Cephalosporin)	0.44	0.18	1.55	1.08	2.21	0.017
β-Lactam antibiotics (Carbapenems)	0.38	0.14	1.46	1.11	1.91	0.006
β-Lactam antibiotics (PTZ)	1.14	0.37	3.12	1.50	6.49	0.002
Tacrolimus	1.07	0.30	2.92	1.63	5.22	< 0.001
Radio-contrast agents	0.92	0.25	2.51	1.55	4.07	< 0.001
Constant	−2.91	0.16	0.06			< 0.001

LOT, Length of vancomycin therapy. ICU, intensive care unit. PTZ, Piperacillin and tazobactam. VA-AKI, vancomycin-associated kidney injury.

TABLE 4 | Medical costs and outcomes of patients with and without VA-AKI.

	Patients without	Patients with	*p* value
	VA-AKI N = 3187	VA-AKI N = 532	
Treatment costs (thousand US$)	0.4 (0.04)	0.7 (0.1)	<0.001
Consumables costs (thousand US$)	4.3 (0.9)	7.5 (1.1)	<0.001
Total costs (thousand US$)	12.7 (1.3)	19.2 (1.9)	<0.001
Length of hospital stay (day)	2.9 (0.2)	3.3 (0.3)	<0.001
30-day mortality	49 (1.5)	47 (8.8)	<0.001
90-day mortality	71 (2.2)	56 (10.5)	<0.001

Data are described as mean (SD), n (%), or median (IQR). b refers to the calibration of the chi-square test. F refers to Fisher's exact test; VA-AKI, vancomycin-associated kidney injury.

TABLE 5 | Outcomes of VA-AKI patients.

Patient outcomes	Total	Stage 1	Stage 2	Stage 3	*p* value
	N = 532	N = 343	N = 100	N = 89	
30-day mortality n (%)	47 (8.8)	25 (7.3)	7 (7.0)	15 (16.9)	0.014
Receive dialysis n (%)	38 (7.1)	8 (2.3)	4 (4.0)	26 (29.2)	<0.001
Renal recovery n (%)	312 (58.6)	211 (61.5)	56 (56.0)	45 (50.6)	<0.001
Full recovery n (%)	218 (41.0)	161 (46.9)	30 (30.0)	27 (30.3)	0.001
Partial recovery n (%)	94 (17.7)	50 (14.6)	26 (26.0)	18 (20.2)	<0.001
Failure to recover n (%)	220 (41.4)	132 (38.5)	44 (44.0)	44 (49.4)	<0.001

insufficient monitoring rate of the target population, inappropriate TDM start times, and an insufficient rate of achieving steady-state concentrations. The TDM guidelines for vancomycin was issued by the American Society of Health-System Pharmacists in 2009 (Rybak et al., 2009), updated in 2020 (Rybak et al., 2020), and issued by the Chinese Pharmacological Society in 2016 (Ye et al., 2016). However, a survey of vancomycin TDM involving 214 medical institutions in China revealed that vancomycin-monitoring technology, while adequately advanced, was not standardized for monitoring time or target populations in clinical practice (Zhou et al., 2019). This may be due to clinicians in China having high-work loads leading to time constraints and distractions (Jiang et al., 2019).

A complete diagnosis of AKI by SCr measurements and standardized vancomycin TDM is necessary for its management. The most current 2020 guidelines recommend using Bayesian-derived AUC monitoring rather than trough concentrations (Rybak et al., 2020). Several studies have shown that pharmacists who lead or participate in vancomycin medication management programs are conducive in improving the effective use of vancomycin and reducing the mean duration of vancomycin therapy and medical expenses (Willis and Jhaveri, 2018; Dadzie et al., 2019; Porter et al., 2019). Therefore, clinical pharmacists may be able to reduce both the workload of doctors and medical expenses (Willis and Jhaveri, 2018). Thus, hospital administrators should consider increasing their investment in clinical pharmacists to reduce the incidence of VA-AKI.

One distinct feature of this study was the high proportion of concomitant nephrotoxic medication use (86.2%) compared with the 28–71% reported in hospitals from the United States (Hidayat et al., 2006; Choi et al., 2017). Previous investigations have indicated that a combination of vancomycin and nephrotoxic agents is associated with nephrotoxicity (Castanheira et al., 2016; Yang, 2017). Uekl et al. showed that the number of combined nephrotoxic agents (OR, 1.590, p = 0.010) was significantly related to nephrotoxicity (Ueki et al., 2020). In accordance with these results, we also observed a significantly higher incidence of VA-AKI in patients given combined multiple nephrotoxic drugs, especially combinations of more than two drugs.

Another distinct finding of this Chinese VA-AKI study was the high proportion of the combined use of carbapenems (especially mipenem and meropenem) with vancomycin, rather than piperacillin-tazobactam (59.3 vs. 1.7%). Vancomycin and piperacillin-tazobactam are among the most commonly prescribed antibiotics in hospitals in the United States, and this particular combination of antibiotics may be empirically useful due to the broad Gram-positive activity of vancomycin and broad Gram-negative activity of piperacillin-tazobactam (Carreno et al., 2018). Both piperacillin-tazobactam and carbapenems have broad Gram-negative activity and are recommended in clinical practice guidelines in China (Ran et al., 2019; Wu and Ren, 2020). We speculate that one reason for more combinations with carbapenems is that piperacillin-tazobactam requires a skin test before administration in China, while carbapenem antibiotics do not. The concern is that

TABLE 6 | Multivariable logistic regression of factors for mortality of VA-AKI patients.

	B	S.E.	OR	95% CI. for OR		*p* value
				Lower	Upper	
Gender (male)	1.116	0.528	3.053	1.084	8.597	0.035
Age ≥60 (years) vs. <60 (years))	1.141	0.425	3.130	1.361	7.198	0.007
Admission to the ICU	−0.881	0.416	0.414	0.183	0.936	0.034
Cardiac surgery	−0.814	0.427	0.443	0.192	1.023	0.057
AKI Stage 1 (reference)						0.020
AKI Stage 2	0.079	0.562	1.082	0.359	3.258	0.888
AKI Stage 3	1.21	0.448	3.352	1.392	8.071	0.007
Full renal recovery (vs. fail to full renal recover)	−1.572	0.564	0.208	0.069	0.627	0.005
Constant	−3.115	0.652	0.044			0.000

ICU, intensive care unit; VA-AKI, Vancomycin-associated kidney injury.

TABLE 7 | Multivariable Logistic Regression of Factors for full renal recovery of VA-AKI patients.

	B	S.E.	OR	95% CI. For OR		*p* value
				Lower	Upper	
Cancer	0.895	0.311	2.447	1.331	4.499	0.004
Admission to the ICU	−0.469	0.206	0.626	0.417	0.938	0.023
Shock or concomitant vasopressors	−0.643	0.219	0.526	0.342	0.807	0.003
AKI Stage 1 (reference)						0.001
AKI Stage 2	0.777	0.278	2.174	1.260	3.749	0.005
AKI Stage 3	0.793	0.283	2.210	1.270	3.846	0.005
Constant	0.294	0.188	1.342			0.117

ICU, intensive care unit; VA-AKI, vancomycin-associated kidney injury.

penicillin-based antibiotics may cause severe allergic reactions such as anaphylactic shock (Yang, 2017). Therefore, the People's Republic of China Pharmacopoeia Clinical Medication Instructions require skin tests before using penicillin (Yang, 2017). Hence, carbapenem antibiotics are preferred as they are more convenient. Another possible reason for more combinations with carbapenems is the higher prevalence of extended-spectrum beta-lactamase (ESBL) in China, compared to United States. One study that collected 15,588 *Enterobacteriaceae* isolates from 63 hospitals in the United States from 2012 to 2014, found a prevalence of ESBL-producing strains of 13.6% for *Escherichia coli*, 17.4% for *Klebsiella pneumoniae*, 10.8% for *Klebsiella. oxytoca* and 5.7% for *Proteus mirabilis* (5.7%) (Castanheira et al., 2016). In contrast, in 2014 the China Antimicrobial Surveillance Network collected 78,955 Enterobacteriaceae isolates from 15 general hospitals and two children's hospitals and found that the prevalence of ESBL-producing strains was 55.3% for *E. coli*, 22.9% for *K. pneumoniae* and *K. oxytoca*, and 24.7% for *P. mirabilis* (Hu et al., 2016). Carbapenem antibiotics produce strong antibacterial activity against ESBL-producing strains and are currently the most effective and reliable antibacterial drugs for the treatment of various infections caused by ESBL-producing Enterobacteriaceae bacteria (Zhou et al., 2014).

The combination of vancomycin plus piperacillin-tazobactam increases the odds of inducing AKI, thus vancomycin plus carbapenems may contribute to a lower rate of VA-AKI in China (Balci et al., 2018; Carreno et al., 2018; Ide et al., 2019; Avedissian et al., 2020; Ciarambino et al., 2020). Our multiple regression analysis showed that both carbapenem and piperacillin-tazobactam antibiotics were independent risk factors for VA-AKI, and the OR value of piperacillin-tazobactam was higher than carbapenems (OR = 3.12 vs. OR = 1.46), which is consistent with previous reports (Ide et al., 2019). The potential mechanism underlying the enhanced toxicity of this combination remains uncertain (Gomes et al., 2014). has suggested that subclinical interstitial nephritis caused by piperacillin-tazobactam in combination with the oxidative stress of vancomycin might lead to increased renal injury (Gomes et al., 2014). (Burgess and Drew, 2014) has posited that piperacillin-tazobactam might reduce vancomycin clearance, resulting in increased exposure in the kidney and, hence, further injury (Burgess and Drew, 2014). Therefore, from the perspective of reducing VA-AKI, the combined use of carbapenem antibiotics, rather than piperacillin-tazobactam should be considered a better choice.

Our study had several strengths. First, the sample size was relatively large, and the population was geographically widely distributed. Second, in terms of nephrotoxic drugs, we included categories that were as comprehensive as possible. Third, we gathered data regarding associated medical costs, which has rarely been addressed in the literature. This study also had some limitations. Due to the retrospective design, we were only able to show associations between vancomycin and AKI and not causality. In addition, urine output was not assessed and this may have affected the rates of identified AKI. Furthermore, as trough levels were not drawn for every patient, we were unable to evaluate the potential effect of vancomycin concentration on the development of AKI, which is a well-known risk factor for nephrotoxicity.

VA-AKI is associated with a higher medical expenses and risk of mortality. We carried out this longitudinal study to further analyze the factors that affect the prognosis of patients with VA-AKI, which was rarely involved in previous studies. Our research shows that full renal recovery is an independent protective factor for mortality. Approximately 70% of patients died with impaired renal function, and we speculate that the deaths of these patients may be related to AKI. Compared with unchangeable factors such as gender and age, renal recovery is a risk factor that can be improved, so it is

the focus of efforts to reduce the mortality of patients. Only 41.2% of the patients with VA-AKI recovered renal function during hospitalization, which is lower than the 58–81% reported in developed countries (Pritchard et al., 2010; Lacave et al., 2017). Once the patient develops AKI, we recommend prompt and active treatment. Admission to ICU helps improve the patient's full renal recovery reduce mortality. We speculate that patients in the ICU can receive more comprehensive monitoring and timely treatment. Higher AKI stages are independent risk factors of failure to full renal recovery and mortality, which is consistent with previous studies (Forni et al., 2017). In conclusion, it may be necessary to suspend vancomycin or adjust the dosage in a timely manner for the renal recovery (Rybak et al., 2020), especially for patients with high KDIGO AKI stages.

CONCLUSION

Lack of a serum creatinine measurement for the diagnosis of AKI and lack of standardization of vancomycin therapeutic drug monitoring should be improved. Patient concomitant with piperacillin-tazobactam are at higher risk. Full renal recovery was associated with a significantly reduced morality.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Requests to access the datasets should be directed to the corresponding Author.

ETHICS STATEMENT

The study was approved by the Ethics Committee of Zhongshan Hospital of Fudan University (Approval No: B2019-194R). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

PK, DX, LX and LQ made contributions to the conception and design of the study. PM, CC and CZ acquired the data. PK, WW, XQ analyzed the data. PM drafted the article and LQ made contributions to revising it critically for important intellectual content. All authors contribute to final approval of the version to be submitted.

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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.2021.632107/full#supplementary-material.

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Lack of Awareness of the Impact of Improperly Disposed Of Medications and Associated Factors: A Cross-Sectional Survey in Indonesian Households

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Introduction: Disposal of unused medications through environmentally unsafe routes is common in Indonesia. The lack of awareness of the impact of improperly disposed of medications is a significant contributing factor. The objectives of this study were to identify factors associated with lack of awareness of the impact of improperly disposed of unused medications and to assess the associations of awareness with medication disposal practices among the general population in Indonesia.

Patients and methods: An observational cross-sectional survey was conducted using nonprobability sampling in Bandung, Indonesia, from November 2017 to January 2018 among respondents who were older than 18 years, had used any medication in the past, were literate, and had signed an informed consent document. Disposal practices and awareness regarding the impact of improperly disposed of unused medications were collected using an online- and a paper-based pre-validated questionnaire. The paper-based questionnaires were distributed to respondents in public places such as city center, markets, and religious places. Binary logistic regression was performed to assess associations of sociodemographic and other related factors with a lack of awareness. Odds ratios (ORs) with 95% confidence intervals (CIs) are reported.

Results: Of 497 participating respondents, 433 and 64 respondents filled an online- or a paper-based questionnaire, respectively. Most respondents were female, aged between 18 and 30 years, and students/university students. Of 497 respondents, more than half (53.1%) were not aware that improper medication disposal could harm the environment and population health. Most respondents (79.5%) had never received information about proper medication disposal practices. The education level, the number of stored medications at home, and previous education about medication disposal practices were significantly associated with awareness of proper practices. In the multivariate analysis, only those with previous education about medication disposal practices were less likely to report a lack of awareness (OR: 0.043; 95% CI: 0.02–0.09). Respondents with

a lack of awareness tended to dispose of their unused medications in the garbage or shared them with friends or relatives.

Conclusion: There is a clear need to increase awareness of the importance of proper medication disposal practices, in particular among the student population of Bandung city, Indonesia. Healthcare providers can play an important role by educating this specific population on the proper disposal of unused medications.

Keywords: unused medications, awareness, medication disposal practices, pharmaceutical waste, risk factors

INTRODUCTION

The prevalence of unused medications in general population households has substantially increased recently, which can lead to medication wastage (Makki et al., 2019). The prevalence of unused medications was 89.3% (N = 337) and 85.2% (N = 263) in Saudi Arabia and Ethiopia, respectively (Kassahun and Tesfaye, 2020; Wajid et al., 2020). Furthermore, 2 of 3 prescribed medications were reported unused in households in the United States (Law et al., 2015). Unused medications refer to medications that are deteriorated, discontinued, expired, or unintended for any future use because of adverse effects, nonadherence, alteration of dosage, or improved condition (Seehusen and Edwards, 2006; Ruhoy and Daughton, 2008; Law et al., 2015). In developed countries such as the United States, the most commonly reported unused medications were treatments for chronic conditions such as diabetes, hypertension, hyperlipidemia, heart disease, and antipsychotic agents (Law et al., 2015). Meanwhile, analgesics, antibiotics, and herbal medicines were the most reported unused medications in developing countries, such as Indonesia and Nigeria (Auta et al., 2011; Insani et al., 2020).

Improper disposal of unused medications has been reported as a global issue (Tong et al., 2011; Paut Kusturica et al., 2017). The reasons underlying improper disposal of unused medications are lack of policies for returning unused medications and lack of awareness of the impacts of improper medication disposal, such as higher healthcare costs and environmental harm (Wasserfallen et al., 2003; Tong et al., 2011; Bekker et al., 2019; Makki et al., 2019; Insani et al., 2020). For example, a study in the United States showed that improperly disposed of antibiotics may lead to drug-resistant bacteria in soil that can then infect humans (Ghosh and LaPara, 2007). Furthermore, previous studies in France and Pakistan showed adverse effects of improperly disposed of pharmaceutical waste in fish and vultures (Oaks et al., 2004; Sanchez et al., 2011). The environmental impact of improper medication disposal is expected to be higher in countries with poorly functioning waste management schemes such as the Middle Eastern, Asian, and African countries (Paut Kusturica et al., 2017). In a previous study among 497 respondents in Indonesia, most people disposed of unused medications in their household garbage (82.1%), whereas only less than 1% returned unused medications to pharmacies (Insani et al., 2020). Therefore, efforts to reduce improper medication disposal practices are urgently needed.

In 2015, the Indonesian government initiated a community empowerment program called the "Smart Use of Medication Movement" (*Gerakan Masyarakat Cerdas Menggunakan Obat*), which promoted the rational use of medications, including a proper disposal practice of unused medications (Hermansyah et al., 2020). This program encourages community pharmacists to provide general education on how to obtain, use, and store medications and dispose of unused medications. In particular, unused medication disposal should follow predefined regulations to prevent being retrieved or reused prior to destruction (Hermansyah et al., 2018). Nevertheless, nationwide implementation of this program is lacking.

The issue of medication disposal practices among the general population in Indonesia has not been studied comprehensively, despite its potential importance. To strengthen the pharmaceutical waste management program in Indonesia, insights are needed into the association between sociodemographic and other related factors and a lack of awareness of the impact of improperly disposed of unused medications. Therefore, the primary objective of this study was to identify factors associated with a lack of awareness of the impact of improperly disposed of medications among the general population in Bandung, Indonesia. The secondary objective was to assess the associations of awareness with medication disposal practices among this population.

PATIENTS AND METHODS

We reported our study according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for a cross-sectional study (Von Elm et al., 2007; **Supplementary Table S1**; **Supplementary data**).

Study Design, Setting, and Data Collection

An observational cross-sectional survey was conducted in Bandung, Indonesia, from November 2017 to January 2018. Bandung is the administrative capital of the West Java province and the third most populous city in Indonesia after Jakarta and Surabaya (Tarigan et al., 2016). Data were collected from respondents who met the following inclusion criteria: older than 18 years, used any medication in the past, literate, and signed an informed consent document; we excluded respondents with incomplete data. The Health Research Ethics Committee of Universitas Padjadjaran, Indonesia approved the study protocol (No. 1155/UN6. C.10/PN/2017).

TABLE 1 | Demographic characteristics of the respondents (N = 497).

Characteristic	N (%)
Gender	
Male	131 (26.4)
Female	366 (73.6)
Age, years	
18–30	424 (85.3)
31–40	19 (3.8)
41–49	38 (7.7)
50–59	16 (3.2)
Last education level	
Primary school	4 (0.8)
Junior high school	10 (2.0)
Senior high school	316 (63.6)
Diploma/bachelor's degree	150 (30.2)
Postgraduate degree	17 (3.4)
Occupation	
Students/university students	343 (69.0)
Employed	115 (23.1)
Unemployed	39 (7.8)
Income, Indonesian rupiah	
<1,000,000	229 (46.1)
1,000,000–3,000,000	180 (36.2)
3,000,000–5,000,000	43 (8.7)
>5,000,0000	45 (9.0)
Number of medications stored at home	
None	22 (4.4)
1–5	327 (65.8)
6–10	84 (16.9)
>10	64 (12.9)

We used a structured questionnaire in Bahasa Indonesia which was developed based on theoretical frameworks of behavior used in previous studies (West et al., 2016; Bashaar et al., 2017). We tested the questionnaire's validity and reliability with 20 respondents who were not familiar with the study prior to the actual data collection to assess the applicability and clarity of the questionnaire as well as to make necessary adjustments. These respondents were excluded in the main analysis. We made minor revisions to some of the wording and the final version of the questionnaire showed adequate validity (correlation of each question to the total score >0.349) and reliability (Cronbach's $\alpha = 0.854$).

Data were collected by either an online- or a paper-based questionnaire using nonprobability sampling. The online-based questionnaire was hosted online using Google form and the link was distributed through social media. Meanwhile, the paper-based questionnaires were distributed to respondents in public places such as city center, markets, and religious places. The questionnaire consisted of two sections: respondents' sociodemographic characteristics (age, gender, highest education level, occupation, and income) and information on unused medication disposal (e.g., what respondents did with unused medications, whether respondents have ever received information about proper medication disposal practices, the number of medications stored at home, and awareness regarding medication disposal practices) (Insani et al., 2020). Unused medications refer to deteriorated, discontinued, expired, and other medications unintended for future use (Yimenu et al., 2020). The term "medication" encompassed prescribed medications, over-the-counter medications, supplements, vitamins, and herbal medicines. We measured respondents' awareness regarding medication disposal practices by asking the following question: "Are you aware that improper medication disposal could harm the environment and population health? (yes/no)" (Bashaar et al., 2017).

Sample Size Calculation

We calculated the sample size using Slovin's formula (Almeda et al., 2010). To obtain a 95% confidence interval (CI) and a margin error of 0.05, a minimum sample size of 400 respondents was needed.

Data Analysis

We used descriptive statistics to report respondents' characteristics and performed chi-square tests to assess univariate associations of dichotomous or nominal factors with their lack of awareness. We included the potential factors that were associated with a lack of awareness in the univariate analyses at a significance of $p < 0.25$ in the initial multivariate models and tested for multicollinearity to check the correlations among the potential factors. Using manual backward elimination, we obtained odds ratios (ORs) with 95% CIs using binary logistic regression analysis. We checked the completeness, accuracy, and consistency of the collected data before entering the data into the statistical software, SPSS version 23.0 (IBM Corp. Armonk, NY, United States).

RESULTS

Baseline Characteristics

Of 497 participating respondents, 433 and 64 respondents filled an online- or a paper-based questionnaire, respectively. Most respondents were female, aged between 18 and 30 years, and students/university students (**Table 1**). More than half of respondents (53.1%) reported lacking awareness of proper medication disposal practices, and less than 21% of respondents had ever received information about proper medication disposal (**Table 1**). Among those who had received such information, the most common source had been public

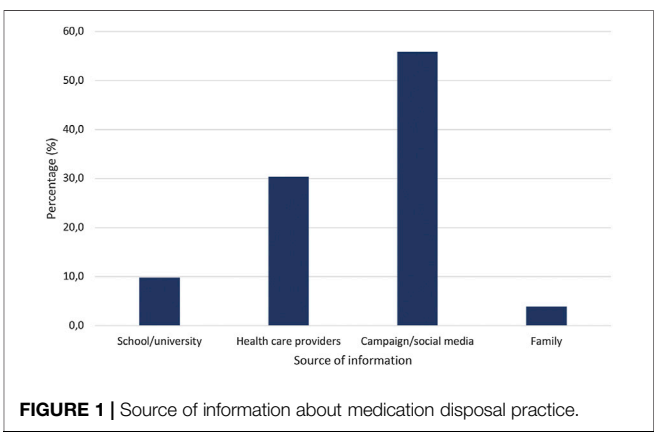

FIGURE 1 | Source of information about medication disposal practice.

TABLE 2 | Univariate association with lack of awareness of proper medication disposal practices (N = 497).

Factor	Aware that improper medication disposal could harm the environment and population health		
	Yes, N (%)	No, N (%)	*p*
Gender (N)			0.368
Male (131)	57 (24.5)	74 (28.0)	
Female (366)	176 (75.5)	190 (72.0)	
Age-group, years (N)			
18–30 (424)	192 (82.4)	232 (87.9)	0.351
31–40 (19)	11 (4.7)	8 (3.0)	
41–49 (38)	22 (9.4)	16 (6.1)	
50–59 (16)	8 (3.4)	8 (3.0)	
Highest education level (N)			0.053*
Primary school (4)	4 (1.7)	0	
Intermediate/secondary school (10)	5 (2.1)	5 (1.9)	
Senior high school (316)	140 (60.1)	176 (66.7)	
Diploma/bachelor's degree (150)	72 (30.9)	78 (29.5)	
Postgraduate degree (17)	12 (5.2)	5 (1.9)	
Occupation (N)			0.554
Students/university students (343)	156 (67.0)	187 (70.8)	
Employed (115)	56 (24.0)	59 (22.3)	
Unemployed (39)	21 (9.0)	18 (6.8)	
Income, Indonesian rupiah (N)		0.428	
<1,000,000 (229)	112 (48.1)	117 (44.3)	
1,000,000–3,000,000 (180)	76 (32.6)	104 (39.4)	
3,000,000–5,000,000 (43)	23 (9.9)	20 (7.6)	
>5,000,0000 (45)	22 (9.4)	23 (8.7)	
Have received information about medication disposal practices (N)			<0.001*
Yes (102)	94 (40.3)	8 (3.0)	
No (395)	139 (59.7)	256 (97.0)	
Number of medications stored at home (N)			0.187*
None (22)	14 (6.0)	8 (3.0)	
1–5 (327)	147 (63.1)	180 (68.2)	
6–10 (84)	37 (15.9)	47 (17.8)	
>10 (64)	35 (15.0)	29 (11.0)	

**Included into multivariate analysis.*

TABLE 3 | Factor associated with lack of awareness of proper medication disposal practices.

Factor[a]	Odds ratios[b] (95% CI)
Have received information about medication disposal practices	
Yes	0.043 (0.02–0.09)
No	References

[a]*Goodness of fit:* p = *0.362,* R-*squared, 23.6%.*
[b]*Final multivariate model.*

campaigns or social media (55.9%), followed by information from health care providers (30.4%; **Figure 1**).

Factors Associated With a Lack of Awareness of Proper Medication Disposal Practices

Results from the multicollinearity test showed that no factors correlated highly with each other (variance inflation factors >1). From the univariate analysis, education, the number of medications stored at home, and previous education about medication disposal practices were the potential factors that were associated with a lack of awareness (**Table 2**). In the final multivariate model, only those with previous education about medication disposal practices were less likely to report a lack of awareness (OR: 0.043; 95% CI: 0.02–0.09; **Table 3**). For the goodness of fit of the final multivariate model, *p* was 0.362 and *R*-squared was 23.6% (**Table 3**).

Associations Between Awareness and Medication Disposal Practices

We observed a significant association between the respondents' awareness of the impact of improperly disposed of medications and their actual disposal practices. Among respondents who were not aware that disposing of unused medications improperly could harm the environment and population health (N = 264), 56.6% disposed of their unused medications in their household garbage (**Table 4**). Similarly, respondents who were unaware of the harmful impacts of improper medication disposal shared their unused medications with friends or relatives (53.8%). In contrast, among the respondents who reported being aware of the negative impacts of improper medication disposal (N = 233), 76.9% disposed of their unused medications by flushing them down the toilet or the sink (**Table 4**).

TABLE 4 | Associations between awareness and actual unused medication disposal practices.

	Actual practices of disposal of unused medications						*p*
	Threw away in household garbage (N = 408)	Flushed down the toilet or sink (N = 26)	Burned the medications (N = 20)	Shared with friends and/or relatives (N = 286)	Returned it to pharmacy (N = 1)	Did not know (N = 40)	
Aware that improper medication disposal could harm the environment and population health							0.001
Yes (N = 233)	177 (43.4%)	20 (76.9%)	15 (75.5%)	132 (46.2%)	1 (100%)	18 (45.5%)	
No (N = 264)	231 (56.6%)	6 (23.1%)	5 (25.5%)	154 (53.8%)	0	22 (55.0%)	

DISCUSSION

More than half of the respondents in our study were not aware of the impacts of improper medication disposal on the environment and on population health. Respondents who had previous education on medication disposal practices were less likely to report a lack of awareness, and respondents with a lack of awareness tended to dispose of their unused medications in the garbage or shared them with friends or relatives.

In our study, despite the respondents' high education levels, they reported a lack of awareness of the impacts of improperly disposed of medications. We found a higher lack of awareness in our study than what previous researchers found in a Swedish population (Persson et al., 2009). Researchers in that previous study reported that information campaigns increased awareness among the Swedish population as indicated by the higher rate of returning medications to pharmacies and the lower rate of discarding them in the garbage (Persson et al., 2009). Our study findings support education as a critical means of raising awareness of the impact of improper medication disposal practices. Therefore, improving the public's medication disposal practices will require involving healthcare providers in educating the general population about good practices through campaigns and health promotions using various media.

We further observed that gaps exist in medication disposal awareness and practices. Although 46.9% of the respondents in our study were aware that improper medication disposal could harm the environment and population health, a significant number (76.9%) disposed of their unused medications by flushing them down the toilet or sink in their households. This might be because of a misunderstanding that wastewater treatment will remove most of the medications from the environment and ecosystem (Ong et al., 2020). However, it is instead the case that medications disposed of in the public sewage system can contaminate local water systems through groundwater, streams, lakes, and rivers (Jones et al., 2004). Previous studies have reported that proper medication disposal practices are still lacking even among respondents who were aware of the impacts of improper disposal (Sonowal et al., 2017; Ariffin and Zakili, 2019). However, it is still important to reduce the lack of awareness, which can be achieved by engaging respondents with appropriate and accessible information (Bond et al., 2012). In a larger context, the Indonesian government should introduce more nationwide programs such as awareness raising campaigns with various media to educate the general population. In the United States, social media, radio, and television have been used to increase population awareness of the proper disposal of unused opioid pain medications (U.S. Food and Drug Administration (FDA), 2020). Other study in the United States observed that pharmacists receiving the educational intervention were more likely to recommend proper methods of medication disposal during patients' counseling (Jarvis et al., 2009). Counseled patients were more likely to properly return unused medications to pharmacies and healthcare providers (Seehusen and Edwards, 2006). Moreover, previous studies in the United Kingdom and Sweden showed that information campaigns to increase awareness resulted in a higher rate of returning medications to pharmacies (O'Sullivan et al., 1996; Persson et al., 2009).

To our knowledge, this is the first thorough evaluation of factors associated with lack of public awareness of proper disposal of unused medications and the associations between the individuals' awareness and their actual medication disposal practices among Indonesian respondents. However, some limitations need to be mentioned. Awareness may overestimate the actual respondents' knowledge on unused medication disposal practices. In addition, we used a self-report questionnaire which could have introduced recall and social desirability bias and, in turn, led to overestimating respondents' awareness and their actual disposal practices. We could not verify the information we collected from the respondents, and direct observation such as through in-home inventory of unused medications would allow for objective assessments of unused medication disposal practices. However, this study approach was hindered by resource constraints and privacy concerns. Furthermore, we could not calculate the response rate due to lack of data regarding the number of potentially eligible respondents who refused to participate in this study. We also could not draw causal inferences regarding the temporal associations between previous education and lack of awareness, as well as between lack of awareness and actual medication disposal practices because of the cross-sectional design. The overall association of the final multivariate model was relatively low, suggesting that other unmeasured factors might have been associated with awareness of proper medication disposal practices, such as beliefs about good practices (Seehusen and Edwards, 2006). Furthermore, most of the respondents in our study were university students and thus might not be representative of the general population. Therefore, we advise caution in interpreting and extrapolating our results beyond Bandung city, Indonesia. Finally,

generalization of our results may be limited because this was a survey from one location in Indonesia.

Strengthening the current state of unused medication disposal practices in Indonesia will require an organized method for collecting unused medications from the general population and disposing of them properly through practical policies such as pharmacy-based take-back medication programs. Such programs were shown to improve medication disposal practices in developed countries such as the United States (Yang et al., 2015), Australia (Bettington et al., 2018), and the United Kingdom (Mackridge and Marriott, 2007). The availability of such program may alleviate medication misuse and environmental damage associated with improper disposal of unused medications. In addition, efforts should be ongoing to raise awareness through a wide variety of educational interventions to improve medication disposal practices in Indonesia. Healthcare providers could discuss proper medication disposal during counseling and distribute written materials with medications. Future research should focus on qualitative research to provide more in-depth guidance for developing such strategies. Furthermore, a better understanding of the knowledge and attitudes relating to medication disposal of different groups would give a more comprehensive overview of medication disposal practices. Such findings might support research on how to effectively educate the population on proper medication disposal practices.

CONCLUSION

Of the 497 respondents to our survey, half reported a lack of awareness of the impacts of improper medication disposal practices. There is a clear need to increase awareness of the importance of proper disposal practices, in particular among the student population of Bandung city, Indonesia. Healthcare providers can play an important role by educating this specific population on the proper disposal of unused medications.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran, Indonesia (No. 1155/UN6.C.10/PN/2017). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SA: Analyzed and interpreted the data, and wrote the paper. WI: Analyzed and interpreted the data. EH: Analyzed and interpreted the data. NQ, SJ, NN, WS, and VG: Conceived and designed the study, performed the study, and analyzed and interpreted the data. RA: Conceived and designed the study, and analyzed and interpreted the data.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.630434/full#supplementary-material

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OPINION
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Collaboration Between Health-Care Professionals, Patients, and National Competent Authorities Is Crucial for Prevention of Health Risks Linked to the Inappropriate Use of Drugs: A Position Paper of the ANSM (Agence Nationale de Sécurité du Médicament et des Produits de Santé)

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All stakeholders, from patients to health-care professionals and regulatory agencies, agree to promote "proper drug use" and to fight against "misuse" (Garfinkel and Bilek, 2020; Nothelle et al., 2017). Misuse is a concept covering different situations which are summarized in **Figure 1**, including unintentional medication errors, poisoning, or recreational use. Such situations will not be considered in our discussion. For intentional prescriptions with therapeutic goals, defining the boundary between appropriate and inappropriate use could be challenging. Intentional off label use for therapeutic purposes can be appropriate in a specific clinical context if supported by a recommendation, consensus, or scientific data. Inappropriate use is therefore defined as unjustified intentional use, and this situation is one of the main concerns tackled by the ANSM (the French drug agency) and calls for collective action.

The appropriateness of medication use should be considered as a continuum from the conditions with the most favorable benefit–risk balance to conditions with an unfavorable balance. Yet, the more the drug use deviates from the profile defined by the summary of product recommendations, the less it is expected to benefit the individual's health status and the more it exposes the patient to unacceptable adverse effects. From a medical point of view, a case-by-case assessment is required, taking into account all the individual patient characteristics requiring treatment and assessing comedications and comorbidities. For these reasons, medical practice requires that experimental and evidence-based data be applied to the uniqueness of the patient. This is a challenging situation explaining why the appropriate use of a drug, even with the best intention, remains a difficult choice, especially in frail populations.

Inappropriate medication use is an important source of adverse effects (Eguale et al., 2016). Very rare (<1/10,000) or rare (<1/1,000) undesirable effects, which are barely identified in clinical trials

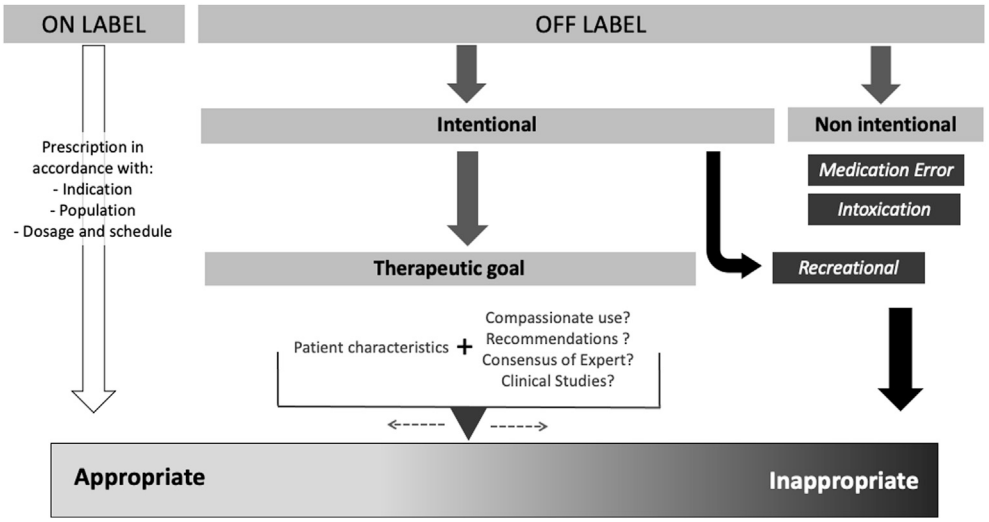

FIGURE 1 | Scale of appropriate vs. inappropriate use.

and almost impossible to detect at an individual level, are heavily represented as a health problem in the population as millions of patients are exposed to these medications over the years.

The gray area of inappropriate use is then difficult to perceive at an individual level. It is not limited to an abrupt switch between right and wrong prescriptions but could also be linked to a progressive drift in practices, leading to new risks for certain populations, in certain clinical contexts or with certain associations. It is also in this reflection that self-medication must take place. It is a direct source of inappropriate use or a factor that favors it indirectly through the risk of interactions that may not be perceptible to the prescribers.

In this context, fighting against inappropriate use and its adverse consequences is a matter of the individual relevance of the medical prescription, but global actions are also required to control them. The needs and wishes of the patient, the physician's experience, and the population expertise of the authorities should not be opposed. Based on recent experiences, the ANSM and its expert group of advisors are advocating for a more integrated vision between the individual scale and the population approach.

In recent years, several risky health situations linked to inappropriate use of drugs have been highlighted. Consequently, health organizations such as the ANSM endorsed recommendations to limit inappropriate uses of medications. For instance, the third and fourth generations of oral contraceptives have been massively prescribed despite their higher relative risk of thromboembolic accident than the 1st and 2nd generations (Emmerich et al., 2014). This has led the ANSM to warn against the former and to recommend the latter as the first line contraceptives. Valproic acid, which has a well-known risk of teratogenicity, was commonly used by women at a childbearing age, forcing the ANSM to ban its use in certain cases (Casassus, 2017). Paracetamol is hugely used with prescriptions of high doses in France (Hider-Mlynarz et al., 2018), increasing the risk of liver toxicity. This situation led to specific measures to provide information on the dosage and to control the availability of the product in pharmacies. Inappropriate prescription of antibiotics was tackled by several awareness campaigns in France and other countries but is still a major concern for health authorities, with the emergence of extensively drug-resistant bacteria (World Health Organization, 2017).

Other initiatives have been launched by health authorities to limit the risks caused by inappropriate use of specific drugs. They are commonly based on communication strategies or regulatory action toward laboratories, but few are focused on the global issue. Some initiatives suggest focusing on particular medications and offering toolkits for prescribers (Hodgson, 2015). The most frequently selected medications according to practitioner opinions included antibiotics, proton pump inhibitors, opioids, statins, cholinesterase inhibitors, and non-steroidal anti-inflammatory drugs (Hazard et al., 2020).

These initiatives are of major importance, but may a common perception of issues related to inappropriate use still be lacking between patients, health-care professionals, and authorities? How could we build a long-term strategy beyond specific topics and crisis situations?

New perspectives should be implemented now:

- **Better anticipation**. It involves the early identification of situations where inappropriate use would lead to health risk. Such situations are characterized by the expected level of exposure in the population, the context of use and the patient profile (e.g., vulnerable populations, patients exposed to polymedication, etc.), and the pharmacological characteristics of the drug (pharmacodynamic effects, adverse effects, therapeutic margin, variability of effects, drug

interactions, etc.). Taking into account these characteristics should make it possible to map risks linked to inappropriate drug use for the purpose of prevention. Detection of inappropriate use should involve all actors. Benchmarking on the use of drugs within different countries will also be a valuable tool to tackle potential misuse of drugs.

- **Better collective commitment including educational programs and co-construction of action plans with stakeholders.** Because it could be difficult to immediately assess whether the use of a particular drug is inappropriate or not, each actor needs to be responsible for its rational use. Discussion between patients and health professionals (prescribers and pharmacists) and use of prevention tools should help to identify and limit uncertain situations to decreased risks. A public debate with all the stakeholders should foster exchanges on the different perceptions and the co-construction of prevention tools in order to sensitize the entire population. The debate could lead to the development of tools to promote prevention such as computerized systems, institutional communication, or guidelines (Dalton et al., 2018; Monteiro et al., 2019). The need for new communication channels is already evident, and the best options should be chosen based on the recipients' opinions. We can then plan specific prevention and information strategies for the known situation of inappropriate use and launch nonspecific pedagogical programs. Helping patients and professionals to understand the risks associated with the use of a drug also involves training and education (Foucaud et al., 2013). The ANSM wishes to involve academics and patient partners to co-construct training programs, in particular for patients with chronic diseases or professionals and future health professionals. The main goal of such programs should be to change patients' and health-care professionals' perception on the appropriate and inappropriate use of health products.

The ANSM now integrates these different perspectives in its strategy of transparency and openness to health-care professionals and the general population. In particular, patients participate as ex officio members in all expert committees, and the ANSM has integrated advisor groups including physicians and patient partners into its organization to improve co-construction of health policies on health products. Even if dialogs already existed, of course, between the agency and the stakeholders, the aim is now to organize an entanglement throughout all our reflections. This sharing of experiences and points of view, from bedside to institution, will be the cornerstone of the collective culture of appropriate use that we all wish to promote.

AUTHOR CONTRIBUTIONS

All authors: conceptualization, analysis, writing (review and editing), and validation. SV and PM: additional writing (original draft) and supervision. All authors agree to be accountable for the content of the work.

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BRIEF RESEARCH REPORT
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Leveraging the Variability of Pharmacovigilance Disproportionality Analyses to Improve Signal Detection Performances

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Background: A plethora of methods and models of disproportionality analyses for safety surveillance have been developed to date without consensus nor a gold standard, leading to methodological heterogeneity and substantial variability in results. We hypothesized that this variability is inversely correlated to the robustness of a signal of disproportionate reporting (SDR) and could be used to improve signal detection performances.

Methods: We used a validated reference set containing 399 true and false drug-event pairs and performed, with a frequentist and a Bayesian disproportionality method, seven types of analyses (model) for which the results were very unlikely to be related to actual differences in absolute risks of ADR. We calculated sensitivity, specificity and plotted ROC curves for each model. We then evaluated the predictive capacities of all models and assessed the impact of combining such models with the number of positive SDR for a given drug-event pair through binomial regression models.

Results: We found considerable variability in disproportionality analysis results, both positive and negative SDR could be generated for 60% of all drug-event pairs depending on the model used whatever their truthfulness. Furthermore, using the number of positive SDR for a given drug-event pair largely improved the signal detection performances of all models.

Conclusion: We therefore advocate for the pre-registration of protocols and the presentation of a set of secondary and sensitivity analyses instead of a unique result to avoid selective outcome reporting and because variability in the results may reflect the likelihood of a signal being a true adverse drug reaction.

Keywords: pharmacovigilance, disproportionality analyses, signal detection, drug safety, Transparency

INTRODUCTION

Spontaneous reports are a valuable data source to complete the pre-marketing safety profile of medical products, notably for rare or long latency adverse drug reactions (ADRs) (Hartmann et al., 1999; Hauben and Bate, 2009). With the massive increase in new reports received each year by pharmacovigilance centers around the world (e.g. more than 2,700,000 new reports were recorded in the WHO's pharmacovigilance database in 2019), traditional approaches such as manual review needed to be complemented. Automated screening tools using quantitative methods have thus been developed to detect signals of disproportionate reporting (SDR), i.e. a higher proportions of reporting of ADRs for a studied drug as compared to the other drugs in the database. They allow broad screening to trigger further investigations, to detect more complex dependencies and to prioritize potential signals (Bate and Evans, 2009; Hauben and Aronson, 2009).

These methods include frequentist, Bayesian and machine learning approaches (Hauben and Bate, 2009; Harpaz et al., 2012). In addition, several models (subgroup, stratification or adjustments) can be used to overcome the multitude of biases related to spontaneous reporting rates of ADR, such as media alerts, selective reporting according to ADR severity, or time since the drug was first marketed (Seabroke et al., 2016; Wisniewski et al., 2016; Sandberg et al., 2020). They also permit to overcome disparities in drug usage and pharmacovigilance systems, or to account for risk factors of developing an ADR (e.g. sex, age, underlying conditions) (Raschi et al., 2018; Sandberg et al., 2020). Several studies have assessed and compared the performances of such methods and models, which did not reveal significant differences for signal detection (Harpaz et al., 2013; Candore et al., 2015; Pham et al., 2019). As a result, no consensus exists to date on the best analyses and no gold standard has been defined (Wisniewski et al., 2016). In this context, a large heterogeneity exists in the modalities retained for signal detection from spontaneous reporting, especially regarding the complementary analyses that can be performed to explore the robustness of the detected statistical signals. The variety of the methodological choices that are made may lead to substantial variability in results and, when these appear conflicting in the literature, lead to increase the complexity of their interpretation (Khouri et al., 2021). In this context, we hypothesized that performing a set of standardized analyses relying on different techniques could help appraising the robustness of a signal.

METHODS

In this study, we used the Observational medical outcomes partnership (OMOP) gold standard reference set to assess the diagnostic performances of a set of seven models of two widely used frequentist (Reporting Odds Ratio) and bayesian (Bayesian confidence propagation neural network) disproportionality methods applied to the WHO pharmacovigilance database, Vigibase®.

Reference Set

The OMOP reference set have been established to facilitate methodological research in drug safety and to allow comparison of signal detection performances of disproportionality analyses. The gold standard consists of 165 true and 234 false drug-event pairs originating from a systematic literature review and natural language processing of structured product labels (Ryan et al., 2013). The reference set spans 181 unique drugs covering antibiotics, nonsteroidal anti-inflammatory drugs, antidepressants, antihypertensives, antiepileptics and glucose lowering drugs. The specific outcomes (acute renal injury, myocardial infarction, acute liver injury, gastrointestinal bleeding) have been selected because they are considered as high priority events in pharmacovigilance for different reasons (Ryan et al., 2013). Acute myocardial infarction and upper gastrointestinal bleeding possess high background rates in the general population, with a different proportion of iatrogenic etiologies identified. Acute kidney and liver injury are important outcomes for post-market drug surveillance as they are the main pathways for drug metabolism and elimination, and because patients with pre-existing conditions are often excluded from phase 3 clinical trials (Trifirò et al., 2009).

Data Source

All data used for disproportionality analyses were extracted from the WHO pharmacovigilance database, VigiBase, from January 1, 1968 to December 31, 2019. Gathering reports from more than 130 member countries, Vigibase is the largest pharmacovigilance database containing more than 21 million individual case safety reports (ICSRs) submitted by pharmaceutical manufacturers, health professionals, or consumers through national pharmacovigilance systems (Lindquist, 2008).

We identified the four outcomes in Vigibase by using a collection of MedDRA Preferred Terms (PT) or standardized MedDRA queries (SMQ) to match the broader definitions used in the reference set (**Supplementary Table S1**). (Reich et al., 2013; Ryan et al., 2013)

Signal Generation

Two disproportionality methods were used in this study, the Reporting Odd Ratio (ROR) used by the European Medicines Agency, and the Bayesian confidence propagation neural network, used by the Uppsala Monitoring Center on behalf of the WHO. A SDR was considered significant if the lower boundary of the 95% confidence interval of ROR (ROR_{LB}) was ≥1 and the number of observed drug-event combinations ≥3; or if the lower boundary of the IC 95% confidence interval (IC_{LB}) was >0 (Bate et al., 1998; European Medicines Agency and EudraVigilance Expert Working Group, 2006. Guideline on the use of statistical signal detection methods in the EudraVigilance data analysis system. Available on: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/draft-guideline-use-statistical-signal-detection-methods-eudravigilance-data-analysis-system_en.pdf, 2006).

We used seven disproportionality models for which the results were very unlikely to be influenced by actual differences in absolute risks of ADR: Model 1: only suspect reports

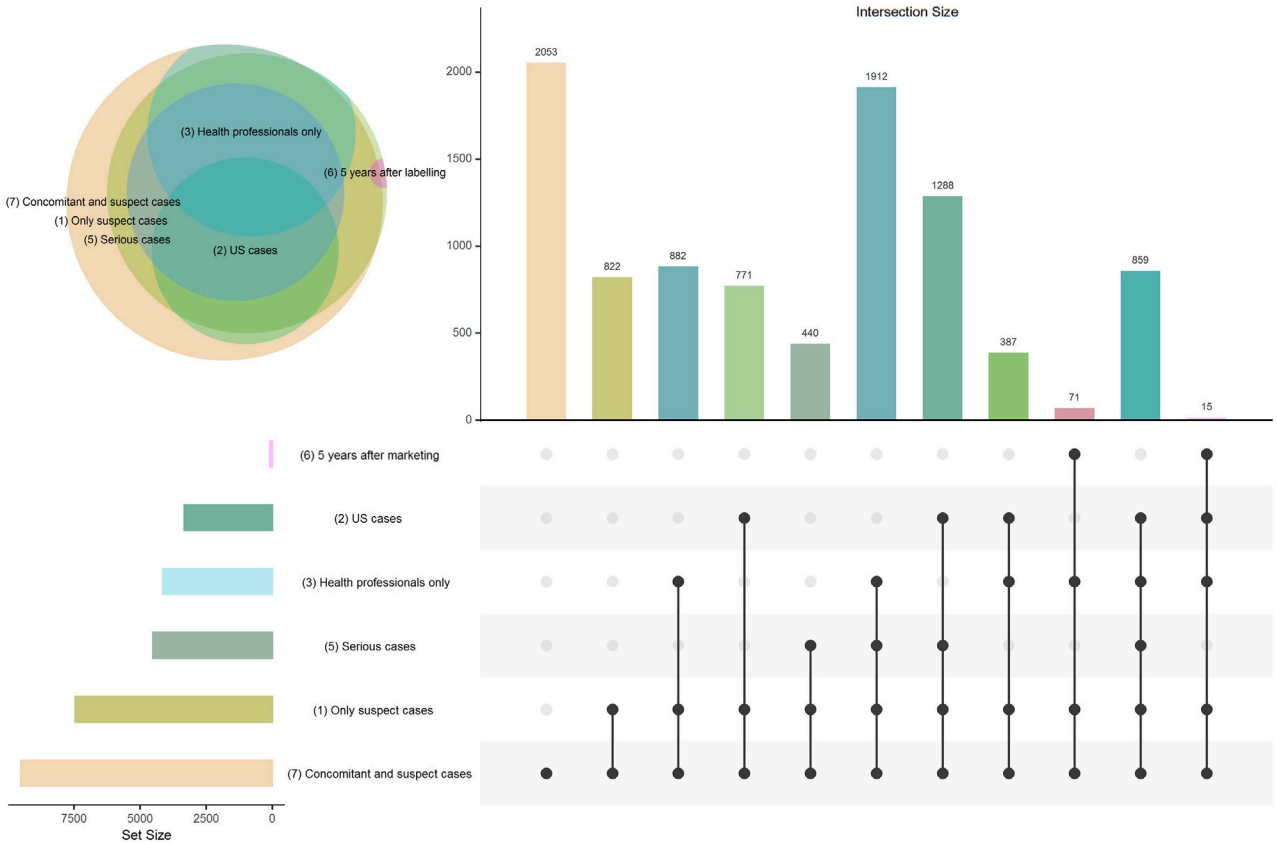

FIGURE 1 | Venn diagram and Upset plot presenting the distribution of cases included in each disproportionality analysis and the overlap between each of the models. Model 4 (subgroups by therapeutic area) is not displayed in the figure because only the comparators were modified in this model (cases correspond to all suspect cases).

included; Model 2: to assess the influence of pharmacovigilance systems between countries we restricted reports to a specific country (United States); Model 3: restricting reports to those submitted by health care professionals only (physicians or pharmacists); Model 4: restricting the database to the drug's corresponding therapeutic area (ATC code level 3) to account for difference non-cases populations; Model 5: including only serious cases; Model 6: included only cases reported within 5 years after the drug's marketing approval date to account for reporting variability according drug time on the market; and Model 7: included suspected and concomitant drugs.

Evaluation

The performances of the disproportionality models were evaluated through sensitivity, specificity and area under the curve (AUC). ROC curves were plotted for each model. To understand the contribution of the number of positive SDRs alongside the disproportionality values we built logistic regression models both with and without inclusion of the number of positive SDR. In addition, we plotted the predictive capacities (marginal means) of the models according to the number of positive SDR. We postulated that the number of drug-event pairs could impact the disproportionality results; we thus included this variable in the logistic regression models.

Lastly, we calculated and compared median ROR_{LB} and IC_{LB} values, and the median number of positive SDR between true and false drug-event pairs through Mann-Whitney-Wilcoxon tests. A two-sided p value < 0.05 was considered significant.

Statistical analyses were performed with R (version 3.6.1). The protocol, data and R codes underlying this article could be found on Open Science Framework (osf.io/a7j3z/)

RESULTS

Data and Signal Generation Results

The distribution of included cases in each analysis and the results of disproportionality analyses for the seven models are presented in **Figure 1** and **Table 1** respectively. Over the 399 drug-event pairs, signals could not be examined for four drugs relating to thirteen events in the reference dataset (3.26% of the set) as these drugs were not found in VigiBase. As recommended, ROR values were not computed

TABLE 1 | Disproportionality analyses and signal generation results for the lower bound of the 95% confidence intervals of the reporting odds ratio (ROR_{LB}) and of the information component (IC_{LB}) for the seven selected models. SDR: Signal of disproportionate reporting. Model 1: only suspect cases; Model 2: subgroup by country (United States); Model 3: health professionals only; Model 4: subgroup by therapeutic area; Model 5: serious cases only; Model 6: 5 years after drug approval; Model 7: suspected and concomitant drugs.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
ROR_{LB}							
N missing (%)	49 (12.3%)	77 (19.3%)	71 (17.8%)	49 (12.3%)	75 (18.8%)	207 (51.9%)	19 (4.8%)
Median	0.48	0.53	0.43	0.61	0.43	0.65	1.16
Q1—Q3	0.15–1.75	0.17–1.97	0.12–1.59	0.28–1.28	0.18–1.62	0.21–2.42	0.59–2.06
Min—Max	0.00–22.39	0.01–57.55	0.00–16.70	0.00–46.57	0.01–22.28	0.00–13.20	0.01–16.74
N positive SDR	122 (33.3%)	120 (30.1%)	107 (26.8%)	117 (29.3%)	104 (26.1%)	73 (18.3%)	212 (53.1%)
N misclassified SDR							
True ADR	59 (35.7%)	60 (36.4%)	71 (43.0%)	86 (52.1%)	73 (44.2%	99 (60%)	40 (24.2%)
False ADR	17 (7.3%)	16 (6.8%)	14 (5.9%)	39 (16.6%)	13 (5.6%))	8 (3.4%)	88 (37.6%)
IC_{LB}							
N missing (%)	13 (3.3%)	13 (3.3%)	13 (3.3%)	13 (3.3%)	13 (3.23%)	13 (3.3%)	13 (3.3%)
Median	−1.57	−1.72	−2.14	−0.99	−2.04	−9.99	0.13
Q1—Q3	−3.44–0.46	−4.69–3.67	−4.38–0.16	−3.07–0.13	−4.16–0.03	−10.27–−1.19	−0.94–0.99
Min—Max	−15.45–4.29	−15.70–5.08	−15.40–3.76	−17.24–4.73	−15.90–4.11	−14.50–3.40	−12.70–3.92
N positive SDR	120 (30.1%)	112 (28.1%)	106 (26.6%)	110 (27.6%)	97 (24.3%)	65 (16.3%)	204 (51.1%)
N misclassified SDR							
True ADR	60 (36.4%)	66 (40.0%)	72 (43.6%)	88 (53.3%)	79 (47.8%)	106 (64%)	42 (25.4%)
False ADR	16 (6.8%)	14 (6.0%)	14 (6.0%)	34 (14.5%)	12 (5.1%)	7 (3.0%)	82 (35.0%)

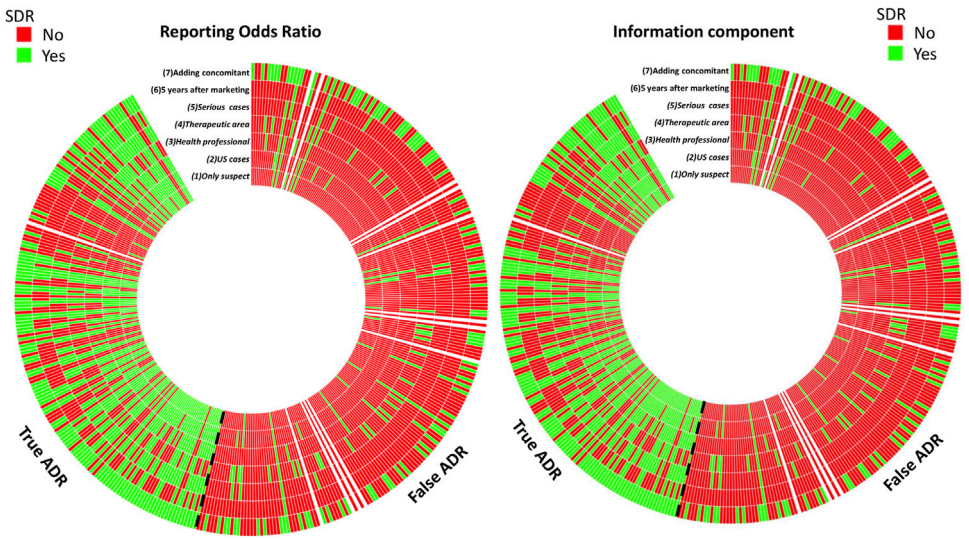

FIGURE 2 | Number of positive and negative signals of disproportionate reporting (SDR) with ROR and IC methods. Each line represents a disproportionality model and each column represents a drug-event pair. A SDR was deemed significant if $ROR_{LB} > 1$ and $n > 3$ for the ROR method, and $IC_{LB} > 0$ for the IC method.

when the number of exposed cases was lower than 3. This led to lack of ROR value for 4.8–51.9% of the 399 drug-event pairs for the model including concomitant drugs and the model including only ICSRs within 5 years of the drug's approval, respectively. The ROR_{LB} values varied from 0.01 (gastrointestinal bleeding and neostigmine) to 57.6 (acute liver injury and propylthiouracil) and IC_{LB} values from −17.2 (acute liver injury and miconazole) to 5.1 (acute liver injury and propylthiouracil). **Figure 2** presents the SDR generated by the two methods for the 7 models. Overall, we noted high variability in the results and in the number of detected SDRs among the models.

Evaluation and Comparison of Model Performances

The sensitivity, specificity, AUC and ROC curves corresponding to the seven models are presented in **Supplementary Figure S1** and **Supplementary Table S2**. Overall, in all disproportionality methods, model 4 (subgroup by therapeutic area), model 6 (within 5 years of drug approval) and model 7 (suspected and

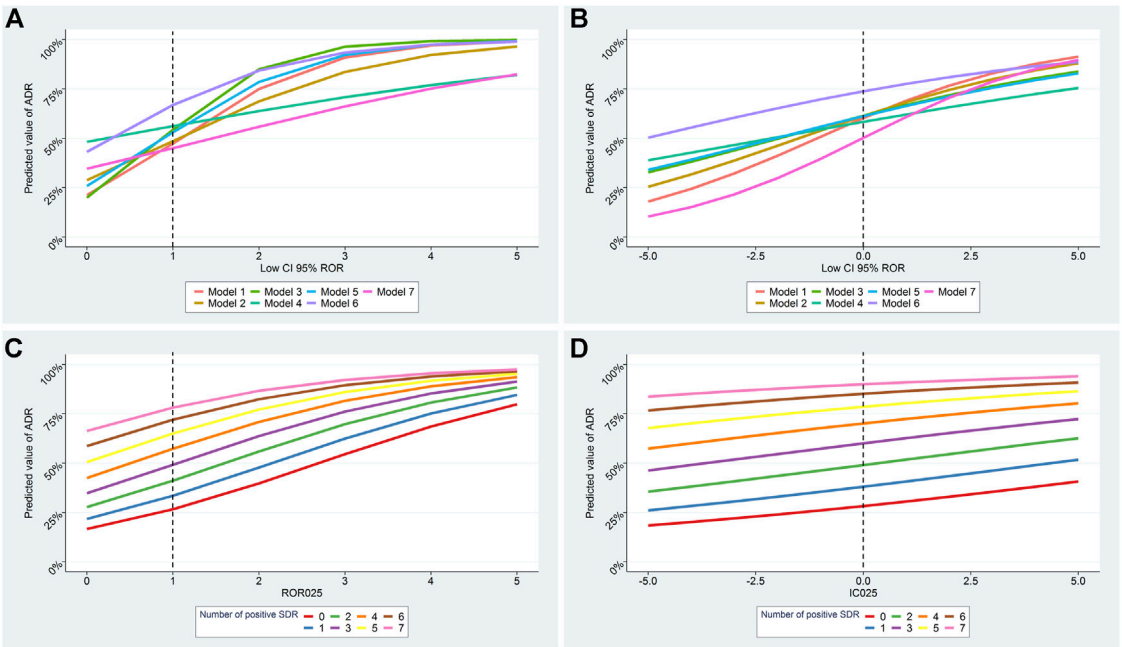

FIGURE 3 | Predicted probability for a signal of disproportionate reporting (SDR) to correspond to a true ADR according to the lower boundary of disproportionality values and to the number of positive SDR. Results of all models according to number of positive SDR for ROR_{LB} and IC_{LB} are presented in **A, B** respectively. Results of model 1 according to the number of positive SDR for ROR_{LB} and IC_{LB} are presented in **C, D**. Model 1: only suspect cases included; Model 2: subgroup by country (United States); Model 3: reporting by health professionals only; Model 4: subgroups by therapeutic area; Model 5: serious cases only; Model 6: within 5 years of drug approval; Model 7: suspected and concomitant drugs.

concomitant drugs) showed limited performances compared to model 1 (including only suspect reports), model 2 (subgroup by country (United States)), model 3 (reports by health professionals) and model 5 (serious cases only). The number of positive and misclassified SDR according to disproportionality methods and models are also presented in **Table 1**. The number of positive SDR generated ranged from 73 to 212 and 65 to 204 for ROR_{LB} and IC_{LB} respectively. Moreover, the proportion of misclassified SDRs was lower for model 1 and model 2 and systematically higher for true than for false ADR (**Table 1**).

Of importance, with the ROR method only 37 of the 165 true ADR displayed a SDR with all models, and 125 of the 234 false ADR for negative SDRs. The results were similar with the IC method for which 33 of the 125 true ADRs systematically displayed a signal and 130 of the 234 false ADRs did not (**Figure 2**).

Comparison of Disproportionality Results Between True and False ADRs

Median ROR_{LB} values were 1.67 (0.68, 3.91) and 0.39 (0.20, 0.72) for true and false ADRs respectively ($p < 0.01$). Median IC_{LB} values were 0.09 (−1.37, 1.28) and −3.62 (−5.82, −2.22) for true and false ADR respectively ($p < 0.01$). The median number of positive SDR significantly differed between false and true ADR groups, 0 (0, 1) and 5 (1, 6) respectively, in both frequentist and Bayesian methods (**Supplementary Table S3**).

Using the Number of Positive SDRs to Improve a Model's Predictive Capacities

To investigate whether the number of positive SDR was predictive of a true ADR independently of the disproportionality estimates we performed logistic regression models with and without the inclusion of the number of positive SDR. In all models the number of positive SDR remained independently and significantly predictive of an ADR (**Supplementary Table S4**). The lower boundary of ROR and IC were no longer significant after adjustment on the number of positive SDR in 5 of the models. The predictive capacities of all methods and models are plotted in **Figure 3**, **Supplementary Figures S2, S3**. The number of positive SDR strongly impacted the predictive capacities of all models notably for lower boundary values close to the threshold of signal detection for ROR and IC.

DISCUSSION

To our knowledge, this is the first study assessing the variability of disproportionality analyses using several models and methods. We showed that the number of positive SDR in a standardized set of 7 models can serve as a significant predictor of a true ADR. Importantly, this property of the number of positive SDR was independent of disproportionality values and could therefore be used to further investigate the plausibility of a signal.

Our study also underlines the necessity of reporting multiple secondary or sensitivity analyses when studying a drug-event association through disproportionality analyses. Indeed, almost 60% of all drug-event pairs displayed both positive and negative SDR regardless of their truthfulness. This finding highlights the potential risk of reporting bias in disproportionality analyses studies, especially since in this study we used a single threshold to define a signal, whereas this is not the case in all studies (Candore et al., 2015). This risk is particularly important in the pharmacovigilance field where the data are openly accessible to researchers, no protocols are pre-registered and no gold standard methods have yet been established (Wisniewski et al., 2016).

Performing a set of analyses, rather than only one, is all the more important as their variability seems to be associated with the probability of being a true finding. The impact of this variability is particularly important when disproportionality values are close to the threshold of signal detection ($ROR_{LB} > 1$ or $IC_{LB} > 0$), for which the probability of a result being true may vary from 30 to 80% depending of the number of positive SDR. Unexpectedly, the disproportionality values were no longer significant when adjusted on the number of positive SDR in 5 of the 7 models for both methods, stressing the relevance of this metric. In this study, we pre-defined a set of analyses for which the results are almost exclusively impacted by the random variability in ADR reporting rates, unrelated to ADRs or patients characteristics. However, we cannot exclude that real differences in the ADR relative risk may exist for some individual drugs according, for example, to the reporting country (Sandberg et al., 2020). Further work is thus needed to select the best set of analyses.

Although this study did not primarily aim to compare the performance of disproportionality analysis models and methods, it supplements the knowledge on this topic. Importantly, we observed very similar results between frequentist and Bayesian methods, in accordance with some previous studies (Candore et al., 2015; Pham et al., 2019). This might indicate that our results could be generalized to other methods. In addition, we found comparable performance between four models: all suspect reports included, limiting reports from a given country, including only reports by health professionals, and including only serious cases, with a lower proportion of misclassified SDR when using the first two methods. In contrast, models restricting comparison to a given therapeutic area, including only cases reported within 5 years of drug approval, or including concomitant reports, were inferior whatever the method. An earlier study conducted by Seabroke et al. likewise showed an appreciable benefit associated with subgrouping by reporter qualification or by country of origin, but little advantage using severity (Seabroke et al., 2016). This discrepancy might be explained by a slightly different definition of a positive signal (subgroup analyses were examined in each strata and a SDR was found significant if the criteria were met in any of the strata). In 2013 Harpaz et al. compared the performances of several disproportionality methods using the FDA adverse event reporting database using the same reference set (Harpaz et al., 2013). They also found a better specificity than sensitivity of the reported odds ratio but the overall performances were inferior to those in our study. That may be due to the greater number of cases included in our study (21 million vs. 5 million) and broader definitions of outcomes, consistent with other studies that found higher performances using larger databases and similar event definitions (Reich et al., 2013; Caster et al., 2020).

Our study had some limitations. The reference dataset used here is one of the largest to date that incorporates both SPCs and evidence from literature reviews. However, Hauben et al. suggested that a small part of false drug-event pairs may be misclassified, which could impact the performances of disproportionality analyses (Hauben et al., 2016). Nonetheless, the comparisons between models and methods are unlikely to have been affected by this bias. Moreover, to standardize the models we had to make choices (e.g. 5 year after drug approval for model 6, United States country for model 2 or defining the therapeutic area by the ATC level 3 in model 4). These standardized definitions may not be relevant for some drug-event pairs (e.g. for drugs not commercialized in the United States or belonging to an heterogenous ATC class) and have to be further adapted to the nature of the studied drug and ADR. The findings of our study may not be generalizable to other databases due to differences related to the database background and the medical products covered. Nevertheless, several studies have highlighted the similitudes and overlaps between SDR found from pharmaceutical company databases and international pharmacovigilance databases (Candore et al., 2015; Vogel et al., 2020). Finally, while it can be assumed that the results are applicable to other drug events pairs, the predicted probabilities calculated in this study are not extrapolable.

CONCLUSION

To conclude, this study shows the wide variability of disproportionality analysis results depending on the method and model specifications, thus opening the door for selective reporting of results. We therefore advocate for the pre-registration of protocols and the presentation of a set of secondary and sensitivity analyses instead of a unique result to limit reporting bias and because variability in the results may reflect the likelihood of a signal being a true adverse drug reaction.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: The protocol, data and R codes underlying this article could be found on Open Science Framework (osf.io/a7j3z/).

AUTHOR CONTRIBUTIONS

CK designed the research, analyzed the data and wrote the manuscript. TN extracted the data and performed the research. All authors contributed to the interpretation of the results and revising 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.2021.668765/full#supplementary-material

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Impact of Total Epinephrine Dose on Long Term Neurological Outcome for Cardiac Arrest Patients: A Cohort Study

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Introduction: Although epinephrine is universally acknowledged to increase return of spontaneous circulation (ROSC) after cardiac arrest, its balanced effects on later outcomes remain uncertain, causing potential harm during post-resuscitation phase. Recent studies have questioned the efficacy and potential deleterious effects of epinephrine on long-term survival and neurological outcomes, despite that the adverse relationship between epinephrine dose and outcome can be partially biased by longer CPR duration and underlying comorbidities. This study explored the long-term effect of epinephrine when used in a cohort of patients that underwent cardiac arrest during cardiopulmonary resuscitation.

Methods: The data were originally collected from a retrospective institutional database from January 2007 to December 2015 and are now available on Dryad (via: https://doi.org/10.5061/dryad.qv6fp83). Use of epinephrine was coded by dose (<2 mg, 2 mg, 3–4 mg, ≥5 mg). A favorable neurological outcome was defined using a Cerebral Performance Category (CPC) 1 or 2. The association between epinephrine dosing and 3-months neurological outcome was analyzed by univariate analysis and multivariate logistic regression.

Results: Univariate and multivariate analysis demonstrated a negative association between total epinephrine dose and neurological outcome. Of the 373 eligible patients, 92 received less than 2 mg of epinephrine, 60 received 2 mg, 97 received 3–4 mg and 124 received more than 5 mg. Compared to patients who received less than 2 mg of epinephrine, the adjusted odds ratio (OR) of a favorable neurological outcome was 0.8 (95% confidence interval [CI]: 0.38–1.68) for 2 mg of epinephrine, 0.43 (95% confidence interval [CI]: 0.21–0.89) for 3–4 mg of epinephrine and 0.40 (95% confidence interval [CI]: 0.17–0.96) for more than 5 mg of epinephrine.

Conclusion: In this cohort of patients who achieved ROSC, total epinephrine dosing during resuscitation was associated with a worse neurological outcome three months after cardiac arrest, after adjusting other confounding factors. Further researches are needed to investigate the long-term effect of epinephrine on cardiac arrest patients.

Keywords: epinephrine dose, neurological outcome, cardiac arrest patients, cohort study, multivariate analysis

INTRODUCTION

Standard-dose epinephrine for adult cardiac arrest is defined as 1 mg given intravenously every 3–5 min until return of spontaneous circulation (ROSC) regardless of cardiac arrest rhythm (Bossaert et al., 2015) by current American Heart Association and European Resuscitation Council guidelines. Epinephrine can effectively increase aortic blood pressure via its alpha-adrenergic vasopressor activity, which contributes to coronary perfusion and subsequently helps achieve ROSC during chest compression (Link et al., 2015). On the other hand, adverse effects including impaired cerebral microvascular flow (Ristagno et al., 2009) and myocardial depression (Angelos et al., 2008) are observed in laboratory. Likewise, epinephrine dosing is also associated with coagulation (Larsson et al., 1989), impaired tissue oxygen utilization and lactate clearance (Rivers et al., 1994) in humans.

For the past few decade, as resuscitation interventions have become more successful, there is an increasing need to reconsider the patient-centered outcomes such as functional status and quality of life in addition to returning of pulses (Becker et al., 2011). Although epinephrine is associated with a greater likelihood of ROSC, this early potential benefit for the heart doesn't guarantee good patient outcomes, as the vast majority of patients resuscitated from cardiac arrest present in coma or with altered level of consciousness (Geocadin et al., 2019). Recent studies have questioned the efficacy and potential deleterious effects of epinephrine on long-term survival and neurological outcomes (Ong et al., 2007; Mayor, 2014).

In a large observational study of OHCA patients in Japan, prehospital epinephrine administration was significantly associated with increased chance of ROSC before hospital arrival but decreased likelihood of survival and worse functional status one month after the event (Hagihara et al., 2012). Admittedly, the total dose of epinephrine administered is proportional to how long a patient remains in cardiac arrest, resulting in higher doses for patients who fail to respond to initial treatment (Callaway, 2013). Therefore, adverse relationship between epinephrine dose and outcome can be partly attributed to "resuscitation time bias" and underlying comorbidities (Matos et al., 2013). Further work from a randomized clinical trial demonstrated that more survivors had severe neurological impairment in the epinephrine-treated group (Perkins et al., 2018), although the between-group difference in the percentage of a favorable neurological outcome at hospital discharge was not statistically significant when compared to placebo group.

We sought to explore the long-term effect of epinephrine when used in cardiopulmonary resuscitation. In this secondary analysis, we described the association between epinephrine dosing during cardiac arrest and 3-months neurological functions among a cohort of patients that underwent cardiac arrest.

METHODS

Study Population

This is a secondary analysis of a retrospective study (Iesu et al., 2018) where the dataset was collected by Iesu et al. and is now available on Dryad (via: https://doi.org/10.5061/dryad.qv6fp83). The former study was originally performed in the Department of Intensive Care at Erasme Hospital, Brussels (Belgium) and was approved by the local Ethical Committee (Comite´ d'Ethique Hospitalo-Facultaire Erasme-ULB) while waiving the need for informed consent considering its retrospective nature. In the original study, the data were collected from a retrospective institutional database (January 2007 to December 2015), where patients admitted after in-hospital cardiac arrest (IHCA) and out-of-hospital cardiac arrest (OHCA) with a Glasgow Coma Scale (GCS) < 9 were included. Exclusion criteria were missing data on liver function or death less than 24 h after ICU admission. All patients were treated with therapeutic hypothermia, targeting a body temperature between 32 and 34°C for 24 h, according to a standardized institutional post-resuscitation management protocol that has been extensively described elsewhere (Tujjar et al., 2015; Iesu et al., 2018).

Data Collection

In the original study, Iesu et al. collected data on demographics, comorbidities (including diabetes, hypertension, chronic renal failure, chronic heart failure, and previous neurological diseases) and first aid information [bystander CPR, time to ROSC (the arrival of emergency medical care), total epinephrine dose and non-shockable rhythm] in all patients. Lactate and glucose level on admission, shock, the length of ICU stay, ICU death and hospital death was recorded, as with the proportion of IHCA and OHCA. Information about whether patients underwent ECPR, quality or duration of CPR was not explicitly stated in the original database or manuscript. Aiming to analyze the association between epinephrine dosage and neurological functions of survivors, cerebral performance categories score (CPC) was employed from the original study to assess neurological outcomes three months after cardiac arrest (1 = no or mild neurological disability, 2 = moderate neurological disability, 3 = severe neurological impairment, 4 = vegetative state, 5 = death). The neurological outcome was defined as favourable with CPC 1–2 and unfavourable with CPC 3–5 (Jennett and Bond, 1975).

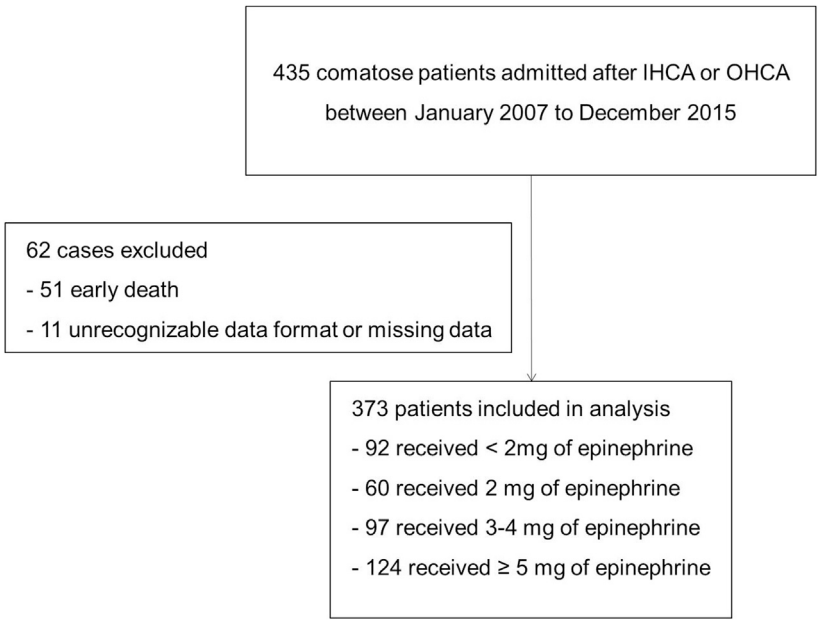

FIGURE 1 | Flowchart of patient selection. Abbreviation: IHCA, in-hospital cardiac arrest; OHCA: out-of-hospital cardiac arrest.

The CPC evaluation was performed during follow-up visits or by telephone interview with the general practitioner.

Statistical Analysis

All the analyses were performed using the statistical software packages R (http://www.R-project.org, The R Foundation) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA). p-value < 0.05 (two-tailed) was considered statistically significant.

In descriptive statistics, continuous variables were presented as mean ± standard deviation (normal distribution) or median [Q1-Q3] (skewed distribution). Classified variables were presented as number and percentage. Chi-square test (for categorical variables), One-Way ANOVA test (for continuous variables with normal distribution), or Mann-Whitney U test (for continuous variables with skewed distribution) were employed to calculate the significance among different epinephrine dosage groups.

First, univariate analysis was conducted to investigate the correlations between different factors (age, gender, epinephrine dosage, etc) and neurological status three months after cardiac arrest. Second, multivariate linear regression was employed to calculate the independent effect of epinephrine dose on neurological outcome. In this step, three adjust models were employed: 1) model 1: no covariates were adjusted; 2) model 2: only adjusted for age and sex; 3) model 3: specific covariates were adjusted as potential confounders if they change the estimates of epinephrine dosage (X) on neurological outcome three months after cardiac arrest (Y) by more than 10% or significantly associated with the neurological outcome (Y). The following covariates were selected a priori on the basis of established associations and/or plausible biological relations and tested: age, gender, chronic renal failure, previous neurological disease, OHCA, witness arrest, bystander CPR, time to ROSC, non-shockable rhythm, baseline lactate, baseline glucose, TTM, ICU length of stay.

Coefficient of each variable in univariate and multivariate model was presented in **Supplementary Appendix Table A1**. Assumption check for multivariate logistic regression model was presented in **Supplementary Appendix Table A2**. Specifically, age and baseline lactate were found curvilinear, and were further checked by generalized addictive model for their effect value. The detailed information were listed in **Supplementary Appendix Table A3**.

RESULTS

From 435 patients in total, 61 of those were excluded for the reason of early death (n = 51) or missing data on liver transaminases, coagulation or total bilirubin (n = 10), according to Iesu et al. (Iesu et al., 2018); additionally, in this manuscript, 1 patient was excluded because of unrecognizable data format in Excel downloaded from Dryad. 373 patients were included in the final analysis based on the inclusion and exclusion criteria (**Figure 1**).

Baseline Characteristics of Selected Participants

Descriptive statistics of the study population were provided in **Table 1**. Generally, the cohort was 61.8 ± 15.4 years of age, and 72.1% of them were male. 61.0% of the patients had OHCA, 85.5% had a witnessed CA, 59.0% had a non-shockable

TABLE 1 | Clinical characteristics of patients at the time of hospital admission.

Variable	Statistics (N = 373)	Epinephrine dosage groups				
		<2 mg (N = 92)	2 mg (N = 60)	3–4 mg (N = 97)	≥5 mg (N = 124)	*p* value [a]
Age (year)	61.8 ± 15.4	65.47 ± 15.09	62.60± (16.38)	61.92 ±(14.95)	58.62 ±(14.98)	0.013
Gender(male)	269 (72.1%)	63 (68.48%)	40 (66.67%)	78 (80.41%)	88 (70.97%)	0.180
Diabetes	90 (24.1%)	24 (26.09%)	17 (28.33%)	27 (27.84%)	22 (17.74%)	0.234
Hypertension	159 (42.6%)	46 (50.00%)	27 (45.00%)	38 (39.18%)	48 (38.71%)	0.329
Previous neurological disease	54 (14.5%)	19 (20.65%)	10 (16.67%)	15 (15.46%)	10 (8.06%)	0.064
Chronic renal failure	62 (16.6%)	18 (19.57%)	9 (15.00%)	17 (17.53%)	18 (14.52%)	0.767
Chronic heart failure	78 (20.9%)	16 (17.39%)	12 (20.00%)	17 (17.53%)	33 (26.61%)	0.281
OHCA	180 (61.0%)	45 (48.91%)	25 (41.67%)	58 (59.79%)	79 (64.23%)	0.013
Witnessed arrest	319 (85.5%)	85 (92.39%)	55 (91.67%)	85 (87.63%)	94 (75.81%)	0.002
Bystander CPR	253 (67.8%)	78 (84.78%)	44 (73.33%)	63 (64.95%)	68 (54.84%)	<0.001
Time to ROSC (min)	18.1 ± 14.1	7.28 ± 6.25	10.30 ± 6.33	17.26 ± 10.40	30.51 ± 13.95	<0.001
Non-shockable rhythm	220 (59.0%)	56 (60.87%)	35 (58.33%)	51 (52.58%)	78 (62.90%)	0.461
Baseline lactate (mEq l^{-1})	6.3 ± 3.3	6.35 ± 3.68	5.67 ± 2.65	6.43 ± 3.66	6.32 ± 3.05	0.525
Baseline glucose (mg dl^{-1})	234.8 ± 125.3	254.95 ± 153.64	225.73 ± 114.15	227.62 ± 116.06	229.86 ± 113.18	0.362
TTM	78 (84.78%)	49 (81.67%)	93 (95.88%)	111 (89.52%)	78 (84.78%)	0.024
ICU stay (day)	7.8 ± 9.7	7.41 ± 8.44	7.83 ± 8.21	7.97 ± 9.05	8.38 ± 11.69	0.913
ICU death	153 (51.9%)	36 (39.13%)	23 (38.33%)	55 (56.70%)	80 (64.52%)	<0.001
Hospital death	164 (55.6%)	40 (43.48%)	27 (45.00%)	59 (60.82%)	86 (69.35%)	<0.001
Favorable neurological outcomes	148 (39.7%)	50 (54.35%)	30 (50.00%)	34 (35.05%)	34 (27.42%)	<0.001

Abbreviations: CPR, cardiopulmonary resuscitation; ICU, intensive care unit; OHCA, out-of-hospital cardiac arrest; ROSC, restoration of spontaneous circulation; TTM, targeted temperature management.

[a]*p* value is calculated as a result of group comparison among epinephrine dosage groups.

TABLE 2 | Univariate analysis for factors and their association with neurological outcomes three months after cardiac arrest.

Variable	Unfavorable neurological outcomes (n = 225)	Favorable neurological outcomes (n = 148)	Univariate analysis (odds ratio, 95% CI)	*p* value
Age (year)	63.64 ± 15.96	59.03 ± 14.09	0.98 (0.97, 0.99)	0.005
Gender (male)	160 (71.11%)	109 (73.65%)	1.13 (0.71, 1.80)	0.611
Hypertension	94 (41.78%)	65 (43.92%)	1.10 (0.72, 1.67)	0.656
Diabetes	60 (26.67%)	30 (20.27%)	0.69 (0.42, 1.13)	0.14
Chronic heart failure	50 (22.22%)	28 (18.92%)	0.82 (0.49, 1.38)	0.456
Chronic renal failure	41 (18.22%)	21 (14.19%)	0.74 (0.42, 1.32)	0.307
Previous neurological disease	41 (18.22%)	13 (8.78%)	0.43 (0.22, 0.84)	0.014
OHCA	125 (55.80%)	82 (55.41%)	0.99 (0.65, 1.51)	0.977
Bystander CPR	139 (61.78%)	114 (77.03%)	2.06 (1.29, 3.29)	0.003
Witnessed arrest	183 (81.33%)	136 (91.89%)	2.59 (1.31, 5.10)	0.006
Time to ROSC (min)	20.09 ± 14.86	15.03 ± 12.25	0.97 (0.96, 0.99)	0.001
Epinephrine dosage (mg)	4.75 ± 4.01	3.03 ± 2.84	0.86 (0.80, 0.92)	<0.001
Non-shockable rhythm	159 (70.67%)	61 (41.22%)	0.29 (0.19, 0.45)	<0.001
Baseline lactate (mEq l^{-1})	6.42 ± 3.46	6.00 ± 3.10	0.96 (0.90, 1.03)	0.238
Baseline glucose (mg dl^{-1})	222.22 ± 105.08	253.93 ± 149.22	1.002 (1.000, 1.004)	0.02
TTM	205 (91.11%)	126 (85.14%)	0.56 (0.29, 1.06)	0.08
ICU stay (day)	7.00 ± 10.45	9.39 ± 8.34	1.03 (1.00, 1.05)	0.026

rhythm, and 14.5% had pre-existing neurological diseases. The ICU length of stay was 4 [2–9] days, 55.6% patients died in hospital and 39.7% patients had a favourable neurological outcome.

Univariate Analysis

We reclassified the baseline data according to neurological status before conducting univariate analysis and the overall results were presented in **Table 2**. Compared to those with unfavorable neurological outcomes, patients with favorable neurological outcomes were younger, had shorter time to ROSC and less total epinephrine dose, and were less frequently to suffer from previous neurological disease or shock during hospital stay, while they were more likely to experience a witnessed CA, a bystander CPR and a shockable rhythm.

Employing univariate linear regression, we found that previous neurological diseases, witnessed CA, bystander CPR, non-shockable rhythm, total epinephrine dosing, shock during ICU stay and baseline glucose were associated with neurological outcomes three months after CA. Among them, previous neurological diseases, total epinephrine dosage, and shock were negatively associated with neurological outcomes, while witnessed CA, shockable rhythm, bystander CPR and baseline glucose were positively correlated with neurological outcomes.

TABLE 3 | Logistic regression analysis for the association between total epinephrine dosage and neurological outcomes.

Epinephrine dosage (mg)	Favorable neurological outcomes (odds ratio, 95% CI, *p* value)		
	Non-adjusted	Adjust I	Adjust II
<2 mg	1.0	1.0	1.0
2 mg	0.84 (0.44, 1.61) 0.600	0.77 (0.40, 1.51) 0.452	0.80 (0.38, 1.68) 0.561
3–4 mg	0.45 (0.25, 0.81) 0.008	0.38 (0.21, 0.70) 0.002	0.43 (0.21, 0.89) 0.024
≥5 mg	0.32 (0.18, 0.56) <0.001	0.25 (0.14, 0.45) <0.001	0.40 (0.17, 0.96) 0.041

No confounding factors were adjusted in non-adjusted model. Age and gender were adjusted in Adjust I model. Age, gender, chronic renal failure, previous neurological disease, OHCA, witness arrest, bystander CPR, time to ROSC, non-shockable rhythm, baseline lactate, baseline glucose, TTM, ICU stay days were adjusted in Adjust II model.

Multivariate Regression Analysis

Aiming to calculate the independent effect of epinephrine dosage on neurological outcomes, three models were constructed based on multivariate logistic regression, with their effect values (Odds ratio, OR) and 95% confidence intervals (CI) listed in **Table 3**. The model-based effect value can be interpreted as how epinephrine dosage changes the likelihood of favorable neurological outcomes. For instance, in unadjusted model, the effect value for 2 mg epinephrine dosing group is 0.84, implying that compared to patients administrated with less than 2 mg of epinephrine, the likelihood of those administrated with 2 mg of epinephrine achieving favorable neurological outcomes decreases 16%.

However, unadjusted model is limited due to its univariate nature, and other factors that simultaneously impact neurological prognosis after CA must be taken into consideration. Specifically, in this manuscript, time to ROSC (min) and age were statistically significant among different epinephrine dosing groups. The former can be easily interpreted from a medical perspective, as patients with longer time to ROSC are treated with more epinephrine shots. Two more models were provided after adjusting different confounding factors, as presented in **Table 3**. In the fully adjusted model (model II), the results indicate that the patients who received more than 5 mg of epinephrine were 60% less likely to achieve a favorable neurological outcome than those administered <2 mg as measured by Cerebral Performance Category (CPC). Moreover, neurological outcomes were not significantly different in patients who received <2 mg of epinephrine vs. those received 2 mg. Further, the effect of epinephrine dosage on 3-months neurological outcomes is consistent among OHCA/IHCA groups and shockable/non-shockable rhythm groups, as revealed by stratification analysis in **Supplementary Appendix Table A4**.

DISCUSSION

In this secondary analysis, total epinephrine dosing during resuscitation was associated with a worse neurological outcome three months after OHCA or IHCA, after adjusting other confounding factors.

Although epinephrine is universally acknowledged to increase ROSC after cardiac arrest, its balanced effects on later outcomes remain uncertain, with potential harm during post-resuscitation phase. In a large observational study by Hagihara et al., the chance of achieving 1-month survival and favorable neurological outcomes (defined as a CPC of 1–2) were remarkably reduced in epinephrine-treated group (Hagihara et al., 2012). In another large cohort of patients who achieved ROSC, the adverse association of prehospital epinephrine administration and chance of survival was observed regardless of resuscitation duration or in-hospital interventions performed (Dumas et al., 2014). Data from randomized clinical trials have also failed to provide a conducive effect of epinephrine on longer-term outcomes. Olasveengen et al. have concluded that CPC score at discharge and 1-year survival were not significantly improved in patients that received intravenous administration of epinephrine (Olasveengen et al., 2009). Perkins et al. demonstrated that survivors from epinephrine group were more likely to display severe neurologic impairment (defined as a score of 4 or 5 on the modified Rankin scale) compared to the placebo group (Perkins et al., 2018).

These paradoxical phenomenons may be related to epinephrine's mechanism of action. By activating α-adrenergic receptors, epinephrine robustly augments coronary perfusion during CPR to increase the likelihood of ROSC. However, at the same time, the blood flow to all other organs, including cerebrum, is reduced to support this temporary benefit for coronary perfusion. This effect may even persist after the return of pulses and eventually incur a metabolic debt from the body and brain that are detrimental to long-term outcomes despite the improvement in ROSC(Sigal et al., 2019). Moreover, the clinical effect of epinephrine is likely to hinge on timing, dosing, and patients' conditions. Compared with previous work, our study has adjusted the effect of baseline glucose, baseline lactate, previous neurological diseases, chronic heart failure, chronic renal failure, coronary artery diseases or other confounding factors that would interfere with long-term neurological outcomes, improving accuracy and robustness of the adverse association between epinephrine dosage and neurological outcomes.

In this study, both OHCA and IHCA patients were included but the final analysis revealed no differences in outcomes between OHCA and IHCA patients. Theoretically, IHCA patients would receive more timely emergency treatments compared to OHCA patients, and may consequently achieve a better outcome; We speculated that it may be due to the OHCA group included were of a better physical condition, as they were younger (even though not statistically significant) and had fewer comorbidities such as

hypertension, diabetes, chronic heart failure and chronic renal failure (see **Supplementary Appendix Table A5**).

For the past few decades, induced hypothermia and integrated plans of care have successfully improved the survival to hospital discharge among cardiac arrest patients (Callaway, 2012). One hypothesis is that these post-resuscitation interventions help alleviate the potential damage caused by epinephrine administration. These practices have raised expectations beyond restoration of spontaneous circulation but also functional status and quality of life after discharge (Callaway, 2012). In this study, we can't conclude that TTM is significant associated with 3-months outcome based on the p value in univariate analysis. Further, in the revised Adjust II model, TTM remained as a potential confounder in evaluating the association between epinephrine dosage and 3-months neurological outcome, indicating that the negative correlation between epinephrine dosage and 3-months neurological outcome was reserved in spite of TTM intervention.

These findings should inspire further investigation on the most appropriate scheme of treatment rather than incriminating epinephrine itself. The standard 1-mg dose of epinephrine for CPR was initially defined basing on dog models (Redding and Pearson, 1962; Redding and Pearson, 1968) and has been applied on adult patients without weight or interspecies adjustment ever since. In a recent study, lower-dose epinephrine administration was not associated with OHCA outcomes in terms of survival to hospital discharge and favorable neurological status (Fisk et al., 2018). On the other hand, our results indicate that the patients who received less than 2 mg of epinephrine were more likely to present a favorable neurological outcome than those administered >5 mg as measured by Cerebral Performance Category (CPC). In addition, neurological outcomes were not significantly different in patients who received <2 mg of epinephrine vs. those received 2 mg. Similar conclusions were also reached in a large observational study using data from Penn Alliance for Therapeutic Hypothermia (PATH) registry (Larsson et al., 1989). Generally, prolonged resuscitation is accompanied by repeated and increased doses of epinephrine, but this continued administration seems detrimental to functional neurological status.

Strengths of our study include analyzing a patient-oriented outcome endpoint in evaluating epinephrine treatment effects from a long term perspective, and create equipoise about the current standard of resuscitation care. After employing strict statistical adjustment to residual confounders, the findings of this observational study are consistent with previous studies, and can provide more evidence and inspire further investigation on the most appropriate scheme of CA emergency treatment.

This study is limited by its observational nature, failing to establish causal relationships between epinephrine dosing and neurological outcomes and is prone to "resuscitation bias," where the estimates of intra-cardiac arrest interventions will be biased toward a harmful effect (Andersen et al., 2018). Also, the research subjects were comatose patients (Glasgow Coma Scale, GCS <9) admitted after IHCA or OHCA from Department of Intensive Care at Erasme Hospital, Brussels (Belgium). Therefore, there is a certain deficiency in the universality and extrapolation of research. As a secondary analysis, we had limited access to sample size and detailed information, such as quality or duration of CPR, which are important variables when assessing intra-cardiac arrest interventions (Matos et al., 2013; Wengenmayer et al., 2017). Nevertheless, such covariates can be tricky to quantify under specific circumstances, and are also absent from previous studies investigating the impact of epinephrine dose on cardiac arrest survival and neurological outcomes (Callaway, 2013; Dumas et al., 2014; Tujjar et al., 2015). Further studies are therefore needed to validate the impact of total epinephrine dose on long-term neurological outcomes.

CONCLUSION

In this cohort of patients who underwent OHCA or IHCA, we observed an adverse association between total epinephrine dosing during resuscitation and neurological status three months after the event, after adjusting other confounding factors. This negative effect of epinephrine was not eliminated by targeted temperature management (TTM). These finding suggest that further researches are needed to investigate the long-term effect of epinephrine on cardiac arrest patients.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

XS: Conceptualization, methodology, and writing. JY and QP: Data collection. YL, LL, and HC: Writing, Reviewing and editing.

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SUPPLEMENTARY MATERIAL

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Genetic Variants in *PTGS1* and *NOS3* Genes Increase the Risk of Upper Gastrointestinal Bleeding: A Case–Control Study

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Objective: To assess the association between *PTGS1* and *NOS3* variant alleles and the risk to develop upper gastrointestinal bleeding (UGIB) secondary to complicated peptic disease.

Methods: A case–control study was conducted in a Brazilian complex hospital from July 2016 to March 2020. *Case:* Patients with UGIB diagnosis. *Control:* Patients admitted for surgery not related to gastrointestinal disorders. Variables: UGIB (outcome), genetic variants in *PTGS1* and *NOS3* genes (independent), and sex, age, schooling, ethnicity, previous history of gastrointestinal disorders, *Helicobacter pylori* serology, comorbidity, drug therapy, and lifestyle (confounding). The single-nucleotide polymorphisms (SNPs) of the *PTSG1* gene (rs1330344, rs3842787, rs10306114, and rs5788) and *NOS3* gene (rs2070744 and rs1799983) were determined using the real-time polymerase chain reaction. *Helicobacter pylori* serology was determined through the chemiluminescence technique. Logistic regression models were built and deviations of allelic frequencies from *Hardy–Weinberg* equilibrium were verified.

Results: 200 cases and 706 controls were recruited. Carriers of the AG genotype of rs10306114 (OR: 2.55, CI 95%: 1.13–5.76) and CA + AA genotypes of rs5788 (OR: 2.53, CI 95%: 1.14–5.59) were associated with an increased risk for the UGIB development. In nonsteroidal anti-inflammatory drugs (NSAIDs) users, the six variants evaluated modified the magnitude of the risk of UGIB, whereas in low-dose aspirin (LDA) users, an increased risk of UGIB was observed for four of them (rs1330344, rs10306114, rs2070744, and rs1799983). Personal ulcer history (p-value: < 0.001); *Helicobacter pylori* infection (p-value: < 0.011); NSAIDs, LDA, and oral anticoagulant use (p-value: < 0.001); and alcohol intake (p-value: < 0.001) were also identified as independent risk factors for UGIB.

Conclusion: This study presents two unprecedented analyses within the scope of the UGIB (rs10306114 and rs2070744), and our findings showing an increased risk of UGIB in the presence of the genetic variants rs10306114 and rs5788, regardless of the drug exposure. Besides, the presence of the evaluated variants might modify the magnitude of the risk of UGIB in LDA/NSAIDs users. Therefore, our data suggest the need for a personalized therapy and drug use monitoring in order to promote patient safety.

Keywords: drug-related side effects and adverse reactions (MeSH D064420), genetic polymorphism, peptic ulcer disease, pharmacovigilance (MeSH), aspirin (MeSH)

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INTRODUCTION

Genetic variants in the *PTGS1* and *NOS3* genes may be important in the pathogenesis of UGIB, since these genes encode isozymes (e.g., *COX-1* and *COX-2*) and substances (e.g., nitric oxide) involved directly in several physiological processes in the platelet aggregation and gastrointestinal tract (Wallace and Miller, 2000; Brzozowski et al., 2005).

The main role of *COX-1* is platelet activation (Palma-Barqueros et al., 2020) and the production of prostaglandins through the arachidonic acid pathway. Prostaglandins, in turn, as well as nitric oxide, produced by the *NOS3* gene, mediate several gastric protection mechanisms, such as inhibition of gastric acid secretion and stimulation of mucus secretion and blood flow (Lanas, 2008; Agúndez et al., 2015).

The presence of genetic variants that affect the functionality of the *PTGS1* and *NOS3* genes may be clinically associated with a risk of bleeding (Lanas, 2008; Agúndez et al., 2015; Palma-Barqueros et al., 2020) and an increased sensitivity to certain drugs, such as low-dose aspirin (LDA) (Shiotani et al., 2014, 2015) and nonsteroidal anti-inflammatory drugs (NSAIDs) (Figueiras et al., 2016).

LDA is widely prescribed in the prevention and treatment of cardiovascular diseases due to its benefits and cost-effectiveness (Kochar et al., 2010), and a recently conducted meta-analysis suggests a protective effect of LDA against in some cancers (e.g., gastric, esophageal, and colorectal) (Qiao et al., 2018). In Brazil, it is estimated that 24.8% of population uses LDA for primary prevention of cardiovascular events and 34.3% for secondary (Vianna et al., 2012).

Despite the clinical benefits, LDA and NSAIDs have been reported as independent risk factors for upper gastrointestinal bleeding (UGIB) due to their gastro-erosive potential and for the irreversible inhibition of the *COX-1* and *COX-2* isozymes, which compromises the platelet aggregation cascade and increases bleeding risks (Lanas et al., 2015). Besides, a synergism in the potential for gastric damage and risk of UGIB was observed in LDA users with *Helicobacter pylori* infection (Huang et al., 2002) and/or carriers of genetic variants that modulate the platelet aggregation (Maree et al., 2005).

In this context, considering that UGIB is a digestive emergency associated with high morbidity (Quan et al., 2014) and seven out of every hundred patients admitted and underwent upper digestive endoscopy (UDE) in a tertiary hospital were diagnosed with UGIB (Forgerini et al., 2021c), studies have been assessing the influence of genetic variants on this risk, especially in LDA and NSAIDs users.

Through a recent systematic review (2020) (Forgerini et al., 2021a), three studies that evaluated single-nucleotide polymorphisms (SNPs) in the *PTGS1* and *NOS3* genes and the UGIB risk were identified (van Oijen et al., 2006; Wu et al., 2016; Mallah et al., 2020).

The first study was conducted by van Oijen et al. (2006), and the unique SNP (rs3842787) in the *PTGS1* gene assessed was not associated with the risk of UGIB (OR: 0.75, CI 95%: 0.19–3.01) (van Oijen et al., 2006). In 2016, Wu *et al.* evaluated this same SNP and two others (rs1330344 and rs3842788), and association data were only reported for the rs1330344 variant, which identified an increased risk of UGIB in homozygous patients (OR: 2.17, CI 95%: 1.01–4.66) (Wu et al., 2016). Finally, Mallah et al. (2020) evaluated three SNPs in the *PTGS1* gene (rs1330344, rs384287, and rs5788) and one SNP in the *NOS3* gene (rs1799983). The risk of UGIB was identified in LDA users and in the presence of CT + TT (rs1330344) (OR: 4.02, CI 95%: 1.11–14.54) and GT + TT genotypes (rs1799983) (OR: 7.690, CI 95%: 2.4–23.7) (Mallah et al., 2020).

Hence, to our knowledge, no study was conducted in the Brazilian population nor evaluated the SNPs rs10306114 in the *PTGS1* gene and rs2070744 in the *NOS3* gene (Forgerini et al., 2021a). Therefore, it was intended to evaluate the influence of those four SNPs already as described in the literature in addition to the two unpublished ones (rs10306114 and rs2070744) and the risk of UGIB.

METHODS

Study Design, Setting, and Ethical Aspects

A case–control study, matched by sex, age (±5 years), and recruitment date (3 months), was conducted in the hospital complex of Clinical Hospital of the Ribeirão Preto Medical School of the University of São Paulo, Brazil.

This study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice of the International Conference on Harmonization. Besides, the study was also registered in the Registro Brasileiro de Ensaios Clínicos (REBEC–number: RBR-3hstqm).

The report of this study was based on Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (von Elm et al., 2008).

Participants

Participants were recruited for the case and control groups from July 2016 to March 2020. Considering the control group must be representative and recruited from the same "study base" as the case participants, participants of both groups were recruited from the same hospital complex (Setia, 2016).

Definition of Case and Control Groups

Patients admitted to the complex hospital with UGIB secondary to complicated peptic disease defined as the presence of endoscopically proven ulcers, bleeding erosions or perforated ulcers, and symptoms of hematemesis, dark vomiting or in coffee grounds, melena and/or hematochezia were included. Patients with excludable endoscopic diagnoses (e.g., esophageal varices, gastric neoplasia, and *Mallory–Weiss* tears) and patients with in-hospital UGIB were excluded. Secondary exclusion criteria included patients who did not undergo UDE within 48 h of hospital admission, discharge from hospital 15 days prior to the current admission, serious health condition, death, and those patients whose interview within the 15-day period preceding admission was not possible.

Being identified as a case, the patient was invited to participate in the study, and control participants were collected, matched by

sex, age (±5 years), and hospital admission time (the maximum period of up to three months after the UDE date of the case participant). Control group participants were recruited from the preoperative unit of the same hospital complex, among patients who underwent surgery not related to gastrointestinal disorders (i.e., cataracts, inguinal/umbilical hernias, plastic, and prostate adenomas).

Patients under 18 years old; history of neoplasia, coagulopathies, immunodeficiency syndrome, and cirrhosis (history of disease); narcotics; carriers of a nasogastric or percutaneous tube; and nonresidents of the region of the study for at least three months were excluded.

Data Collection and Variables

Four researchers (M.F, G.U, P.C.M, and T.R.N) conducted the recruitment of the participants in the case and control groups, using a questionnaire previous designed, validated (Figueiras et al., 2016), and adapted to be applied in Brazil. The data obtained during the interview with patients and/or family members were confirmed in secondary sources, such as the electronic medical record, medical prescriptions, and laboratory tests.

The interviewers evaluated the interviews on a scale from zero to 10, considering the reliability of the information recalled. The interview consisted of low reliability with scores below six points, and a reference value was adopted following the study by Figueiras et al. (2016).

Dependent variable: diagnosis of UGIB secondary to complicated peptic disease through UDE or exploratory laparotomy and clinical symptoms.

Independent variable: the presence of genetic variants in *PTGS1* and *NOS3* genes.

Confounding variables: sex, age, ethnicity, schooling, body mass index, previous personal history of gastrointestinal disorders (ulcer, dyspepsia, and bleeding), familiar history of ulcer, comorbidities (cardiovascular, blood, renal, kidney and respiratory diseases; high blood pressure; diabetes *mellitus*; dyslipidemia; depression; arthritis; and arthrosis), *Helicobacter pylori* infection, drug therapy in use (LDA, NSAIDs, oral anticoagulants, proton pump inhibitors, H2 receptors antagonists, and antidepressants), lifestyle habits (smoking, alcohol, and coffee consumption), and reliability of interviews.

An index date was defined as the day on which signs and symptoms of UGIB started for the cases and the date of the interview for the controls (Figueiras et al., 2016). To assess drug exposure, an etiological window of seven days prior to the index day was considered. Besides, LDA use was defined as the use of aspirin in doses lower than 150 mg and for a minimum period of three months.

The lifestyle habits in the two months preceding the index date were recorded. The smoking habit was stratified into nonsmoker, smoker and ex-smoker. For smokers, the amount of tobacco consumed was stratified as "moderate" (1–15 cigarettes/day) and "heavy" (>15 cigarettes/day).

Alcohol consumption in the two months prior to the index date was stratified into alcohol consumption and nonconsumption. Among the participants who consumed alcohol, the consumption was measured through the average amount in grams of alcohol consumed per day, being 0; >0 to ≤30 g of alcohol/day; and> 30 g of alcohol/day.

Smoking and alcohol consumption were stratified according the study of Figueiras et al. (2016).

Coffee consumption was stratified as consume or no consume. In those patients who consumed coffee, the consumption was categorized according to the frequency (daily, weekly, and monthly) and amount (mL = 0 ml; 0 < mL ≤ 100; and >100 ml).

Collection of Biological Sample

After the interview, approximately 5.0 ml of whole blood was collected in ethylenediaminetetraacetic acid from the participants in the case and control groups for further analysis of the proposed genetic variants and the search for antibodies for *Helicobacter pylori* infection (IgG).

Genotyping

The genomic DNA obtaining and quantification as well as genotyping were carried out at Centro de Medicina Genômica of Clinical Hospital of the Ribeirão Preto Medical School of the University of São Paulo and Laboratory of Pathology of the Ribeirão Preto Medical School of the University of São Paulo.

Genomic DNA extraction was carried out using the Maxwell® 16 Blood DNA Purification Kit (Promega, Madison, WI, United States) and the DNA quantification through the assay with Qubit ™ dsDNA HS Assay Kit (Applied Biosystems, Foster City, United States).

The genotyping technique was led in the StepOnePlus real-time PCR thermocycler (Applied Biosystems, Foster City, United States) using TaqMan Drug Metabolism Genotyping Assay technology (Applied Biosystems, Foster City, United States). For the *PTGS1* gene, the following assays were used: rs1330344 [C > T], assay ID C_31956972_10; rs10306114 [A > G], assay ID C_30090763_10; rs3842787 [C > T], assay ID C_30252576_20; rs5788 [C > A], and assay ID C_11722597_20. For the *NOS3* gene, the following assays were used: rs1799983 [G > T], assay ID C_3219460_20; and rs2070744 [C > T], assay ID C_15903863_10.

The real-time PCR conditions were performed with the following protocol: 1 min at 60°C, 10 min at 95°C, 40 cycles of denaturation for 15 s at 95°C, 40 cycles of annealing for 1 min at 60°C, and final extension for 1 min at 60°C. For quality control of genotyping, 10% of the samples were reanalyzed randomly.

Helicobacter pylori Serology

The serology of *Helicobacter pylori* in order to determine IgG antibodies was performed on frozen plasma samples through the chemiluminescence technique. The test results were stratified into reagent (≥1.10 U IgG/mL), non-reagent (≤0.90 U IgG/mL), and inconclusive (0.91–1.09 U IgG/mL).

Data Tabulation

The variables collected through the interviews were typed weekly in the Research Electronic Data Capture (REDCap) database, in addition to the results of genetic analyzes and laboratory tests for *Helicobacter pylori* infection.

Statistical Analysis

The statistical distribution was tested using the Kolmogorov–Smirnov test, and considering that normality was identified, parametric statistical tests were used. Quantitative data were described through the mean and standard deviation, and qualitative data were described through absolute and relative frequency. Continuous variables were analyzed using the T-*Student* test and categorical variables using the chi-square test (BioEstat 5.0). Deviations of allelic frequencies from *Hardy–Weinberg* equilibrium were verified using the chi-square test (BioEstat 5.0).

The association between *PTGS1* and *NOS3* variant alleles and the risk to develop UGIB was estimated by odds ratio (OR) adopting a level of statistical significance of 0.05 for decision-making and building confidence interval with 95% (CI 95%) using unconditional logistic regression models (Kuo et al., 2018) in SPSS 20.0 software (IBM Company, Chicago, IL, United States).

The unconditional logistic regression models were designed for the dependent binomial variable (case or control), and participants with wild phenotype (the absence of genetic variant) and not users of LDA or NSAIDs were established as the reference category. Besides, considering the genetic variants, the participants were stratified in wild *vs.* heterozygous and homozygous genotype as well as wild *vs.* variant genotype (heterozygous + homozygous for variant allele).

A bivariate analysis was performed according to the outcome (UGIB) and all the predictive covariates of this study. From the bivariate analysis, variables with p-value ≤ 0.20 were selected for the logistic regression model. The regression model was built with all the predictive variables selected in the bivariate analysis using the methods of Insert (inclusion of all variables concomitantly).

In addition to the main logistic model, two other models were built. The first considered only LDA users and the second NSAIDs users. Therefore, the influence of the presence of genetic variants in these two subgroups was assessed.

Data Transparency

This study is part of a larger study that aims to assess genetic variants associated with the risk of UGIB secondary to complicated peptic ulcer in the Brazilian population. The full-methodology protocol is available at Open Science Framework (OSF) (Forgerini et al., 2021b).

RESULTS

Two thousand eight hundred eighty-three UDE tests were conducted for 2,557 patients admitted to the complex hospital of Clinical Hospital of the Ribeirão Preto Medical School of the University of São Paulo.

As primary exclusion (n = 2,268), patients under 18 years old (n = 194), excludable endoscopic diagnoses (n = 1,828), in-hospital UGIB (n = 91), admission not due to UGIB (n = 79), those with a history of disease (n = 70), carriers of a nasogastric or percutaneous tube (n = 4), and nonresidents of the region of the study for at least three months (n = 2) were excluded. Secondary exclusion criteria included (n = 109) patients whose interview within the 15-day period preceding admission was not possible (n = 55), patients who did not undergo UDE within 48 h of hospital admission (n = 30), death (n = 9), discharge from hospital 15 days prior to the current admission (n = 8), and serious health condition (n = 7).

Therefore, through the daily monitoring and analysis of the UDE conducted during the period of this study, 180 patients were recruited for the case group. Besides, another 20 patients were also included after hospital admission for perforated gastric or duodenal ulcer and later endoscopic analysis (ICD10 K251 and K261). Thus, 200 patients were recruited to the case group and were paired with 706 controls.

Considering the epidemiological profile of the participants, most were men (case group: 72.5% and control group: 72.5%), self-declared white (case group: 67.3% and control group: 73.5%), and with a mean age of 60.1 (±16.3) and 59.9 (±15.8) years, respectively. Most of the patients were reagent for *Helicobacter pylori* infection (case group: 76.3% and control group: 57.6%), noncurrent smokers (case group: 70.1% and control group: 84.7%), abstain (case group: 51.5% and control group: 55.5%), and consumed coffee (case group: 85.0% and control group: 90.8%).

No interview received a score lower than six points, and most received scores higher than eight points (54.5% in the case group and 68.4% in the control group). The average of the interview scores was 7.60 (standard deviation: ± 1.26) in the case group and 8.27 (standard deviation: ± 1.36) in the control group. The baseline characteristics of the participants are described in **Table 1**.

The *Hardy–Weinberg* equilibrium was observed in both groups (case and control) for the six SNPs assessed, and the frequency of genetic variants in *PTGS1* and *NOS3* genes is described in **Table 2**.

Regardless of LDA and NSAIDs use, an increased risk of UGIB was observed in carriers of AG genotype *vs.* AA of rs10306114 (OR: 2.55, CI 95%: 1.13–5.76) and in carriers of CA + AA genotypes *vs.* CC of rs5788 (*PTGS1* gene) (OR: 2.53, CI 95%: 1.14–5.59) (**Table 3**).

Considering the *PTGS1* gene, LDA users showed an UGIB risk about four times higher in those carriers of the CT + TT genotypes *vs.* CC of rs1330344 (OR: 3.73, CI 95%: 2.00–6.95) and carriers of the AG + GG *vs.* AA of rs10306114 (OR: 4.15, CI 95%: 1.28–13.49). In NSAIDs users, the four variants evaluated were associated with the risk of UGIB (rs1330344, rs3842787, rs10306114, and rs5788), and this risk ranged from 2.71 in carriers of the CA + AA genotypes *vs.* CC of rs5788 (CI 95%: 1.251–5.88) to 5.69 in carriers of the AG + GG genotypes *vs.* AA of rs10306114 (CI 95%: 1.46–22.07) (**Table 4**).

Regarding the *NOS3* gene, LDA users showed an UGIB risk about four times higher in those carriers of the CT + TT genotypes *vs.* CC of rs2070744 (OR: 3.66, CI 95%: 1.90–7.04) and in carriers of the GT + TT genotypes *vs.* GG of rs1799983 (OR: 4.21, CI 95%: 2.00–8.59). A higher risk of UGIB was observed in NSAIDs users than in LDA users: CT + TT genotypes *vs.* CC of rs2070744 (OR: 4.43, CI 95%: 2.37–8.26)

TABLE 1 | Baseline characteristics of the participants of case (n = 200) and control (n = 706) groups.

Variable	Case (%) n = 200	Control (%) n = 706	p-value[a]
Sex (men)	145 (72.5)	512 (72.5)	0.995
Mean age (±SD)	60.2 (±16.3)	59.8 (±15.8)	0.750[b]
Mean schooling (±SD)	6.3 (±4.3)	6.2 (±4.6)	0.750
Ethnicity			0.091[c,d]
White	134 (67.0)	516 (73.1)	
Black	65 (32.5)	186 (26.3)	
Oriental	1 (0.5)	4 (0.6)	
Body mass index (kg/m^2)			0.003[d]
Underweight	10 (5.1)	15 (2.1)	
Normal weight	80 (40.4)	197 (28.0)	
Overweight	60 (30.3)	263 (37.4)	
Obesity I	30 (15.2)	152 (21.6)	
Obesity II	13 (6.6)	55 (7.8)	
Obesity III	5 (2.5)	21 (3.0)	
Missing data	2	3	
Reliability of the interview			<0.001[d]
6–7	91 (45.5)	223 (31.6)	
8–9	77 (38.5)	32 (45.9)	
>9	32 (16.0)	159 (22.5)	
Previous history of gastrointestinal disorders			
Familiar history of ulcer	41 (21.8)	132 (22.3)	0.870
Personal history of dyspepsia	60 (30.2)	291 (41.2)	0.004[d]
Personal history of ulcer	44 (22.1)	64 (9.1)	<0.001[d]
Personal history of bleeding	35 (17.6)	94 (13.3)	0.135[d]
***Helicobacter pylori* serology**			<0.001[d]
Reagent	142 (76.3)	388 (57.6)	
No reagent	44 (23.7)	286 (42.4)	
Plasma sample not satisfactory	14	32	
Comorbidity			
Cardiovascular disease	62 (31.5)	131 (19.1)	<0.001[d]
Blood disease	3 (1.5)	42 (6.0)	0.009[d]
Renal disease	7 (3.5)	36 (5.2)	0.348
Respiratory disease	8 (4.0)	71 (10.1)	0.007[d]
High blood pressure	104 (52.0)	371 (52.5)	0.891
Diabetes *mellitus*	38 (19.1)	158 (22.4)	0.306
Dyslipidemia	21 (10.7)	165 (23.4)	<0.001[d]
Depression	20 (10.1)	81 (11.5)	0.559
Arthrosis	9 (4.5)	48 (6.9)	0.237
Arthritis	3 (1.5)	21 (3.0)	0.3218
Drug therapy in use (ATC)			
Proton pump inhibitors (A02BC)	36 (18.0)	125 (17.7)	0.923
H2 receptors antagonists (A02BA)	03 (1.5)	10 (1.4)	0.930
Oral anticoagulants (B01A)	22 (11.0)	18 (2.5)	<0.001[d]
Low-dose aspirin (B01AC06)	51 (25.5)	90 (12.7)	<0.001[d]
Nonsteroidal anti-inflammatory drugs (M01A)	43 (23.6)	71 (10.2)	<0.001[d]
Antidepressants (N06A)	19 (9.5)	70 (9.9)	0.962
Lifestyle			
Smoking habit			<0.001[d]
Nonsmoker	136 (70.1)	580 (84.7)	
Moderate (1–15 cigarettes/day)	23 (11.9)	52 (7.6)	
Heavy (>15 cigarettes/day)	35 (18.0)	53 (7.7)	

(Continued on following page)

TABLE 1 | (*Continued*) Baseline characteristics of the participants of case (*n* = 200) and control (*n* = 706) groups.

Variable	Case (%) *n* = 200	Control (%) *n* = 706	*p*-value[a]
Alcohol intake (average of grams of alcohol/day)			<0.001[d]
Abstain (0 g)	103 (51.5)	392 (55.5)	
Little (0 > consume ≤30 g of alcohol/day)	71 (35.5)	297 (42.1)	
Moderate (>30 g of alcohol/day)	26 (13.0)	17 (2.4)	
Missing data	1	5	
Coffee consumption			0.260
No	29 (14.5)	65 (9.2)	
Yes	171 (85.5)	641 (90.8)	
Daily coffee consumption	159 (79.5)	599 (84.8)	
Weekly coffee consumption	10 (5.0)	35 (5.0)	
Monthly coffee consumption	2 (1.0)	6 (0.8)	
Amount of coffee consumption per day (ml)			<0.001[d]
mL = 0	30 (15.0)	66 (9.3)	
0 < mL ≤ 100	101 (50.5)	469 (66.4)	
mL > 100	69 (314.5)	171 (24.2)	

[a]p-value *is polychotomous and represents the entire variable.*
[b]p-value *obtained in the T-*Student *test. The other* p-*values were obtained using the chi-square test.*
[c]*Oriental ethnicity was not considered for the chi-square test (number of participants <5) in these categories.*
[d]*Variables with* p-*value ≤ 0.20 and selected for the multivariate model.*
ATC, Anatomical Therapeutic Chemical; SD, standard deviation.

and carriers of the GT + TT genotypes *vs.* GG of rs1799983 (OR: 6.53, CI 95%: 3.10–13.44), respectively (**Table 4**).

In the logistic regression model, personal history of ulcer (OR: 3.82, CI 95%: 1.88–7.75); *Helicobacter pylori* infection (OR: 1.91, CI 95%: 1.16–3.16); LDA (OR: 3.70, CI 95%: 1.88–7.27), NSAIDs (OR: 4.66, CI 95%: 2.57–8.43), and oral anticoagulant use (OR: 10.58, CI 95%: 3.58–31.25); and alcohol intake (0 < consume ≤30 g of alcohol/day: OR: 2.58, CI 95%: 1.41–4.74 and >30 g of alcohol/day: 7.98, CI 95%: 2.98–21.32) were identified as independent risk factors for UGIB (**Table 3**).

Personal history of dyspepsia (OR: 0.40, CI 95%: 0.24–0.69), overweight (OR: 0.54, CI 95%: 0.33–0.89), blood (OR: 0.14, CI 95%: 0.02–0.81) and respiratory disease (OR: 0.18, CI 95%: 0.05–0.57), and dyslipidemia (OR: 0.21, CI 95%: 0.10–0.46) were identified as protection factors for UGIB. Heterogeneity in reliability of the interview was identified between the case and control groups (chi-square < 0.001—**Table 1**), and this variable showed statistical significance in the logistic regression model when scores were eight and nine (OR: 0.57, CI 95%: 0.34–0.95) (**Table 3**).

DISCUSSION

This is an unprecedented study in the Brazilian population, and it assessed the influence of six genetic variants in the *PTGS1* (rs1036114, rs3842787, rs1330344, and rs5788) and *NOS3* genes (rs2070744 and rs1799983) on the risk of UGIB.

The four genetic variants evaluated in the *PTGS1* gene were associated with an increased risk of UGIB. Despite a wide clinical review that reported genetic variants in this gene to appear more associated with the prognosis of a disease than with the risk of developing it (Agúndez et al., 2015), it has also been suggested that carriers of these genetic variants are at an increased risk of bleeding (Halushka et al., 2003; Maree et al., 2005). Indeed, carriers of variants rs10306114 and rs3842787 may have a *COX-1* with less capacity to produce prostaglandins, which can also enhance sensitivity to LDA and NSAIDs (Halushka et al., 2003), and carriers of variants rs1330344 and rs5788 may show a reduction in the platelet aggregation and, consequently, an increased risk of bleeding (Maree et al., 2005).

Except for rs1036114, which is an unprecedented analysis, three other studies assessed the influence of rs3842787, rs1330344, and rs5788 on the UGIB risk (van Oijen et al., 2006; Wu et al., 2016; Mallah et al., 2020). In line with our findings, Mallah and colleagues suggested that rs1330344 was associated with an increased risk of UGIB in LDA users (Mallah et al., 2020), and Wu and colleagues reported rs1330344 as a possible risk factor for the gastric mucosal injury induced by LDA (Wu et al., 2016),whereas two studies did not identify association between genetic variants rs3842787 and rs5788 and UGIB risk (van Oijen et al., 2006; Wu et al., 2016).

Comparing our findings with those of these three studies, it is important to highlight that despite the enriching clinical discussion carried out by van Oijen et al. (2006) and Wu et al. (2016), there are important methodological limitations (van Oijen et al., 2006; Wu et al., 2016). In the study by van Oijen et al., there is a lack of clarity in the recruitment and matching process, in addition to a small sample size (106 cases and 74 controls), while in the study by Wu et al., aspects of unmatched participants, not adjusting to confounding variables, and inclusion of only 13 participants in the UGIB group were

TABLE 2 | Description of frequency of genetic variants in *PTGS1* and *NOS3* genes between the case and control groups and *Hardy–Weinberg* equilibrium.

Variable	Case (%) *n* = 200	Control (%) *n* = 706	*p*-value[a]
PTGS1 gene			
rs1330344 [C > T]			0.095
CC (*wild type*)	28 (14.2)	69 (9.8)	
CT	82 (41.6)	274 (38.9)	
TT	87 (44.2)	362 (51.3)	
CT + TT	169 (85.8)	636 (90. 2)	
HWE (*p*-value)	0.2302	0.1088	
rs10306114 [A > G]			0.011*
AA (*wild type*)	161 (83.9)	631 (91.2)	
AG	30 (15.6)	60 (8.7)	
GG	1 (0.5)	1 (0.1)	
AG + GG	31 (16.1)	61 (8.8)	
HWE (*p*-value)	0.7528	0.7298	
rs3842787 [C > T]			0.699
CC (*wild type*)	157 (79.3)	577 (81.8)	
CT	40 (20.2)	124 (17.6)	
TT	1 (0.5)	4 (0.6)	
CT + TT	41 (20.7)	128 (18.2)	
HWE (*p*-value)	0.3576	0.3335	
rs5788 [C > A]			0.098
CC (*wild type*)	102 (52.3)	422 (60.4)	
CA	76 (39.0)	235 (33.6)	
AA	17 (8.7)	42 (6.0)	
CA + AA	93 (47.7)	277 (39.6)	
HWE (*p*-value)	0.5989	0.2287	
***NOS3* gene**			
rs2070744 [C > T]			0.907
CC (*wild type*)	38 (19.5)	141 (20.2)	
CT	88 (45.1)	321 (46.1)	
TT	69 (35.4)	235 (33.7)	
CT + TT	157 (80.5)	556 (79.8)	
HWE (*p*-value)	0.3012	0.1025	
rs1799983 [G > T]			0.414
GG (*wild type*)	92 (47.4)	359 (51.4)	
GT	87 (44.8)	277 (39.6)	
TT	15 (7.7)	63 (9.0)	
GT + TT	102 (52.5)	340 (48.6)	
HWE (*p*-value)	0.3681	0.3650	

[a]p-value *obtained in the* chi-square test.
HWE, Hardy–Weinberg *equilibrium.*

observed. From another perspective, besides the methodological aspects, the epidemiological profile and population miscegenation of the included participants should be considered. It is well known that the Brazilian population is highly mixed, which will directly influence our findings, since ethnicity and ancestry markers influence the presence and frequency of genetic variants in populations (Ramos et al., 2014; Rodrigues-Soares et al., 2018).

Regarding the *NOS3* gene, the SNPs rs2070744 and rs1799983 have been widely studied as possible risk factors for cardiovascular diseases (Zhang et al., 2012; Nawaz et al., 2015), and rs1799983 was identified as a genetic biomarker of coronary artery disease in a meta-analysis of 132 case–control studies (Li et al., 2019). Meanwhile, little is known regarding the presence of these variants in the pathophysiology of UGIB.

Despite several studies that evaluated the influence of genetic variants in the idiosyncratic response to LDA treatment (Shiotani et al., 2014, 2015; Wang, 2019) and also in interindividual differences in platelet activation in LDA users (Postula et al., 2011), to our knowledge, only one study assessed the rs1799983 within the scope of UGIB (Mallah et al., 2020). Mallah et al. found an increased risk of UGIB in the presence of genetic variation in LDA users (OR: 7.69, CI 95%: 2.40–23.7, *p*-value: < 0.001), corroborating with our findings (OR: 4.21, CI 95% 2.00–8.89, *p*-value: 0.001). The wide confidence interval observed in the study by Mallah et al. may be justified by the low sample size of participants included in the analysis (nine cases and five controls) when compared to the sample of our study (28 cases and 38 controls).

One hypothesis for the identified increased risk of UGIB in LDA users would be that the presence of the genetic variant rs1799983 influences the expression of *NOS3* gene activity and affects serum nitrate levels, which compromises important physiological mechanisms (e.g., gastric protection and platelet aggregation) (Lanas, 2008; Vecoli, 2014). Therefore, the compromise of these mechanisms, in addition to possible gastrointestinal damage associated with LDA use (Lanas et al., 2015), may be a clinical rationale for the increased risk of gastrointestinal bleeding.

Considering LDA is widely prescribed in the prevention of cardiovascular events, an increase on UGIB risk can be an important healthcare issue. In addition, despite well-established benefits of LDA in secondary prevention (Parekh et al., 2013), the risks can outweigh the unclear benefits (e.g., reduction of mortality or incidence of new cardiovascular events) in primary prevention (Collins et al., 2009), especially due to the risks of bleeding (Kochar and Gaziano, 2010). Furthermore, although several studies have been assessing the use of LDA in cancer prevention, a systematic review that included 103,787 participants did not identify a reduction in the incidence of cancer or in the overall cancer mortality (Chubak et al., 2016).

In this context, individualized assessment of the risk/benefit of LDA and NSAIDs use, treatment monitoring, and development of guidelines for the evaluation of genetic variants potentially involved in idiosyncratic responses may be effective strategies in the risk management. In NSAIDs and warfarin users, for instance, international guidelines already recommend the assessment of genetic variants involved in the metabolism of these drugs and potentially associated with possible safety risks (e.g., *CYP2C9* and *VKOCR1*) (Johnson et al., 2011; Swen et al., 2011). Besides, an effective risk communication to improve signal detection of possible adverse drug events related to LDA use can help reduce the harm of medication. However, in Brazil, despite efforts to harmonize pharmacovigilance regulations with the world, several concerns remain, particularly those that impair effective risk communication and signal detection (e.g., a lack of

TABLE 3 | Risk of upper gastrointestinal bleeding secondary to complicated peptic disease after the logistic regression model.

Variable	Logistic regression model		
	Insert method		
	OR	CI 95%	*p*-value
Constant	-	-	0.034
Ethnicity (self-declared black)	1.21	0.71–2.05	0.468
Ethnicity (self-declared oriental)	4.41	0.40–47.93	0.222
Body mass index (underweight)	1.20	0.34–4.25	0.771
Body mass index (overweight)	0.54	0.33–0.89	0.017[a]
Personal history of dyspepsia	0.40	0.24–0.69	<0.001[a]
Personal history of ulcer	3.82	1.88–7.75	<0.001[a]
Personal history of bleeding	1.59	0.82–3.10	0.166
Helicobacter pylori (reagent)	1.91	1.16–3.16	0.011[a]
Cardiovascular disease	1.54	0.82–2.88	0.177
Blood disease	0.14	0.02–0.81	0.028[a]
Respiratory disease	0.18	0.05–0.57	0.003[a]
Dyslipidemia	0.21	0.10–0.46	<0.001[a]
Oral anticoagulants use	10.58	3.58–31.25	<0.001[a]
LDA use	3.70	1.88–7.27	<0.001[a]
NSAIDs use	4.66	2.57–8.43	<0.001[a]
Smoking habit (1–15 cigarettes/day)	1.61	0.75–3.43	0.213
Smoking habit (>15 cigarettes/day)	1.97	0.97–3.87	0.058
Alcohol intake (0 < consume ≤30 g of alcohol/day)	2.58	1.41–4.74	0.002[a]
Alcohol intake (>30 g of alcohol/day)	7.98	2.98–21.32	<0.001[a]
Coffee consumption (0 < mL ≤ 100 ml)	0.48	0.23–1.00	0.051
Coffee consumption (>100 ml)	1.02	0.46–2.23	0.963
Reliability of the interview (scores 8–9)	0.57	0.34–0.95	0.030[a]
Reliability of the interview (score 10)	0.65	0.33–1.27	0.210
rs1330344 (CT genotype)	0.13	0.67–1.89	0.632
rs1330344 (TT genotype)	1.29	0.62–2.62	0.483
rs1330344 (CT + TT genotypes)	1.14	0.64–2.00	0.650
rs10306114 (AG genotype)	2.55	1.13–5.764	0.024[a]
rs10306114 (GG genotype)	-	-	-[b]
rs10306114 (AG + GG genotypes)	0.63	0.31–1.30	0.219
rs3842787 (CT genotype)	1.13	0.62–1.89	0.632
rs3842787 (TT genotype)	1.29	0.67–2.69	0.483
rs3842787 (CT + TT genotypes)	0.63	0.31–1.30	0.219
rs5788 (CA genotype)	1.00	0.61–1.63	0.990
rs5788 (AA genotype)	0.72	0.28–1.81	0.488
rs5788 (CA + AA genotypes)	2.53	1.14–5.59	0.022[a]
rs2070744 (CT genotype)	0.76	0.46–1.25	0.287
rs2070744 (TT genotype)	0.81	0.40–1.63	0.566
rs2070744 (CT + TT genotypes)	0.75	0.47–1.19	0.227
rs1799983 (GT genotype)	0.91	0.54–1.54	0.732
rs1799983 (TT genotype)	1.04	0.58–2.07	0.760
rs1799983 (GT + TT genotypes)	0.99	0.59–1.53	0.385

OR, odds ratio; CI 95%, confidence interval 95%; LDA, low-dose aspirin; NSAIDs, nonsteroidal anti-inflammatory drugs.

[a]*Statistical significance.*

[b]*It was not possible to conduct the analysis due to the small number of participants in each group (one case and one control).*

The category of reference for genetic variants is the wild phenotype.

awareness or incentive to report patient effects and underreporting) (Varallo et al., 2019).

It is important to highlight that in addition an increased risk of UGIB was also identified with other drug class use (e.g., oral anticoagulants), *Helicobacter pylori* infection, and lifestyle habits (e.g., alcohol intake). Hence, to identify patients at higher risk for UGIB or rebleeding, a clinical care pathway for the management of the patients diagnosed with UGIB is needed (Franco et al., 2015). Furthermore, studies have been documenting the effectiveness of the prescription of proton pump inhibitors in patients on LDA therapy or with high risk of UGIB (Lanas et al., 2011) as well as eradication of *Helicobacter pylori* (Lanas and Scheiman, 2007).

Finally, our study has strengths and limitations. To our knowledge, this is the first Brazilian study to assess genetic variants of the *PTGS1* and *NOS3* genes within the scope of the UGIB, in addition to unprecedented analysis of two SNPs (rs10306114 and rs2070744). The following can be mentioned as strengths of this study: the adjusted analysis for well-documented variables for UGIB risk (e.g., NSAIDs, oral anticoagulant, and LDA use; *Helicobacter pylori* infection; and lifestyle habits), inclusion of only biologically unrelated patients in order to reduce possible biases, and data collection through face-to-face interviews. The limitations of this study are the impossibility to analyze the influence of carrying more than

TABLE 4 | Logistic regression model for genetic variants in *PTGS1* and *NOS3* genes in low-dose aspirin and nonsteroidal anti-inflammatory drug users, and risk of upper gastrointestinal bleeding secondary to complicated peptic disease.

Variable	Logistic regression model			
	N case/control	Insert method		
		OR	CI 95%	p-value
LDA users[a]				
PTGS1 gene				
rs1330344 (C > T)				
CT	22/31	5.76	2.50–13.23	<0.001[b]
TT	26/52	2.71	1.27–5.75	0.009[b]
CT + TT genotypes	48/83	3.73	2.00–6.95	<0.001[b]
rs10306114 (A > G)				
AG	8/10	3.95	1.19–13.07	0.024[b]
GG	1/0	—	—	—[c]
AG + GG genotypes	9/10	4.15	1.28–13.50	0.018[b]
rs3842787 (C > T)				
CT	10/15	2.42	0.81–7.21	0.110
TT	1/0	—	—	—[c]
CT + TT genotypes	11/15	2.56	0.88–7.44	0.084
rs5788 (C > A)				
CA	18/33	1.83	0.76–4.39	0.176
AA	3/8	1.58	0.33–7.42	0.558
CA + AA genotypes	21/41	1.77	0.81–3.86	0.151
NOS3 gene				
rs2070744 (C > T)				
CT	27/39	4.00	1.84–8.71	<0.001[b]
TT	16/30	3.20	1.29–7.92	0.012[b]
CT + TT genotypes	43/69	3.66	1.90–7.04	<0.001[b]
rs1799983 (G > T)				
GT	26/29	4.54	2.10–9.82	<0.001[b]
TT	2/9	2.15	0.30–15.43	0.445
GT + TT genotypes	28/38	4.21	2.00–8.89	<0.001[b]
NSAID users[d]				
PTGS1 gene				
rs1330344 (C > T)				
CT	17/30	5.74	2.56–12.85	0.024[b]
TT	15/35	2.47	1.11–5.50	0.027[b]
CT + TT genotypes	32/65	3.70	2.05–6.66	<0.001[b]
rs10306114 (A > G)				
AG	5/8	5.69	1.46–22—07	0.012[b]
GG	0/0	—	—	—[c]
AG + GG genotypes	—	—	—	—
rs3842787 (C > T)				
CT	8/13	5.69	1.86–17.39	0.002[b]
TT	0/0	—	—	—[c]
CT + TT genotypes	—	—	—	—

(Continued on following page)

TABLE 4 | (*Continued*) Logistic regression model for genetic variants in *PTGS1* and *NOS3* genes in low-dose aspirin and nonsteroidal anti-inflammatory drug users, and risk of upper gastrointestinal bleeding secondary to complicated peptic disease.

Variable	Logistic regression model			
	N case/control	Insert method		
		OR	CI 95%	p-value
rs5788 (C > A)				
CA	12/23	2.38	0.98–5.77	0.055
AA	4/5	4.21	0.84–21.03	0.079
CA + AA genotypes	16/28	2.71	1.25–5.88	0.012[b]
NOS3 gene				
rs2070744 (C > T)				
CT	15/35	2.50	1.13–5.54	0.024[b]
TT	16/15	10.99	4.25–28.38	<0.001
CT + TT genotypes	31/50	4.43	2.37–8.26	<0.001[b]
rs1799983 (G > T)				
GT	17/23	4.57	2.01–10.37	<0.001[b]
TT	5/4	37.07	5.67–242.21	<0.001[b]
GT + TT genotypes	22/27	6.53	3.10–13.74	<0.001[b]

N, number of participants of case/control groups; OR, odds ratio; CI 95%, confidence interval 95%; LDA, low-dose aspirin; NSAIDs, nonsteroidal anti-inflammatory drugs.
[a]Analysis adjusted to ethnicity; body mass index; history of ulcer, bleeding and dyspepsia; cardiovascular, blood, and respiratory diseases; Helicobacter pylori serology; reliability of the interview; use of oral anticoagulants and nonsteroidal anti-inflammatory drugs; smoking habit; alcohol intake; and amount of coffee consumption per day.
[b]Statistical significance.
[c]It was not possible to conduct the analysis with the homozygous category for variant allele due to the absence of participants or reduced sample size.
[d]Analysis adjusted to ethnicity; body mass index; history of ulcer, bleeding and dyspepsia; cardiovascular, blood and respiratory disease; Helicobacter pylori serology; reliability of the interview; use of oral anticoagulants and low-dose aspirin; smoking habit; alcohol intake; and amount of coffee consumption per day.
The category of reference for genetic variants is the wild phenotype.

one SNP and the risk of UGIB in addition to the reduced sample size of LDA and NSAIDs users.

In summary, this study presents two unprecedented analyses within the scope of the UGIB (rs10306114 and rs2070744), and our findings suggested an increased risk of UGIB in the presence of the variants rs10306114 and rs5788, regardless of the drug exposure. Besides, the presence of the evaluated genetic variants might modify the magnitude of the risk of UGIB in LDA/NSAIDs users. Hence, patients undergoing chronic LDA and NSAIDs treatment should be monitored in order to reduce harmful medication use, and our findings can contribute with evidence to the scope of pharmacogenomics and personalized therapy, promoting patient safety.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee of the São Paulo State University (protocol number 1.657.615) and Clinical Hospital of the Ribeirão Preto Medical School of the University of São Paulo (protocol number 1.536.886). Besides, the study was also registered in the Registro Brasileiro de Ensaios Clínicos (REBEC-number: RBR-3hstqm). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MF participated in methodology, patients' recruitment, genetic analysis, investigation, formal analysis, data curation, writing—original draft, and writing—review and editing. GU and TN participated in methodology, patients' recruitment, investigation, formal analysis, data curation, and writing—review and editing. SB and AF participated in the genetic analysis, formal analysis, and writing—review and editing. PM participated in methodology, patients' recruitment, formal analysis, data curation, writing—review and editing, project administration, and funding acquisition. All authors contributed in the idealization and writing of this manuscript, and approved the submitted version.

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Relationship Between Mean Vancomycin Trough Concentration and Mortality in Critically Ill Patients: A Multicenter Retrospective Study

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Background: It remains unclear whether the mean vancomycin trough concentration (VTC) derived from the entire course of therapy is of potential benefit for critically ill patients. This study was conducted to explore the association between mean serum VTC and mortality in intensive care units (ICUs).

Methods: 3,603 adult patients with two or more VTC records after receiving vancomycin treatment in the eICU Collaborative Research Database were included in this multicenter retrospective cohort study. Mean VTC was estimated using all measured VTCs and investigated as a continuous and categorical variable. Patients were categorised into four groups according to mean VTC: <10, 10–15, 15–20, and >20 mg/L. Multivariable logistic regression and subgroup analyses were performed to investigate the relationship of mean VTC with mortality.

Results: After adjusting for a series of covariates, logistic regression analyses indicated that mean VTC, as a continuous variable, was positively correlated with ICU (odds ratio, 1.038, 95% confidence interval, [1.014–1.063]) and hospital (1.025 [1.005–1.046]) mortalities. As a categorical variable, mean VTC of 10–15 mg/L was not associated with reduced ICU (1.705 [0.975–2.981]) and hospital (1.235 [0.829–1.841]) mortalities. Mean VTC of 15–20 mg/L was not correlated with a lower risk of hospital mortality (1.370 [0.924–2.029]). Moreover, mean VTCs of 15–20 and >20 mg/L were significantly associated with higher ICU mortality (1.924 [1.111–3.332]; 2.428 [1.385–4.258]), and mean VTC of >20 mg/L with higher hospital mortality (1.585 [1.053–2.387]) than mean VTC of <10 mg/L. Similar results were observed in patients with different Acute Physiology and Chronic Health Evaluation IV score, creatinine clearance, age, and body mass index subgroups.

Conclusion: Mean VTC was not associated with reduced ICU/hospital related mortality. Our results suggested that VTC monitoring might not guarantee vancomycin efficacy for ICU patients.

Keywords: vancomycin trough concentration, intensive care unit, mortality, eICU collaborative research database, observational study

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INTRODUCTION

Vancomycin, a glycolpeptide bactericide that acts by obstructing the synthesis of the bacterial cell wall, was isolated from streptomycin approximately half a century ago (Moellering, 2006). It is widely used to treat Gram-positive bacterial infections, including methicillin-resistant *Staphylococcus aureus* (MRSA) and exhibits time-dependent bactericidal activity with a long post-antibiotic effect (Álvarez et al., 2016; Liu et al., 2011; Rybak, 2006). Therapeutic drug monitoring (TDM) is an adjuvant and practical method used for vancomycin dosing adjustment in intensive care units (ICUs) because of its narrow therapeutic window (Tabah et al., 2015). Based on infection models and clinical pharmacokinetic/pharmacodynamic (PK/PD) studies, the area under the concentration-time curve over 24 h/minimum inhibitory concentration ($AUC_{0-24\,h}$/MIC) has been established as the most predictive index to reflect the clinical and microbiological efficacies of vancomycin (Jung et al., 2014; Moise-Broder et al., 2004). Bacterial clearance, along with improvements in clinical signs and symptoms, has been suggested to be associated with AUC_{0-24h}/MIC ≥ 400 (Makmor-Bakry et al., 2019; Rybak et al., 2020). However, it is difficult to precisely determine multiple serum/tissue vancomycin concentrations during the same dosing interval to calculate the AUC in clinical practice (Rybak et al., 2009).

Serum vancomycin trough concentration (VTC) monitoring before the fourth dose has been suggested as the most practical method for TDM of vancomycin, as a VTC 15–20 mg/L may achieve an AUC_{0-24h}/MIC ≥400 if the MIC is ≤ 1 mg/L (Rybak et al., 2009). The Infectious Diseases Society of America has recommended that the VTC should be maintained above 10 mg/L to avoid the development of resistance, and 15–20 mg/L to improve clinical outcomes (Rybak et al., 2009). The Chinese Pharmacological Society recommended 10–15 mg/L as the target VTC for adult patients, and 10–20 mg/L for adult patients with severe MRSA infections (Ye et al., 2016). However, plenty of studies have demonstrated that VTC did not have a considerable effect on treatment outcomes (Albur et al., 2012; Jung et al., 2014; Liang et al., 2018; Makmor-Bakry et al., 2019) and highlighted a high incidence of inappropriate VTC leading to increased healthcare costs (Seng et al., 2018). As a result, the latest consensus in 2020 advocated vancomycin area under the concentration-time curve (AUC) values obtained by Bayesian modelling as the most accurate approach for managing vancomycin dosing (Rybak et al., 2020). Nevertheless, to our knowledge, those most retrospective and prospective studies only focused on the initial VTC after vancomycin therapy. It remains unclear whether the mean VTC, estimated using all measured VTCs during the entire course of treatment, is beneficial for critically ill patients. Therefore, this multicenter observational study with a large sample was further performed to investigate the association of mean VTC with mortality in critically ill patients.

MATERIALS AND METHODS

Data Source and Study Design

This multicenter observational study was performed using the eICU Collaborative Research Database (eICU-CRD, version 2.0), which is a public de-identified ICU database comprising 200,859 patient unit encounters for 139,367 unique patients admitted between 2014 and 2015. The eICU database is available from https://physionet.org/content/eicu-crd/. Patients were admitted to 1 of 335 units at 208 hospitals located throughout the United States (Pollard et al., 2018). The eICU-CRD includes data on vital signs, laboratory measurements, medications, Acute Physiology and Chronic Health Evaluation (APACHE) components, care plan information, admission diagnoses, time-stamped diagnoses, and treatments. All researchers of this study received the necessary training and obtained permission to access the database.

Patient Selection

Adult patients (≥18 years) receiving vancomycin therapy with a single hospital admission and two or more TDM records on the first ICU stay were included in this study. The exclusion criteria were as follows: 1) patients with an ICU length of stay ≤24 h, 2) patients without records of their ICU discharge status, 3) patients with missing or unqualified covariates for multivariable adjustment, and 4) patients with an outlier value of VTC. The upper and lower fences represented values more and less than Q3 and Q1, respectively, by 1.5 times the interquartile range (IQR). The outlier value of VTC was defined as the value above or below the upper (mean VTC >30.70 mg/L) or lower (mean VTC <1.82 mg/L) fences (IQR was calculated using the formula: Q3—Q1).

Outcomes and Covariates

The outcomes of this study were ICU and hospital mortalities. The variables, mean VTC or serum creatinine (Scr), were calculated by dividing the sum of all collected VTC or Scr by the frequency of monitoring. Mean creatinine clearance (CCl) was determined by the Cockcroft-Gault equation: ([140—age in years] × weight in kg/[72 × mean Scr concentration in mg/dl]) × 0.85 if female (Cockcroft and Gault, 1976). Related treatments (such as dialysis, ventilation, vasodepressor, and vasopressor administration) were included, as these may reflect illness severity and/or affect VTC throughout ICU stay. Variables such as demographic data (e.g., age, sex, and ethnicity), initial body mass index (BMI), APACHE IV score, and comorbidities that could influence mortality (e.g., sepsis, burns, pancreatitis, gastrointestinal bleed, diabetes, heart failure, chronic obstructive pulmonary disease [COPD], hepatic failure, tumour, pneumonia, and renal failure) were also assessed during the first 24 h of ICU admission.

Statistical Analysis

Based on the recommended VTC in a series of guidelines (Rybak et al., 2009; Matsumoto et al., 2013; Ye et al., 2016), we divided the mean VTC into four categories: <10, 10–15, 15–20, and >20 mg/L. Continuous variables were presented as the medians (IQR) and compared using the Kruskal-Wallis H test. Categorical variables were presented as frequencies (percentages) and compared using χ^2 or Fisher's exact tests. A Chord diagram showed the connection between the initial VTC and mean VTC for each patient. Logistic regression models were used to investigate the association of the mean VTC, as a continuous and categorical variable, with ICU and hospital mortalities. To flexibly model and visualise the relationship between mean VTC and mortality, we also

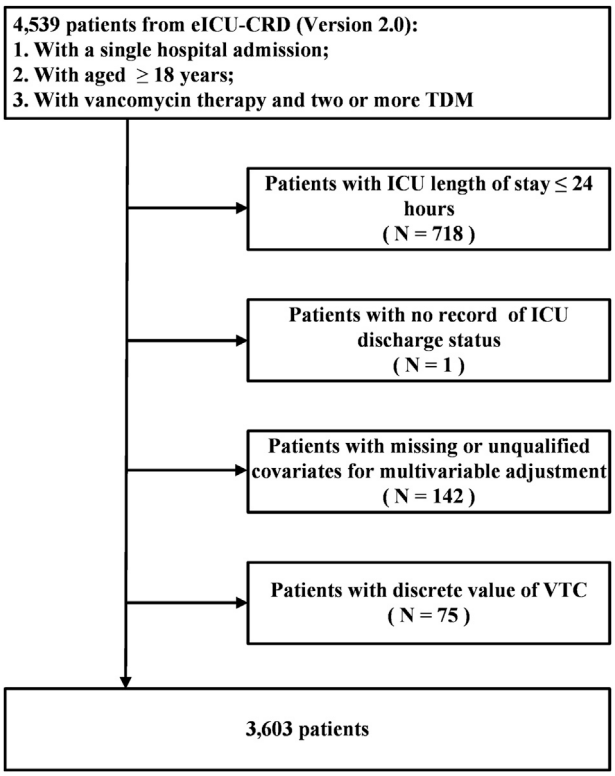

FIGURE 1 | Flow Chart of Participant Selection. Cohort selection and criteria for exclusion, a total of 3,603 patients were included in the analysis. eICU-CRD, eICU Collaborative Research Database; TDM, therapeutic drug monitoring; ICU, intensive care unit; VTC, vancomycin trough concentration.

used restricted cubic splines with four knots at the fifth, 35th, 65th, and 95th percentiles. Interaction and subgroup analyses were conducted to determine whether the relationship between mean VTC and mortality persisted when the severity of the clinical status (≤64, or >64), CCl (≤80, or >80 ml/min), age (≤60, or >60 years), or BMI (≤30, or >30 kg/m^2) changed. In addition, the subpopulation with records of vancomycin dose and duration was abstracted for another analysis. All tests were two-sided, and a p value < 0.05 was considered statistically significant. Data were extracted using the SAS version 9.4 software (SAS Institute, Cary, NC, United States), and all statistical analyses were performed using SPSS 22.0 (SPSS, Inc., Chicago, IL, United States) and Stata 14.0 (Stata Corp., College Station, TX, United States).

RESULTS

Individual Selection and Clinical Characteristics

A total of 4,539 adult patients with a single hospital stay and at least two VTC records at the first ICU admission were extracted from the eICU-CRD. The followings were excluded: 718 patients with a length of ICU stay ≤24 h, 1 patient without a record of ICU discharge status, 142 patients with missing or unqualified covariates for multivariable adjustment, and 75 patients with a discrete VTC. Finally, 3,603 patients were included in this study (**Figure 1**). The patients were divided into the following four groups according to the mean VTC during ICU stay: <10 mg/L (n = 372, 10.3%), 10–15 mg/L (n = 1,165, 32.3%), 15–20 mg/L (n = 1,261 35.0%), and >20 mg/L (n = 805, 22.3%). Age, BMI, mean VTC, number of VTC, APACHE IV score, Scr, CCl, and use of vasodepressor, vasopressor and dialysis were significantly different among the four groups (p < 0.05). Additionally, there was a significant difference in the prevalence of COPD, heart failure, and renal failure (p < 0.05). Groups with a higher mean VTC had higher ICU (4.6, 8.2, 11.6, and 15.4%, respectively; p < 0.001) and hospital (10.5, 14.1, 18.5, and 22.0%, respectively; p < 0.001) mortalities (**Table 1**). The Chord diagram presented the connection between the mean VTC and initial VTC for each patient (**Figure 2**). Among all patients, there were 1,622 (45.0%) patients with the mean VTC and the initial VTC in the same groups. 250 (19.7%) patients with initial VTC <10 mg/L had a mean VTC 15–20 mg/L. Among patients with initial VTC 10–15 mg/L, 458 (44.3%) patients reached a mean VTC within 15–20 mg/L. And 182 (27.8%) patients with initial VTC >20 mg/L had a mean VTC 15–20 mg/L.

Association of Mean VTC With Mortality

The univariable logistic regression model revealed that the mean VTC, as a continuous variable, was positively correlated with ICU (odds ratio, 1.073, 95% confidence interval, [1.051–1.095]) and hospital (1.054 [1.036–1.072]) mortalities. This association was still robust (1.038 [1.014–1.063]; 1.025 [1.005–1.046], respectively) after adjusting for age, sex, ethnicity, BMI, APACHE IV score, CCl, the use of ventilation, dialysis, vasodepressor and vasopressor, and diagnoses at ICU admission (**Table 2**). When mean VTC was considered as a categorical variable, patients with mean VTCs of 10–15, 15–20, and >20 mg/L were associated with higher ICU mortality (1.854 [1.091–3.150]; 2.734 [1.632–4.582]; 3.802 [2.254–6.414]), and those with mean VTCs of 15–20 and >20 mg/L showed a significant increase for hospital mortality (1.935 [1.349–2.776]; 2.407 [1.660–3.489]) compared with those with mean VTC <10 mg/L in the univariable logistic regression analyses. After multivariable adjustment, mean VTC of 10–15 mg/L was not associated with reduced ICU (1.705 [0.975–2.981]) and hospital (1.235 [0.829–1.841]) mortalities. Mean VTC of 15–20 mg/L was not correlated with a lower risk of hospital mortality (1.370 [0.924–2.029]). Moreover, mean VTCs of 15–20 and >20 mg/L were significantly associated with higher ICU mortality (1.924 [1.111–3.332]; 2.428 [1.385–4.258]), and mean VTC of >20 mg/L with increased hospital mortality (1.585 [1.053–2.387]) compared with mean VTC of <10 mg/L (**Table 2**). Restricted cubic splines visually showed that the risks of ICU (A) and hospital (B) mortalities increased with an increasing mean VTC (**Supplementary Figure 1**).

Association of Mean VTC With Mortality in Different Subgroups

We further analyzed the association between mean VTC and mortality in different predefined subgroups: APACHE IV score

TABLE 1 | Baseline characteristics of the study cohort according to mean VTC categories.

Characteristics	Entire population	Mean VTC				p Value
		< 10 mg/L	10–15 mg/L	15–20 mg/L	> 20 mg/L	
	(*N* = 3,603)	(*N* = 372)	(*N* = 1,165)	(*N* = 1,261)	(*N* = 805)	
Age n (%)						<0.001
≤60 years	1,674 (46.5)	209 (56.2)	569 (48.8)	529 (42.0)	367 (45.6%)	
>60 years	1929 (53.5)	163 (43.8)	596 (51.2)	732 (58.0)	438 (54.4%)	
Sex n (%)						0.264
Male	2097 (58.2)	199 (53.5)	690 (59.2)	739 (58.6)	469 (58.3)	
Female	1,506 (41.8)	173 (46.5)	475 (40.8)	522 (41.4)	336 (41.7)	
Ethnicity n (%)						0.303
Caucasian	2,791 (77.5)	277 (74.5)	910 (78.1)	990 (78.5)	614 (76.3)	
Others	812 (22.5)	95 (25.5)	255 (21.9)	271 (21.5)	191 (23.7)	
BMI (kg/m^2) median (IQR)	27.41 (23.25,33.30)	25.90 (22.25,31.60)	26.81 (23.02,32.55)	27.73 (23.48,33.39)	28.86 (24.09,35.13)	<0.001
Mean VTC (mg/L) median (IQR)	15.90 (12.55,19.57)	8.49 (7.25,9.35)	12.77 (11.50,13.89)	17.17 (16.05,18.46)	22.53 (21.00,24.80)	<0.001
Number of VTC median (IQR)	2 (2,3)	2 (2,3)	2 (2,3)	3 (2,4)	2 (2,3)	<0.001
APACHE IV score median (IQR)	64 (48,83)	58 (41,77)	62 (47,80)	64 (48,84)	69 (53,89)	<0.001
Vasodepressor n (%)	1,743 (48.4)	161 (43.3)	541 (46.4)	615 (48.8)	426 (52.9)	0.006
Vasopressor n (%)	1,318 (36.6)	127 (34.1)	380 (32.6)	494 (39.2)	317 (39.4)	0.002
Ventilation n (%)	2,361 (65.5)	240 (64.5)	732 (62.8)	852 (67.6)	537 (66.7)	0.081
Scr (mg/dl) median (IQR)	0.89 (0.66,1.35)	0.68 (0.52,0.90)	0.77 (0.61,1.10)	0.95 (0.70,1.38)	1.17 (0.83,1.89)	<0.001
CCl (ml/min) median (IQR)	96.57 (59.36,144.69)	129.72 (86.58,182.60)	109.82 (70.33,158.53)	91.87 (57.72,133.30)	75.22 (43.51,119.53)	<0.001
Dialysis n (%)	231 (6.4)	5 (1.3)	47 (4.0)	87 (6.9)	92 (11.4)	<0.001
Diagnoses n (%)						
Tumour	304 (8.4)	28 (7.5)	99 (8.5)	104 (8.2)	73 (9.1)	0.831
Hepatic failure	25 (0.7)	0 (0)	8 (0.7)	11 (0.9)	6 (0.7)	0.124
COPD	302 (8.4)	18 (4.8)	110 (9.4)	125 (9.9)	49 (6.1)	0.001
Heart failure	307 (8.5)	13 (3.5)	85 (7.3)	127 (10.1)	82 (10.2)	<0.001
Diabetes	433 (12.0)	36 (9.7)	149 (12.8)	156 (12.4)	92 (11.4)	0.392
Gastrointestinal bleed	285 (7.9)	38 (10.2)	87 (7.5)	100 (7.9)	60 (7.5)	0.354
Pancreatitis	57 (1.6)	4 (1.1)	20 (1.7)	19 (1.5)	14 (1.7)	0.820
Burns	8 (0.2)	2 (0.5)	3 (0.3)	3 (0.2)	0 (0)	0.183
Pneumonia	950 (26.4)	83 (22.3)	315 (27.0)	345 (27.4)	207 (25.7)	0.237
Sepsis	1,276 (35.4)	114 (30.6)	410 (35.2)	466 (37.0)	286 (35.5)	0.169
Renal failure	510 (14.2)	34 (9.1)	153 (13.1)	192 (15.2)	131 (16.3)	0.005
ICU Mortality n (%)	382 (10.6)	17 (4.6)	95 (8.2)	146 (11.6)	124 (15.4)	<0.001
Hospital mortality n (%)	613 (17.0)	39 (10.5)	164 (14.1)	233 (18.5)	177 (22.0)	<0.001

VTC, vancomycin trough concentration; BMI, body mass index; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation; Scr, serum creatinine; CCl, creatinine clearance; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit.

≤64 (n = 1,841) and APACHE IV score >64 (n = 1,762); CCl ≤80 ml/min (n = 1,393) and CCl >80 ml/min (n = 2,210); age ≤60 years (n = 1,674) and age>60 years (n = 1,929); BMI ≤30 kg/m^2 (n = 2,245) and BMI >30 kg/m^2 (n = 1,358). There was no significant interaction stratified by APACHE IV score (ICU mortality, $p_{\text{interaction}}$ = 0.545; hospital mortality, $p_{\text{interaction}}$ = 0.058), CCl (ICU mortality, $p_{\text{interaction}}$ = 0.807; hospital mortality, $p_{\text{interaction}}$ = 0.769), age (ICU mortality, $p_{\text{interaction}}$ = 0.469; hospital mortality, $p_{\text{interaction}}$ = 0.314) and BMI (ICU mortality, $p_{\text{interaction}}$ = 0.636; hospital mortality, $p_{\text{interaction}}$ = 0.627). Notably, mean VTC, as a continuous variable, was significantly correlated with both ICU and hospital mortalities in APACHE IV score >64, CCl ≤80 ml/min, age ≤60 years and BMI ≤30 kg/m^2 subgroups. When mean VTC was used as a categorical variable, similar results were observed across the different subgroups. Compared with patients with mean VTC of <10 mg/L, those with mean VTCs of 10–15 and 15–20 mg/L were not associated with reduced ICU and hospital mortalities in all subgroups (**Table 3**; **Supplementary Tables 1, 2, 3**).

We collected 1,553 patients with the records of vancomycin dose and duration to exclude the impact of vancomycin administration on the prognosis of patients. There were significantly statistical differences in total vancomycin dose, average daily dose, and duration of vancomycin exposure across the four groups (p < 0.05). The multivariable logistic regression model was built to adjust a series of covariates, including average daily dose and duration of vancomycin exposure, which demonstrated that the relationship between mean VTC and mortality still persisted (**Supplementary Table 4**).

DISCUSSION

In this retrospective multicenter cohort study, we recruited a total of 3,603 critically ill patients with two or more VTC monitoring records after vancomycin treatment from 335 different ICUs at 208 hospitals in the eICU-CRD. Mean VTC was calculated by dividing the sum of all collected VTC by the frequency of

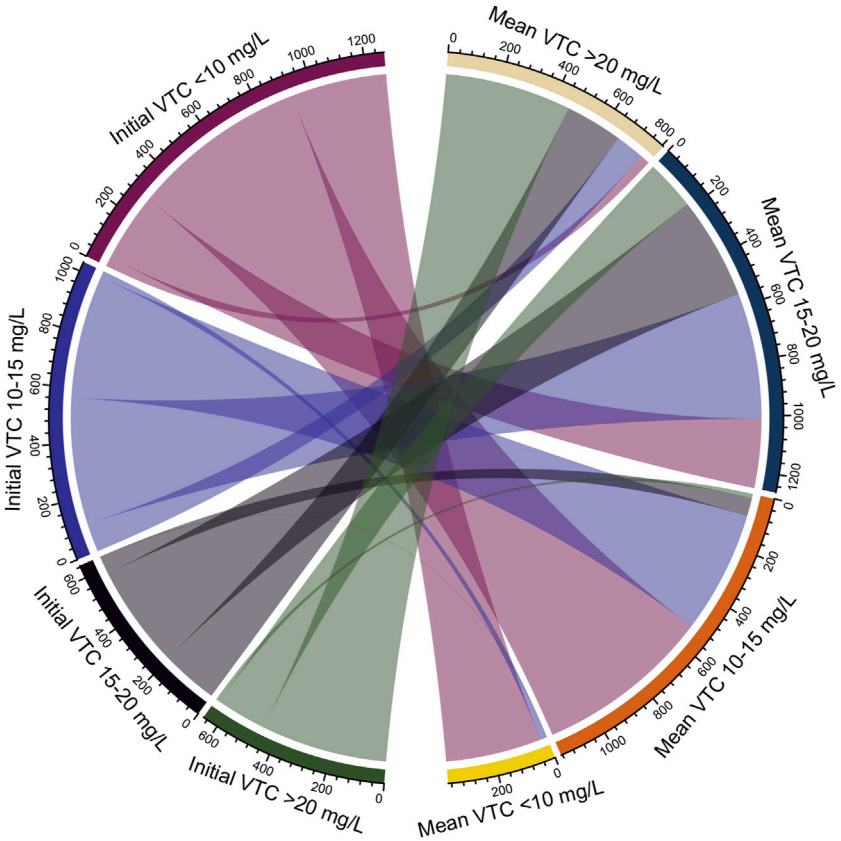

FIGURE 2 | Connection Between the Mean VTC and Initial VTC. A chord diagram presented the difference of the mean VTC with initial VTC for each patient. VTC, vancomycin trough concentration.

TABLE 2 | Logistic analysis for the association of mean VTC with mortality.

	ICU mortality		Hospital mortality	
VTC variable	**OR (95% CI)**	***p* value**	**OR (95% CI)**	***p* value**
Univariable model				
Continuous variable	1.073 (1.051,1.095)	<0.001	1.054 (1.036,1.072)	<0.001
Categorical variable				
<10 mg/L	1		1	
10–15 mg/L	1.854 (1.091,3.150)	0.022	1.399 (0.966,2.026)	0.076
15–20 mg/L	2.734 (1.632,4.582)	<0.001	1.935 (1.349,2.776)	<0.001
>20 mg/L	3.802 (2.254,6.414)	<0.001	2.407 (1.660,3.489)	<0.001
Multivariable model				
Continuous variable	1.038 (1.014,1.063)	0.002	1.025 (1.005,1.046)	0.012
Categorical variable				
<10 mg/L	1		1	
10–15 mg/L	1.705 (0.975,2.981)	0.061	1.235 (0.829,1.841)	0.299
15–20 mg/L	1.924 (1.111,3.332)	0.019	1.370 (0.924,2.029)	0.117
>20 mg/L	2.428 (1.385,4.258)	0.002	1.585 (1.053,2.387)	0.027

Multivariable model: *adjusted for age (category), sex, ethnicity, BMI, APACHE IV score, CCl, the use of ventilation, dialysis, vasodepressor and vasopressor, and diagnoses at ICU admission (tumour, hepatic failure, COPD, heart failure, diabetes, gastrointestinal bleed, pancreatitis, burns, pneumonia, sepsis, and renal failure).*
VTC, vancomycin trough concentration; ICU, intensive care unit; OR, odds ratio; CI, confidence interval; BMI, body mass index; APACHE, Acute Physiology and Chronic Health Evaluation; CCl, creatinine clearance; COPD, chronic obstructive pulmonary disease.

monitoring for each patient. Our study showed that mean VTCs of 10–15, 15–20, and >20 mg/L were not associated with reduced ICU and hospital mortalities for critically ill patients. Patients with mean VTCs of 15–20 and >20 mg/L were found to be exposed to even higher risks of ICU or hospital mortality compared with those with mean VTC of <10 mg/L. The

TABLE 3 | Multivariable analysis for the association of mean VTC with mortality in Apache IV score subgroups.

VTC variable	ICU mortality		Hospital mortality	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
APACHE IV score ≤64				
Continuous variable	0.995 (0.952,1.040)	0.838	0.980 (0.946,1.015)	0.257
Categorical variable				
<10 mg/L	1		1	
10–15 mg/L	1.486 (0.588,3.758)	0.403	0.857 (0.475,1.546)	0.609
15–20 mg/L	1.443 (0.576,3.618)	0.434	0.803 (0.446,1.449)	0.467
>20 mg/L	1.459 (0.549,3.873)	0.449	0.809 (0.423,1.550)	0.523
APACHE IV score >64				
Continuous variable	1.057 (1.027,1.087)	<0.001	1.049 (1.024,1.074)	<0.001
Categorical variable				
<10 mg/L	1		1	
10–15 mg/L	1.724 (0.864,3.438)	0.122	1.552 (0.916,2.629)	0.102
15–20 mg/L	2.053 (1.043,4.040)	0.037	1.889 (1.125,3.172)	0.016
>20 mg/L	2.895 (1.457,5.751)	0.002	2.333 (1.371,3.970)	0.002

Multivariable model: *adjusted for age (category), sex, ethnicity, BMI, CCl, the use of ventilation, dialysis, vasodepressor and vasopressor, and diagnoses at ICU admission (tumour, hepatic failure, COPD, heart failure, diabetes, gastrointestinal bleed, pancreatitis, burns, pneumonia, sepsis, and renal failure).*

VTC, vancomycin trough concentration; APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; OR, odds ratio; CI, confidence interval; BMI, body mass index; CCl, creatinine clearance; COPD, chronic obstructive pulmonary disease.

results indicated that maintaining therapeutic serum VTC between 15 mg/L and 20 mg/L throughout the entire course of vancomycin treatment failed to demonstrate a benefit for ICU and hospital mortalities in ICU patients, and implied that VTC might not be a suitable monitoring indicator to ensure vancomycin efficacy for critically ill patients.

As serum VTC has been suggested as a surrogate marker for the AUC/MIC index to monitor vancomycin efficacy, several studies have been designed to verify the association of VTC with clinical and microbiological outcomes in critically ill patients (Rybak et al., 2009). Surprisingly, most studies found no statistical difference in treatment outcomes according to the VTC level. Two retrospective studies have shown that VTC alone is not a good indicator for vancomycin treatment success among patients with MRSA bacteremia (Jung et al., 2014; Makmor-Bakry et al., 2019). Two other retrospective cohort studies have demonstrated that VTC >15 mg/L fails to improve the outcomes of patients with MRSA infections (Clemens et al., 2011; Hall et al., 2014). Additionally, two prospective, multicenter, observational studies have demonstrated no significant association between VTC level and vancomycin treatment response in a Chinese population diagnosed with gram-positive bacterial infections (Liang et al., 2018; Shen et al., 2018). Moreover, the latest consensus suggests that there are minimal to no data to support the safety and efficacy of a target VTC of 15–20 mg/L in patients with serious MRSA infections (Rybak et al., 2020). However, those aforementioned studies only explored the relationship between single steady-state VTC and outcomes. In a retrospective study of 76 critically ill patients confirmed MRSA infections, *Cheong* reported that the initial VTC was not associated with treatment response, which was consistent with the results of the other studies. Surprisingly, a corrected VTC, calculated as dividing the sum of each measured VTC multiplied by the number of days at that level by the total number of days under treatment, was higher among patients with improved clinical presentation and laboratory results than among those with poor clinical outcomes (Cheong et al., 2012). Because of the lack of large-scale population studies on multiple VTC records after receiving vancomycin therapy, which may represent personalised PK/PD profiles of vancomycin, whether the mean VTC derived from the entire course of therapy is of potential benefit for critically ill patients remains unclear.

In this study, the mean VTC was estimated using all collected VTCs throughout the therapy course, providing an overall level of VTC during the ICU stay. The chord diagram showed 19.7% patients with initial VTC < 10 mg/L, 44.3% patients with initial VTC 10–15 mg/L, and 27.8% patients with initial VTC > 20 mg/L had reached a mean VTC 15–20 mg/L eventually, which indicated that initial sub-therapeutic or excessive VTC had been adjusted to achieve suggested VTC. Therefore, mean VTC could reflect exposure dosage of vancomycin after adjustment for those with lower or higher initial VTC, to some extent. We further found that mean VTCs of 15–20 and >20 mg/L were significantly correlated with higher ICU mortality (1.924-fold and 2.428-fold), mean VTC of >20 mg/L with higher hospital mortality (1.585-fold) than a mean VTC of <10 mg/L. Moreover, mean VTCs of 10–15 and 15–20 mg/L were not associated with reduced ICU/hospital related mortality. Therefore, this present study is one in a growing number of studies demonstrating that maintaining therapeutic VTC might not ensure vancomycin efficacy for critically ill patients. For clinics, our findings suggested that the interpretation of VTC results should be considered cautiously in clinical practice.

The APACHE IV score is useful for assessing the severity of illness and predicting outcomes in ICU patients (Ko et al., 2018; Zimmerman et al., 2006). To diminish the influence of disease severity itself, all individuals were divided into two subgroups for further investigations based on the median first APACHE IV score (≤64 or >64). Vancomycin is eliminated primarily via the renal route, with >80–90% recovered unchanged in urine within 24 h after administration of a single dose (Rybak, 2006). A decrease in the glomerular filtration rate for any cause would

increase the VTC, making the association between mortality and VTC difficult to assess (Cantú et al., 1994). To exclude the influence of renal function on outcomes, we classified the patients into two groups according to CCl (≤80 or >80 ml/min) to assess this relationship. Meanwhile, the age and BMI of patient associated with mean VTC can be very significant for mortality (Haeseker et al., 2016; Tsai et al., 2018). Sensitivity analyses were also performed in patients of different ages (≤60 or >60 years) and BMIs (≤30 or >30 kg/m^2). There is no interaction between mean VTC and those variables for ICU as well as hospital mortalities, which indicated that those variables did not impact the association between mean VTC and mortality. Subgroup analyses also found similar results about the relationships of mean VTC with mortality in all predefined subgroups. Therefore, mean VTC was not associated with improved outcomes regardless of the severity of illness, the degree of renal function, age, or BMI. Based on these results, The AUC/MIC or Bayesian AUC-only estimation may be considered as possible alternative methods for vancomycin administration.

A few underlying mechanisms may explain our results. First, Patel et al. reported that patients with a VTC of 15–20 mg/L could achieve an $AUC_{0\text{-}24}$/MIC ratio of ≥400 when the MIC value is ≤ 1 (Patel et al., 2011). However, several studies have demonstrated a poor correlation between VTC and $AUC_{0\text{–}24}$ because of high inter-individual variability (Bakke et al., 2017; Haeseker et al., 2016; Neely et al., 2014; Tsai et al., 2018). A 3-years, prospective study indicated that 68% of adults who were administered vancomycin with an $AUC_{0\text{-}24}$ ≥ 400 mg h/L had a trough concentration of <15 mg/L (Neely et al., 2018). Additionally, a prospective study of Chinese patients suggested that C_{max}, $AUC_{0\text{–}24}$, and $AUC_{0\text{–}24\ h}$/MIC are not significantly associated with clinical and microbiological outcomes based on multivariable logistic regression analysis (Shen et al., 2018). Second, vancomycin has been associated with nephrotoxicity, which is the most serious common side effect of vancomycin linked to a higher risk of mortality (Jeffres, 2017). A multicenter prospective clinical trial including 288 adult patients indicated that a VTC of >15 mg/L is associated with a 3-fold increased risk of nephrotoxicity (Bosso et al., 2011). Another retrospective study in the 315 vancomycin-treated patients observed that vancomycin nephrotoxicity is independently correlated with higher VTC of >20 mg/L (Park et al., 2018). The result is similar to those of our study, in which mean VTC >15 mg/L was associated with increased ICU or hospital mortality. Therefore, the optimal TDM of vancomycin in ICU patients warrants further investigation.

Our study has some limitations. First, given the retrospective nature of the study, the risk of unmeasured confounding effects and the introduction of bias were unavoidable. However, this multicenter study of VTC included the largest sample size to date, allowing the findings to be generalised. Second, there is no available information on microbiological outcomes and acute kidney injury after vancomycin treatment in eICU-CRD. Nevertheless, mortality may represent clinical outcomes to confirm the prognostic value of VTC, as critically ill patients with gram-positive bacterial infection have high mortality, particularly owing to MRSA infections (Calfee et al., 2014). Third, hospital protocols for the time of VTC monitoring, target VTC, and adjustments of vancomycin were not available due to the retrospective design. However, the sensitivity analysis found the association was persisted in less than half of the patients with records on vancomycin dose and duration. Fourth, we can provide only the association between mean VTC and mortality rather than causality. In the future, a well-designed randomised controlled trial with more treatment information should be considered to evaluate causality between VTC and mortality.

CONCLUSION

In conclusion, mean VTC was not associated with reduced ICU/hospital related mortality, independent of the degree of disease severity, renal function, age, and BMI. Our results indicated that VTC monitoring might not ensure vancomycin efficacy for ICU patients. Therefore, the interpretation of VTC results should be considered cautiously for clinics in clinical practice. The AUC/MIC or Bayesian AUC-only estimation may be considered as possible alternative methods for vancomycin administration, and warrant further investigations in future studies.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://eicu-crd.mit.edu.

ETHICS STAEMENT

As all protected patient health-related information in the eICU had been deleted, the requirement for individual patient consent was waived. The database is released under the Health Insurance Portability and Accountability Act (HIPAA) safe harbor provision. The re-identification risk was certified as meeting safe harbor standards by Privacert (Cambridge, MA) (HIPAA Certification no. 1031219-2).

AUTHOR CONTRIBUTIONS

GW, YH, and JR conceived of the study. GW provided funding for the study. GW, XL and XW provided critical appraisal and revision. JL, RL, YG, XJ, JZ, and JR extracted and collected data from eICU-CRD. YH, XJ, and JL performed the statistical analysis. YH and JR wrote the manuscript. YH, JR, JL, XJ, YG, RL, and JZ revised the manuscript. 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.2021.690157/full#supplementary-material

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Safety Profile of Ibrutinib: An Analysis of the WHO Pharmacovigilance Database

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As ibrutinib has become a standard of care in B-cell malignancies in monotherapy or in combination with other agents, definition of its safety profile appears essential. The aim of this study was to further characterize the safety profile of ibrutinib through the identification of potential safety signals in a large-scale pharmacovigilance database. All serious individual case safety reports (ICSRs) in patients aged ≥18 years involving ibrutinib suspected in the occurrence of serious adverse drug reactions or drug interacting from November 13th, 2013 to December 31st, 2020 were extracted from VigiBase, the World Health Organization global safety database. Disproportionality reporting was assessed using the information component (IC) and the proportional reporting ratio (PRR), with all other anticancer drugs used as the reference group. To mitigate the confounding of age, two subgroups were considered: patients aged<75 years and ≥75 years. A signal of disproportionate reporting (SDR) was defined if both IC and PRR were significant. A total of 16,196 ICSRs were included. The median age of patients was 72.9 years, 42.6% of ICSRs concerned patients aged ≥75 years, and 64.2% male patients. More than half (56.2%) of ICSRs resulted in hospitalization or prolonged hospitalization. Among 713 SDRs, 36 potential safety signals emerged in ibrutinib-treated patients, mainly ischemic heart diseases, pericarditis, uveitis, retinal disorders and fractures. All potential safety signals having arisen in this analysis may support patient care and monitoring of ongoing clinical trials. However, owing to the mandatory limitations of this study, our results need further confirmation using population-based studies.

Keywords: ibrutinib, Bruton's tyrosine kinase inhibitor, drug safety, adverse drug reaction, pharmacovigilance

Abbreviations: ATC, anatomical therapeutic chemical, BCR, b-cell receptor; BTK, bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; EMA, european medicines agency; FDA, US food and drug administration; IC, information component; IC025, credibility interval; ICSR, individual case safety report; IQR, interquartile range; MAP, mitogen-activated protein; MedDRA, medical dictionary for regulatory activities; MCL, mantle cell lymphoma, PDGFR-β, platelet derived growth factor receptor β, PRR, proportional reporting ratio; PT, preferred term; rT3, reverse triiodothyronine; SADR, serious adverse drug reaction; SDR, signal of disproportionate reporting; SmPC, summary of product characteristics; T4, thyroxin; UMC, uppsala monitoring center; VEGF, vascular endothelium growth factor; VEGFR, vascular endothelium growth factor receptor; WHO, world health organization; WM, waldenström macroglobulinemia; 95% CI, 95% confidence interval.

INTRODUCTION

Ibrutinib, a first-in-class, oral, once-daily, Bruton's tyrosine kinase (BTK) inhibitor, has been demonstrated as an effective treatment for chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), Waldenström macroglobulinemia (WM), marginal zone lymphoma and chronic graft versus host disease (Treon et al., 2015; Chanan-Khan et al., 2016; Miklos et al., 2017; Dimopoulos et al., 2018; Burger et al., 2019; Byrd et al., 2019; Moreno et al., 2019; Shanafelt et al., 2019). By targeting BTK, ibrutinib impairs B-cell receptor (BCR) signaling pathway and inhibits B-cell proliferation, survival and migration, leading to significant prolonged survival in high-risk, relapsed or refractory diseases (Herman et al., 2011; Ponader et al., 2012).

Discrepancies in discontinuation rates due to toxicity have been highlighted between initial pivotal clinical trials and real-world studies, where adverse drug reactions (ADRs) were responsible for 51, 29, and 21% of ibrutinib discontinuations in CLL, WM and MCL respectively (Gustine et al., 2018; Mato et al., 2018; Sharman et al., 2020). Higher treatment discontinuation for safety reasons in real-world settings is likely due to differences in patient characteristics. Ibrutinib is prescribed mostly to elderly patients for whom chemo-immunotherapy is unsuitable. As a result, comorbidity burden and co-medications could compromise the safety of ibrutinib in real-life practice. In a previously published cohort study ($n = 102$ patients), patients aged ≥80 years were at higher risk of serious adverse drug reaction (SADR) within the first year of ibrutinib treatment (Allouchery et al., 2021).

Despite increasing use in B-cell malignancies, the post-marketing safety profile of ibrutinib remains unclear. While several studies have assessed the safety of ibrutinib in real life settings, but they have been focused on specific ADRs, such as infectious, bleeding or cardiovascular ADRs (Lipsky et al., 2015; Varughese et al., 2018; Dartigeas et al., 2019; Dickerson et al., 2019; Salem et al., 2019; Frei et al., 2020). Unlike chemotherapy, which is given for a finite number of cycles, ibrutinib is continued until the occurrence of disease progression or unacceptable toxicity. Data on long-term safety of ibrutinib are especially important in light of prolonged ibrutinib exposure but are mainly limited to clinical trial settings. In the 6-years follow-up in the phase 3 RESONATE study of relapsed/refractory CLL ($n = 195$ patients, median ibrutinib treatment duration of 41 months), the most commonly treatment-emergent adverse events of any grade remained consistent with previous reports of patients treated with ibrutinib, and generally decreased over time for patients remaining on ibrutinib therapy, with exceptions for hypertension and bleeding ADRs (Munir et al., 2019).

As ibrutinib has become a standard of care in B-cell malignancies in monotherapy or in combination with other agents, definition of its safety profile appears essential. The aim of this study was to further characterize the safety profile of ibrutinib through identification of potential safety signals in a large-scale pharmacovigilance database.

MATERIALS AND METHODS

Data Source

The Uppsala Monitoring Center (UMC) receives individual case safety reports (ICSRs) of suspected ADRs from national pharmacovigilance systems, which are stored in VigiBase, the World Health Organization (WHO) global database of ICSRs (Lindquist, 2008). In December 2020, VigiBase contained more than 24 million ICSRs from >130 countries. ADRs originate from physicians, pharmacists or other healthcare professionals, patients and pharmaceutical companies.

Each ICSR contains 1) anonymous administrative informations (reporter qualification, date of reporting, country of origin); 2) patient characteristics (sex, age); 3) description of the ADRs coded according to the Medical Dictionary for Regulatory Activities (MedDRA) (Brown et al., 1999), seriousness, time to onset, outcome; 4) drugs involved: international nonproprietary name and coded according to the Anatomical Therapeutic Chemical (ATC) classification, role in the ADRs, indication.

Per the Jardé law in France regarding research involving human participants, this study did not require ethical review or informed consent as it involved an existing anonymized database.

Data Extraction and Selection

All serious ICSRs involving ibrutinib (ATC code: L01XE27) suspected in the occurrence of SADRs or drug interacting from November 13th, 2013 (first authorization in the United States) to December 31st, 2020 were extracted. Exclusion criteria were as follows: missing age, patient aged <18 years, no MedDRA preferred term (PT) and PT "no adverse event."

Data Analysis

A descriptive analysis of included ICSRs was performed. Continuous variables were described by mean and standard deviation, or median and interquartile range (IQR) and categorical variables by number and proportion of subjects in each class.

Disproportionality analyses of spontaneous reporting databases are based on the identification of drug-event pairs reported more often than expected regarding the frequency of reporting of other drug-event pairs, resulting in signals of disproportionate reporting (SDRs) (Montastruc et al., 2011). In the present study, SDR detection was performed using the information component (IC) with its 95% credibility interval (IC_{025}) and the proportional reporting ratio (PRR) with its corresponding 95% confidence interval (95%CI). Specifically developed and validated by UMC, the IC is an indicator value for disproportionate reporting that compares observed and expected values, the objective being to find associations between drugs and ADRs (Bate et al., 1998). The PRR compares the rate of reporting of an event among all reports for a given drug with the rate of reporting of the same event among a control group of drugs (Evans et al., 2001). The IC and

TABLE 1 | Characteristics of included individual case safety reports.

	n = 16,196	
Geographic area, n (%)		
North America	10,832	(66.9)
Europe	4,642	(28.6)
Asia	483	(3.0)
Oceania	145	(0.9)
South America	66	(0.4)
Africa	28	(0.2)
Reporter, n (%)		
Healthcare professional	9,302	(57.4)
Non-health care professional	6,838	(42.2)
Missing data	56	(0.4)
Age, years		
Median, interquartile range	72.9	(65.0–79.1)
Min-max		18–99
Age, years, n (%)		
<75	9,294	(57.4)
≥75	6,902	(42.6)
Sex, n (%)		
Male	10,390	(64.2)
Female	5,640	(34.8)
Missing data	166	(1.0)
Seriousness criterion[a], n (%)		
Death	2,651	(16.4)
Life-threatening	613	(3.8)
Caused/Prolonged hospitalization	9,099	(56.2)
Persistent or significant disability/incapacity	201	(1.2)
Other medically important condition	8,177	(50.5)
Missing data	6	(0.0)
Suspected drugs, n (%)		
Only ibrutinib	13,720	(84.7)
Ibrutinib +1 other drug	1,623	(10.0)
Ibrutinib + ≥2 other drugs	853	(5.3)
Indication, n (%)		
Chronic lymphocytic leukemia	10,546	(65.1)
Mantle cell lymphoma	1,781	(11.0)
Waldenström macroglobulinemia	983	(6.1)
Graft versus host disease	121	(0.7)
Marginal zone lymphoma	92	(0.6)
Other	1,346	(8.3)
Missing data	1,327	(8.2)

ICSR, individual case safety report; min-max, minimum-maximum.
[a]*ICSRs can have more than one seriousness criterion.*

the PRR were calculated for ibrutinib in comparison with all other anticancer drugs (ATC code: L01 "antineoplastic agents"), considering SADRs at the PT level. All other anticancer drugs were used as comparators because 1) the choice of relevant comparators for ibrutinib is complex since ibrutinib is mostly prescribed in elderly patients for whom chemo-immunotherapy is unsuitable; 2) other anticancer drugs prescribed in the same approved indications as ibrutinib, like rituximab or cyclophosphamide, are also used in a broad spectrum of indications in solid tumors or in hematological malignancies; 3) the large number of ICSRs with all other anticancer drugs allows for sufficient sensitivity to detect ibrutinib-associated safety signals. To mitigate the impact of age on safety profile of ibrutinib, two subgroups were considered: patients aged <75 years and ≥75 years. Drug-SADR associations were statistically significant if $IC_{025} \geq 1$, or if the lower limit of 95% confidence interval of the PRR >1, with at least 3 cases of interest reported. A SDR was considered if both measures, i.e., IC and PRR, were significant. SDRs were then assessed carefully by two clinical pharmacologists trained in pharmacovigilance (MA, M-CP-P), according to their clinical relevance and their acknowledgment in the Summary of Product Characteristics (SmPC) approved by the European Medicines Agency (EMA) (European Medicines Agency, 2021) and by the US Food and Drug Administration (FDA) (US Food and Drug Administration, 2020). All SDRs which correspond to unexpected SADRs were classified as potential safety signals. All statistical analyses were performed using SAS software (v9.4, SAS Institute, NC, United States), and disproportionality analysis was performed by the UMC.

RESULTS

Population Characteristics

Among 806,474 patients aged ≥18 years experiencing SADRs associated with anticancer drugs, 16,196 were receiving ibrutinib.

The median (interquartile range, IQR) age of patients was 72.9 (65.0–79.1) years, 42.6% of ICSRs concerned patients aged ≥75 years, and 64.2% male patients. More than half (56.2%) of ICSRs resulted in hospitalization or prolonged hospitalization, 16.4% were fatal and 3.8% were life-threatening. Ibrutinib was the only drug reported as suspected in 84.7% of ICSRs. The indication of ibrutinib treatment was available for 91.8% of ICSRs. The most frequently represented were CLL (65.1%), MCL (11.0%) and WM (6.1%). Two thirds (66.9%) of ICSRs were reported for North America and 28.6% for Europe, and 57.4% were reported by healthcare professionals (**Table 1**).

Disproportionality Analyses

A total of 1,024 SDRs (713 unique drug-event pairs) were reviewed (patients aged <75 years $n = 605$, ≥75 years $n = 419$). The number and proportion of potential safety signals in each group, as well as expected SADRs, non-signals are displayed in **Table 2**. A total of 50 SDRs (4.9%) and 36 unique drug-event pairs were classified as potential safety signals (**Table 3**).

Considering cardiac disorders, significant disproportionality emerged for ischemic heart diseases and bradyarrhythmias (including conduction defects and sinus node dysfunctions), with some differences between patients aged <75 years and

TABLE 2 | Number of assessed signals of disproportional reporting by age group.

Assessed SDRs	<75 years (n = 605)		≥75 years (n = 419)	
	n	%	n	%
Expected[a]	323	53.4	233	55.6
Non-signal[b]	250	41.4	168	40.1
Potential safety signal	32	5.2	18	4.3

SDR, signal of disproportionate reporting.
[a]*Considered well-described in the summary of product characteristics approved by the European Medicines Agency or by the US Food and Drug Administration.*
[b]*SDRs with alternative explanations, or potentially related to the characteristics of ibrutinib-treated patients or B cell malignancies.*

TABLE 3 | Potential safety signals identified in VigiBase, according to age groups.

MedDRA SOC/Sub-group SADRs		Only ibrutinib among suspected drugs %	MedDRA PT	<75 years (n = 9,294)				≥75 years (n = 6,902)			
				n	IC/IC_{025}	PRR (95%CI)		n	IC/IC_{025}	PRR (95%CI)	
	Ischemic heart diseases	84.5	Myocardial infarction	100	1.01/0.71	2.05	(1.68–2.50)	77	NS		NS
			Angina pectoris	26	1.00/0.39	2.07	(1.40–3.06)	8	NS		NS
			Coronary artery occlusion	11	1.26/0.28	2.61	(1.43–4.77)	11	1.41/0.44	3.16	(1.68–5.95)
			Ischemic cardiomyopathy	4	2.02/0.28	7.13	(2.54–19.97)	2	NS		NA
Cardiac disorders	Pericarditis	76.1	Pericardial effusion	81	1.83/1.50	3.75	(3.00–4.68)	53	1.77/1.35	3.96	(2.95–5.30)
			Pericarditis	24	1.63/1.00	3.35	(2.23–5.05)	11	1.71/0.74	4.19	(2.20–8.01)
			Cardiac tamponade	16	2.13/1.34	5.18	(3.12–8.62)	4	NS	3.23	(1.13–9.24)
			Pericardial hemorrhage	14	3.87/3.01	47.40	(23.95–93.82)	9	2.66/1.57	14.52	(6.21–33.96)
	Bradyarrhythmia (including conduction defects and sinus node dysfunctions)	67.3	Sinus bradycardia	11	1.41/0.43	2.94	(1.61–5.38)	5	NS		NS
			Right bundle branch block	7	2.21/0.95	6.77	(3.11–14.72)	5	1.96/0.44	6.99	(2.54–19.23)
			Sinus node dysfunction	6	2.10/0.72	6.35	(2.75–14.66)	4	NS		NS
			Atrioventricular block 1st degree	6	2.49/1.12	10.42	(4.41–24.60)	4	NS	5.24	(1.75–15.68)
			Atrioventricular block 2nd degree	4	1.84/0.10	5.64	(2.04–15.65)	5	NS	3.75	(1.45–9.70)
Ear disorders	Hearing impairment	88.5	Deafness	21	1.27/0.59	2.55	(1.65–3.94)	31	1.54/0.99	3.32	(2.27–4.84)
Endocrine disorders	Hypothyroidism	75.0	Thyroid hormones decreased	5	2.37/0.84	9.96	(3.90–25.46)	3	2.10/0.05	15.73	(3.52–70.28)
	Cataract	85.7	Cataract	52	1.13/0.71	2.25	(1.71–2.96)	67	1.21/0.85	2.50	(1.94–3.22)
	Uveitis	91.3	Uveitis	21	1.16/0.48	2.35	(1.52–3.64)	2	NS		NA
Eye disorders	Glaucoma	80.8	Glaucoma	16	1.64/0.84	3.45	(2.09–5.70)	10	NS		NS
	Retinal disorders	92.9	Retinal tear	6	1.93/0.55	5.28	(2.30–12.11)	0	NA		NA
			Vitreous detachment	5	1.71/0.18	4.46	(1.80–11.01)	0	NA		NA
			Retinal vascular occlusion	4	2.65/0.91	24.63	(7.84–77.32)	0	NA		NA
Injury, poisoning and procedural complications	Fractures	93.4	Hip fracture	20	0.95/0.25	2.01	(1.29–3.13)	83	1.36/1.03	2.79	(2.23–3.51)
			Spinal fracture	18	1.16/0.41	2.35	(1.47–3.76)	18	NS		NS
			Upper limb fracture	10	NS		NS	19	0.83/0.11	1.89	(1.18–3.02)
			Foot fracture	17	1.32/0.55	2.67	(1.65–4.33)	5	NS		NS
			Lower limb fracture	8	NS		NS	14	1.20/0.84	2.58	(1.48–4.48)
			Spinal compression fracture	15	1.31/0.49	2.68	(1.60–4.49)	6	NS		NS
			Lumbar vertebral fracture	3	NS		NS	12	1.74/0.81	4.27	(2.29–7.93)
			Stress fracture	7	1.79/0.53	4.39	(2.04–9.42)	0	NA		NA
Metabolism disorders	Hyponatremia	71.2	Hyponatremia	35	NS		NS	51	0.48/0.06	1.43	(1.08–1.90)
			Blood sodium decreased	28	1.67/1.08	3.42	(2.34–5.00)	39	1.62/1.13	3.53	(2.51–4.95)
Psychiatric disorders	Depression	79.6	Depression	66	0.68/0.31	1.62	(1.27–2.07)	51	1.19/0.77	2.47	(1.85–3.30)
			Depressed mood	20	1.02/0.33	2.13	(1.37–3.33)	16	1.27/0.48	2.73	(1.62–4.59)
Respiratory, thoracic and mediastinal disorders	Pleurisy	82.3	Pleural effusion	247	1.63/1.44	3.21	(2.83–3.64)	221	1.48/1.29	3.08	(2.68–3.54)
			Pleurisy	15	1.42/0.60	2.92	(1.47–4.89)	8	1.22/0.06	2.75	(1.32–5.75)
Vascular disorders	Arterial disorders	92.9	Aortic aneurysm	8	1.70/0.53	3.93	(1.93–8.00)	6	NS		NS

IC, information component; IC_{025}, 95% credibility interval; MedDRA, medical dictionary for regulatory activities; NA, not applicable; NS, not significant; PT, preferred term; PRR, proportional reporting ratio; SADR, serious adverse drug reaction; SOC, system organ class; 95%CI, 95% confidence interval.

≥75 years. Only SDRs of coronary artery occlusion and right bundle branch were found in the older group, but with PRRs similar to those of patients aged <75 years. Ibrutinib was also associated with higher reporting of pericarditis (MedDRA PTs pericarditis, pericardial effusion and pericardial hemorrhage) in both age groups, even though IC and PRR reached statistical significance for cardiac tamponade only for patients aged <75 years. Similarly, regarding vascular SADRs, the SDR of aortic aneurysm was found only in the younger patients.

When focusing on respiratory disorders, a SDR of pleurisy was found for ibrutinib in comparison with other anticancer drugs in the two age groups.

A considerable number of eye disorders related to ibrutinib emerged from our analysis. Cataract was over-reported in both age groups, while uveitis, glaucoma, and retinal disorders (i.e. retinal tear, vitreous detachment and retinal vascular occlusion) were over-reported only in patients aged <75 years.

Overlap of cardiovascular, respiratory and ocular SADRs is shown in **Figure 1**.

We found potential safety signals referred to ibrutinib and fractures. Except for hip fracture, patterns of fractures differ between age groups. Ibrutinib was associated with higher reporting of hip fracture, spinal fracture, foot fracture, spinal compression fracture and stress fracture in patients aged <75 years, and upper and lower limb fractures and lumbar vertebral fracture in patients aged ≥75 years.

Other potential signals of interest were associated with ibrutinib in both age groups, especially deafness, hypothyroidism, hyponatremia and depression.

DISCUSSION

Main Results

We report the largest study to date on the safety profile of ibrutinib through analysis of ICSRs from the WHO pharmacovigilance database. Clinically relevant potential safety signals emerged from our analysis, with some differences between patients aged <75 years and ≥75 years: cardiovascular disorders (including ischemic heart disease, pericarditis, bradyarrhythmia and aortic aneurysm), deafness, hypothyroidism, eye disorders (including cataract, uveitis, glaucoma and retinal disorders), fractures, hyponatremia, depression and pleurisy. Potential underlying mechanisms are summarized in **Figure 2**.

Potential Safety Signals

The cardiovascular safety profile of ibrutinib has been widely described, and includes hypertension (Dickerson et al., 2019), supraventricular arrhythmias, including atrial fibrillation (Leong et al., 2016) and life-threatening ventricular arrhythmias (Lampson et al., 2017; Guha et al., 2018). More recently, ibrutinib-associated stroke (with/without AF/hypertension) and cardiac failure have also been listed in the European SmPC. However, our study provides new findings on ibrutinib-associated cardiotoxicity. These results show SDRs of ischemic heart diseases, e.g. myocardial infarction, angina pectoris, coronary artery occlusion and ischemic cardiomyopathy, mostly in ibrutinib-treated patients aged <75 years. While SDRs of myocardial infarction, angina pectoris and ischemic cardiomyopathy disappeared in patients aged ≥75 years, the SDR of coronary artery occlusion still persists in this subgroup, underlining the potential role of ibrutinib. Growing evidence suggest that ibrutinib-associated cardiac toxicity may be explained by the multiple off-target effects of ibrutinib at clinically relevant concentrations. A recent structure-based drug repositioning identified ibrutinib as micromolar Vascular Endothelium Growth Factor Receptor 2 (VEGFR2) inhibitor (Adasme et al., 2020). Despite antiplatelet effects (Busygina et al., 2018, 2019), ibrutinib, like anti-angiogenic drugs, could produce conditions favoring hypertension and ischemic heart diseases, through the inhibition of nitric oxide formation and endothelial dysfunction (Mourad et al., 2008). Vascular Endothelium Growth Factor (VEGF) blockade may also be implicated in arterial wall injuries, leading to increased risk of artery dissections and aneurysms with antiangiogenic drugs (Oshima et al., 2017; Guyon et al., 2021). Interestingly, ibrutinib was shown to interrupt collagen fibrosis in a murine model of chronic graft-versus-host disease (Dubovsky et al., 2014). Furthermore, Wiedower et al. (2016) reported a cerebral aneurysm in a 46-year-old man treated for CLL, with spontaneous resolution after ibrutinib discontinuation, suggesting the possible direct role of ibrutinib on vascular remodeling. In addition to ibrutinib-induced hypertension, VEGF blockade may also explain the SDR of aortic aneurysm with ibrutinib in patients aged <75 years.

Our study identified bradyarrhythmias including conduction defects and sinus node function disorders, as emerging safety signals in patients aged <75 years receiving ibrutinib. In patients aged >75 years, probably due to low number of cases, only a SDR of right bundle branch was found. Conduction disorders signal over-reporting is concordant with a previous pharmacovigilance study in VigiBase evaluating the cardiovascular safety of ibrutinib (Salem et al., 2019). In a recent case-series of ibrutinib-induced high-grade heart block, 14 out of 18 cases of high degree heart block occurred within 13 months of ibrutinib initiation (Vartanov et al., 2021). All patients required pacemaker placement, and most resumed ibrutinib without recurrence of conduction disorders. It remains unclear whether or not the mechanisms that mediate ibrutinib-induced AF may be responsible for conduction disorders. Increased incidence of AF in ibrutinib-treated patients has been associated with not only with inhibition of C-terminal Src kinase (Xiao et al., 2020), but also with downregulation of phosphoinositide 3-kinase (PI3K)-Akt pathway (McMullen et al., 2014; Jiang et al., 2019). Cardiac fibrosis observed in these experimental murine models could contribute to the promotion of conduction disorders.

An association between pericardial/pleural effusions and ibrutinib has been reported in a few case-reports (Styskel et al., 2019; Miatech et al., 2020; Kidoguchi et al., 2021). This is consistent with emerging potential safety signals of pericarditis/pleurisy in both subgroups. A SDR of pleural effusion was also found for ibrutinib in a study evaluating the risk of pleural effusion with tyrosine kinase inhibitors (Mahé et al., 2018). Underlying mechanisms needs further investigation. Some

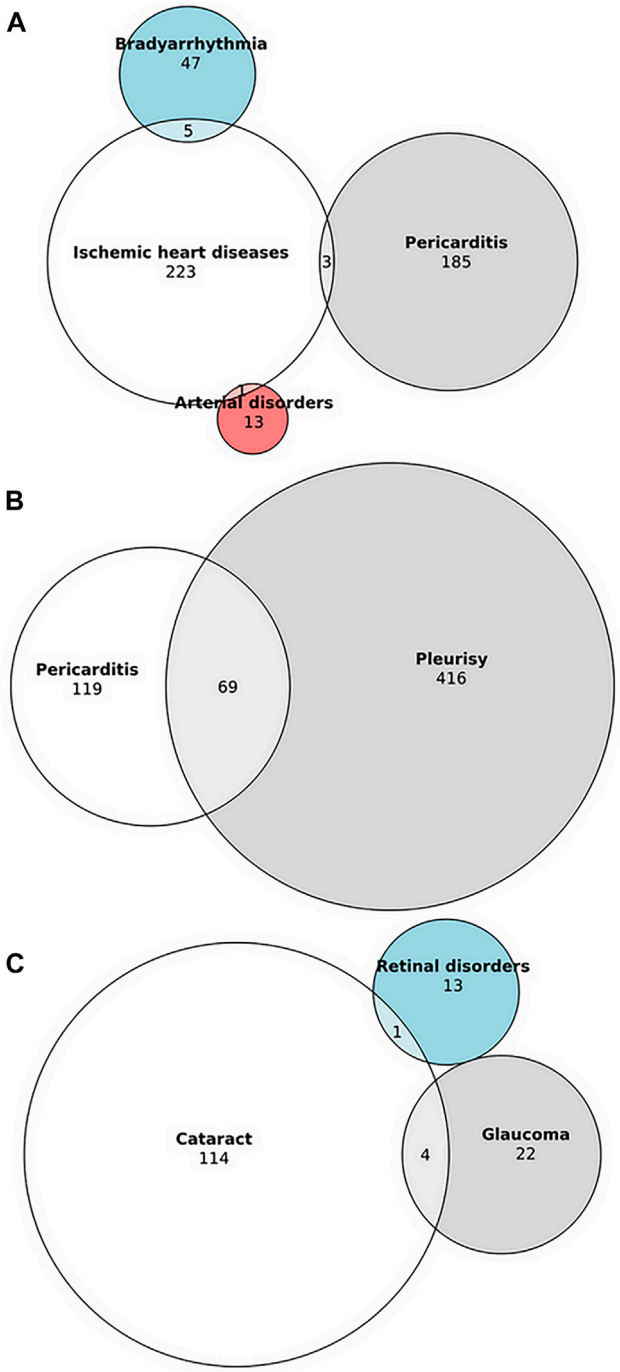

FIGURE 1 | Overlap of cardiovascular, respiratory and ocular serious adverse drug reactions associated with ibrutinib in VigiBase. **(A)** Overlap between ischemic heart diseases, pericarditis, bradyarrhythmia and arterial disorders. **(B)** Overlap between pericarditis and pleurisy. **(C)** Overlap between cataract, glaucoma and retinal disorders. Due to the lack of overlap with other ocular disorders, uveitis is not displayed.

authors have suggested that tyrosine kinase inhibitors could cause serosal inflammation (pleural and pericardial effusions) by their ability to inhibit multiple targets as Platelet Derived Growth Factor Receptor β (PDGFR-β) and Src family tyrosine kinases, which are involved in the maintenance of interstitial fluid tissue pressure and endothelial permeability (Kelly et al., 2009).

Our analysis highlights potential safety signals of ocular disorders: cataract in the two subgroups, uveitis, glaucoma and retinal ADRs (including retinal tear, vitreous detachment, retinal vascular occlusion) in patients aged <75 years. Since original studies on canine models have demonstrated corneal toxicity in animals receiving high doses of ibrutinib (US Food and Drug Administration, 2020), a concern emerged about the risk of cataract formation in ibrutinib-treated patients. Furthermore, blurred vision concerned 10% of ibrutinib-treated patients in the phase III RESONATE study (Byrd et al., 2014). Cataract was found to be one of the most commonly reported grade ≥3 adverse events in the 5-year follow-up of the phase III RESONATE study and was observed in 5.2% of patients (Burger et al., 2019). However, these results remain consistent with the background rate of cataract in the elderly (Asbell et al., 2005). Similarly, the higher reporting of glaucoma in the younger group may result from characteristics of ibrutinib-treated patients.

To date, ibrutinib-induced uveitis has been reported in only 3 case-reports (Arepalli et al., 2021; Bohn et al., 2021; Mehraban Far et al., 2021), in contrast with the 119 ICSRs documented in VigiBase. Selective pressure for Th1-mediated immunity has been proposed as a mechanism for ibrutinib-associated uveitis (Mehraban Far et al., 2021). Concerning retinal disorders, only macular edema has been previously reported with ibrutinib (Saenz-de-Viteri and Cudrnak, 2018; Mirgh et al., 2020). It is noteworthy that ibrutinib can penetrate the blood-brain barrier in mantle cell lymphoma patients (Bernard et al., 2015) and therefore reach the retina by passing through the blood-retinal barrier. Like MEK inhibitors (Huillard et al., 2014), retinal pigment epithelium toxicities may result from inhibition of mitogen-activated protein (MAP) kinases or upregulation of aquaporins in the retinal pigment epithelium (Daruich et al., 2018).

Concomitantly with a well-known risk of fall (US Food and Drug Administration., 2020), ibrutinib was associated with higher reporting of fractures on ibrutinib in both age groups. Except for hip fracture, patterns of fractures differ between age groups. Spinal (including spinal compression), foot and stress fractures reporting was significantly increased in patients aged <75 years, while lower limb, upper limb and stress fractures significantly increased in the older group. A recent retrospective study found a higher risk of spinal fracture in ibrutinib-treated patients for CLL (Laroche et al., 2020). In contrast, some authors have suggested that ibrutinib could be a potential therapeutic agent for certain osteoclast-related diseases, such as osteoporosis and rheumatoid arthritis. Ibrutinib inhibits osteoclast differentiation and function *in vitro* by regulating the expression of osteoclast-associated genes (Shinohara et al., 2014). Moreover, in the same study, oral administration of ibrutinib was shown to protect against bone loss in a murine model of osteoporosis. It should be noted that confounders including B-cell malignancies, or risk factors of fractures (e.g., body mass index, prevalence of smoking, specific comorbidities, serum vitamin D levels, or use of medications)

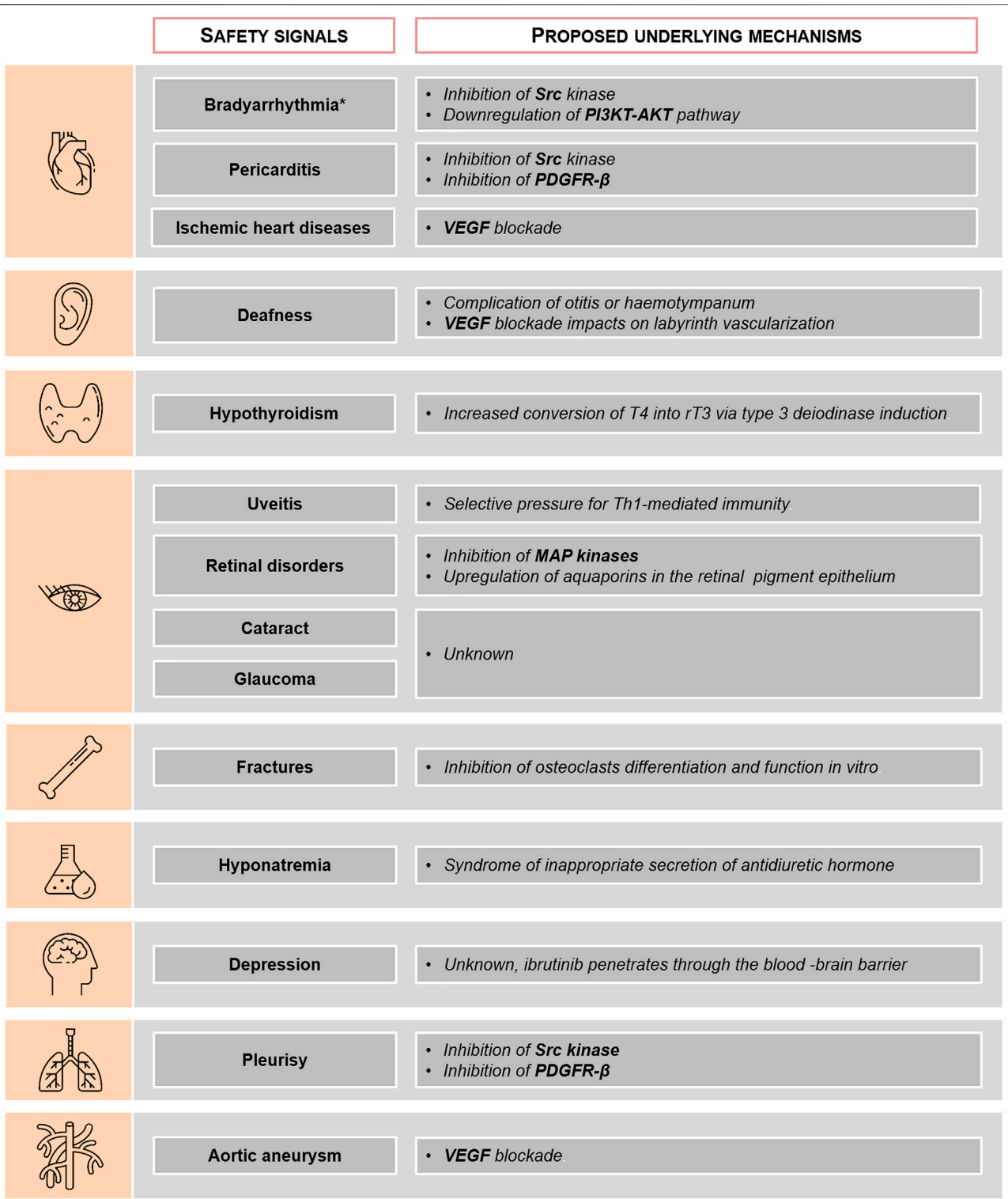

FIGURE 2 | Potential underlying mechanisms for ibrutinib-associated safety signals. *Including conduction defects and sinus node function dysfunctions. PDGFR-β, platelet derived growth factor receptor; MAP, mitogen-activated protein; PI3K, phosphoinositide 3-kinase; rT3, reverse triiodothyronine; T4, thyroxin; VEGF, vascular endothelium growth factor.

could also affect our findings. Since BTK plays a role in bone resorption and metabolism, our results suggest a need for further assessments on the potential occurrence of fractures and osteoporosis in patients treated with ibrutinib.

Regarding endocrine and metabolic disorders, the SDR of hyponatremia is concordant with long-term safety data from the phase 3 RESONATE-2 study, where hyponatremia was found to be one of the most common grade ≥3 adverse events. Tyrosine kinase inhibitors treatment has been associated with syndrome of inappropriate antidiuretic hormone secretion, especially in patients under BCR-ABL inhibitors (Liamis et al., 2016). However, underlying mechanisms for ibrutinib-induced hyponatremia did not emerge from literature.

Nearly a dozen tyrosine kinase inhibitors have been implicated in hypothyroidism and definitive associations are known for 5 (axitinib, imatinib, pazopanib, sorafenib, and sunitinib) (Kust et al., 2016). Only one case-report described increased thyroid-hormone requirements in a thyroidectomized 80-year-old woman while on ibrutinib treatment (Mazori and Skamagas, 2021). The levothyroxine dose required to preserve a normal TSH decreased after ibrutinib discontinuation, indicating that ibrutinib-induced hypothyroidism is reversible. The authors hypothesize that the patient's hypothyroid state was caused by type 3 deiodinase induction, leading to increased conversion of thyroxine into reverse triiodothyronine.

We found that depression was more frequently reported with ibrutinib in both groups, in comparison with anticancer drugs. Only anxiety is listed in the US product information (US Food and Drug Administration, 2020). As underlined above, ibrutinib is able to pass through the blood-brain barrier, and could as a result induce psychiatrics ADRs, such as anxiety or depression.

Lastly, to the best of our knowledge, ototoxicity of ibrutinib has not been previously reported. Because of increased risk of infections (including otitis) and bleeding events (including hemotympanum) in patients treated with ibrutinib, our results should be interpreted with caution. Off-target effects of ibrutinib, in particular VEGF inhibition, may explain ibrutinib-associated deafness. Anti-VEGF agents may induce hearing loss by causing a reduction in local blood flow and/or microvascular thrombosis in the labyrinth (Dekeister et al., 2016; Cheng et al., 2019).

Strengths and Limitations

This work has several important strengths. The present study used the world's largest and most representative spontaneous reporting database (VigiBase), which currently includes more than 124 million ICSRs from >130 countries. Reports from VigiBase represent data in the context of real-world settings, which have not been investigated in clinical trials (Bégaud and Montastruc, 2019; Montastruc et al., 2019). It used a validated method (i.e., disproportionality analysis) which was found to be able to detect unknown or rare safety signals (Montastruc et al., 2019).

From a statistical point of view, the combined use of two complementary disproportionality measures (i.e., PRR and IC) provides the most accurate estimate for drug-SADR associations, especially for associations with few cases. Moreover, given the characteristics of ibrutinib-patients, this study was performed trying to limit confounding of age. Subgroup analyses (i.e. patients aged <75 years and ≥75 years) improve sensitivity and precision and are clearly beneficial over crude analyses in large databases (Seabroke et al., 2016; Sandberg et al., 2020).

Our study has some limitations. Underreporting is an intrinsic limitation to research performed using pharmacovigilance data. It mostly concerns less severe and/or expected adverse drug reactions (Lopez-Gonzalez et al., 2009; Alatawi and Hansen, 2017). Furthermore, González-Rubio et al. (2011) found that ADRs are more often reported for elderly patients. Therefore, the potential impact of under-reporting on this work appears to be low. The use of all anticancer drugs as the reference group should have minimized any potential indication bias, even if differences in cancer types and patient characteristics may account for the discrepancies found between the two age groups. ADR reporting comes from heterogeneous sources in VigiBase, thus raising the possibility of incomplete information for time to onset or comorbidities. Consequently, the impact of risk factors on potential safety signals cannot be ruled out. In addition, the volume of ICSRs for a specific drug may be different between countries according to its extent of use or time of registration.

CONCLUSION

Using a large-scale pharmacovigilance database, we found clinically relevant potential safety signals in patients exposed to ibrutinib, mainly ischemic heart diseases, pericarditis, uveitis, retinal disorders and fractures. Owing to the mandatory limitations of this study, these results need further confirmation using population-based studies. However, all of these potential safety signals should be considered in patient care and in clinical trial designs.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available. The data that support the findings of this study are available from the UMC. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of the UMC. Requests to access the datasets should be directed to marion.allouchery@univ-poitiers.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Concept and design: MA, FS and M-CP-P. Acquisition, analysis or interpretation of data: MA, FS, and M-CP-P. Drafting of the manuscript: MA, FS and M-CP-P. Critical revision of the manuscript for important intellectual content: all authors. Supervision: FS and M-CP-P.

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Risk of Malignancy and Tuberculosis of Biological and Targeted Drug in Patients With Spondyloarthritis: Systematic Review and Meta-analysis of Randomized Controlled Trials

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Objective: Concerns exist regarding the potential development of malignancy and tuberculosis in patients with spondyloarthritis (SpA) treated with biologics. We assessed the extent to which biologic therapy may increase the risk of malignancy and tuberculosis in patients with SpA by meta-analysis to derive estimates of sparse harmful events occurring in Randomized Controlled Trials (RCTs).

Methods: A systematic literature search was conducted in PubMed, EMbase, Web of Science, the Cochrane Library, and China Biology Medicine disc for RCTs evaluating the risk of sparse harmful events of biologic therapy in patients with SpA from inception through August 9, 2021. We calculated a pooled Peto OR for malignancy and tuberculosis in biologics-treated patients vs. placebo patients. The risk of bias on the included RCTs was assessed by using Cochrane Risk of Bias tool.

Results: In total, 63 studies were included in this meta-analysis, and 83 patients and 7 patients developed malignancy and tuberculosis, respectively. Overall, the risk of malignancy and tuberculosis was increased in SpA patients treated with biologics compared to placebo (malignancy: Peto OR: 2.49, 95%CI: 1.61–3.87, $p < 0.001$; tuberculosis: Peto OR: 5.98, 95%CI: 1.29–27.76, $p = 0.022$). Remarkably, compared to placebo, there was higher risk of malignancy for IL-17 inhibitors (Peto OR: 3.68, 95%CI: 1.20–11.30, $p = 0.023$) and small molecule targeted drugs (Peto OR: 3.08, 95%CI: 1.37–6.90, $p = 0.043$) in peripheral SpA, and for TNF receptor-Fc fusion protein in axial SpA (Peto OR: 7.18, 95%CI: 1.21–42.69, $p = 0.030$). Besides, the risk of tuberculosis was higher for anti-TNFα antibody in axial SpA (Peto OR: 6.17, 95%CI: 1.03–37.13, $p = 0.046$).

Conclusion: This meta-analysis showed an elevated risk of malignancy in patients with peripheral SpA treated with biologics, especially for IL-17 inhibitors, and small molecule targeted drugs, a slightly increased risk of malignancy in TNF receptor-Fc fusion protein in axial SpA, and increased risk of tuberculosis in patients with axial SpA treated with anti-TNFα antibody. These findings need to be validated by studies with larger population and longer follow-up.

Keywords: spondyloarthritis, biologic therapy, malignancy, tuberculosis, systematic review and meta-analysis

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INTRODUCTION

Spondyloarthritis (SpA) is a series of chronic inflammatory conditions that have a range of manifestations, including predominantly axial SpA (radiographic axial SpA (axSpA) and non-radiographic axial SpA (non-axSpA)) and peripheral SpA (enteropathic arthritis, reactive arthritis, and psoriatic arthritis). People with predominantly axSpA may have additional peripheral symptoms, and vice versa. Treatment with non-steroidal anti-inflammatory drugs (NSAIDs) or conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs), or a combination of both, can usually ameliorate disease activity and retard joint damage, thereby improving quality of life of patients with SpA. However, in a sizeable proportion of patients with SpA, NSAIDs or csDMARDs fail or are not tolerated. For these patients not responding to NSAIDs or csDMARDs, biologics or small molecular targeted drugs can provide clinically important improvement via targeting specific inflammatory mediators in inflammatory pathways, alleviating inflammation, and thus better controlling symptoms and structural destruction. Concerns have been raised about the safety of biologics or small molecular targeted drugs, especially with regard to malignancies and tuberculosis, because they can interfere with the immune system.

Cancer is a common event in individuals with rheumatic diseases. A study suggested that about 20% of individuals with rheumatoid arthritis will be diagnosed with malignancy during their remaining lifetimes (Cush and Dao, 2012). When analyzing this risk, some confounding factors have to be taken into account. For example, treatments for the rheumatic diseases that act on the immune system, such as biological agents, may play a significant role in favoring cancer. At present, most of the researches on tumorigenicity of biologics are on TNF-α inhibitors for the treatment of rheumatoid arthritis, but few are in SpA. The use of biologics was not significantly associated with an increased risk of malignancy in individuals with rheumatoid arthritis who were included in randomized controlled trials (RCTs) for at least 6-months duration, compared with other csDMARDs or with placebo (Lopez-Olivo et al., 2012). Because of the different pathogenesis between rheumatoid arthritis and SpA, it is necessary to investigate the occurrence of malignancy in the treatment of SpA with biologics.

Tuberculosis is an important infectious disease worldwide, which is related to a significant morbidity and mortality, especially in developing countries. More and more biologics are used in the treatment of individuals with SpA, which is a matter of great concern, particularly for the individuals having previously suffered from tuberculosis. Studies in Taiwan have shown a 2.28-fold increase in the risk of tuberculosis in patients with rheumatoid arthritis than that in the general population (Liao et al., 2015), while patients treated with TNF-α inhibitors were at higher risk of developing tuberculosis than those treated with other medications (Lim et al., 2016). Hence, the link between biological agents and the occurrence of tuberculosis is certainly an area of concern.

At present, many RCTs have reported the safety of biologics and small molecular targeted drugs in the treatment of SpA. However, RCTs are inadequate for detecting and quantifying rare events, such as malignancies and tuberculosis. A meta-analytic approach is considered useful to overcome the inherent limitations of individual RCTs in the assessment of safety outcomes. The main objective of the systematic review is to summarize and contextualize the risk of malignancies and tuberculosis accompanying biologics and small molecular targeted drugs use in RCTs and long-term extension studies using meta-analysis.

MATERIALS AND METHODS

We strictly followed PRISMA (the Preferred Reported Items for Systematic Reviews and Meta-analyses) guidelines and the recommendations from the Cochrane Collaboration to conduct this systematic review and meta-analysis.

Data Sources and Searches

An experienced medical librarian and information specialist was invited to conduct a comprehensive literature search with input from the study team. The following electronic bibliographic databases: PubMed, EMbase, the Cochrane Library, Web of Science, and China Biology Medicine disc (CBM), were searched from inception through August 9, 2021. No limits were applied to race, sex, or language, except for human subjects. The details of search strategies for electronic database were showed in **Supplementary Appendix A1**. Other resources were hand-searched, including Websites and bibliographic references from systematic reviews and RCTs of interest, for additional citations not identified through the original search strategy.

Selection of the Trials

Study inclusion was assessed by two pairs of independent reviewers (SM and XJ, LW and YW). Disagreements were discussed and resolved by consensus and, when needed, a third reviewer acted as an adjudicator (LH) until a consensus was reached.

Eligible Trials Were Required to

Type of study design: Randomized controlled trials (RCTs) that have been published in peer-review journals and had at least one 24-weeks follow-up.

Type of patients: Patients (≥18 years old) with SpA including axial SpA and peripheral SpA confirmed by physician/specialist according to the 1984 modified New York criteria and ASAS classification criteria for SpA.

Type of intervention and comparator: Studies comparing any biologics (TNF-α inhibitors, IL-17 inhibitors, IL-6 inhibitors, IL-23 inhibitors, and small molecule targeted drugs and so on) against non-biologics (placebo, NSAIDs or csDMARDs).

Outcomes: Reporting at least one outcome of malignancy or tuberculosis.

Data Extraction and Risk of Bias Assessment

Two reviewers (SM and XJ) independently individual trial data, and two additional reviewers (LW and YW) cross-checked the

extracted results. Disagreements were discussed and solved through consensus, and a third reviewer acted as an adjudicator (LH) if necessary. From each selected trial, we collected general information (e.g. authors' name, publication year, country, and study design), study population (e.g. age of patients, gender distribution), and intervention characteristics (details of intervention and control, duration of intervention and follow-up). Primary outcome data was number and type of malignancies and tuberculosis.

All included trials were assessed for risk of bias by two reviewers (SM and XJ) with version 2 of the Cochrane Risk of Bias Assessment tool. The following domains of individual trials were assessed: random sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other biases (including carryover, extreme baseline imbalance, and funding). We assessed the risk of bias using the categories of yes (low risk of bias), no (high risk of bias), and unclear (lack of information or uncertainty about the potential for bias).

Data Synthesis and Analysis

We identified the number of patients with at least one malignancy or tuberculosis, based on the analysis of adverse event in individual trial. The number of patients with SpA receiving at least one dose of study drug represented the denominator of our outcome measurement.

Our study protocol required the use of a fixed-effect model for meta-analysis due to its superior performance while pooling the clinical trials with few or rare events, comparing to random-effect model, and the results were expressed as Peto odds ratio (OR) and associated 95%CI. Peto OR is preferred for few or rare events, and it is not necessary to have corrections for zero cell counts in a single group. We stratified by biologics classes to explore how different biologics classes affect the risk of malignancy and tuberculosis infection. Since there were fewer cases of malignancy and tuberculosis, we could not compare the dose or frequency of administration. Therefore, for the clinical trials that investigated different doses and frequencies of the same biologics class, we pooled cases of malignancy or tuberculosis across the different doses and frequencies for analysis. The I (Lopez-Olivo et al., 2012) statistic was used to assess heterogeneity across RCTs. If the I (Lopez-Olivo et al., 2012) value was over 50%, substantial heterogeneity was present, and then the possible causes needed be investigated.

Meta-analysis was conducted using "meta" package on R 3.6.2 software. p-value < 0.05 was considered as statistically significant in all tests.

RESULTS

Literature Selection and Trial Characteristics

A total of 13,103 unique citations were identified through electronic bibliographic databases and hand-searching. There were 8,925 records that were potentially relevant to our topic in our first selection round, of which 166 were deemed eligible for full review. Finally, a total of 63 trials met inclusion criteria for our systematic review and meta-analysis. All the included studies were reported in Chinese or English. The details of study selection process were showed in **Supplementary Figure S1**.

The 63 RCTs containing 19,291 patients with SpA, were published between 2003 and 2021. Among these RCTs, 27 investigated axSpA and 36 investigated peripheral SpA. A majority of RCTs were two-arms clinical trials, with follow-up periods ranging from 12 to 144 weeks (**Supplementary Table S1**).

Risk of Bias Assessments

All studies provided sufficient details of randomization. Although most of the clinical trials declared that they were double-blind, more than half of the trials indicated inadequate in the method of allocation concealment. In all studies, the co-interventions and baseline characteristics were similar between the biologics group and control group, and control groups (placebo and) were grouped together. In some patients who previously received placebo or csDMARDs, switching from place to active medication might introduce a potential risk of bias. In some trials (28/58), the method of imputation of no response, with "advancement penalty", was used to address this potential risk of bias. The risk of bias graph of assessment for all the included RCTs was demonstrated in **Figure 1**. Risk of bias summary was shown in **Supplementary Figure S2**.

Malignancy

Published data in 63 retrieved RCTs reported 69 malignancies in 11,281 patients receiving at least one dose of biologics (0.6%) and 14 malignancies in 7,913 control patients (0.2%). Of the 83 malignancies, 55 were solid tumors (ie, prostate cancer, gastric adenocarcinoma), 17 were skin cancer (ie, melanomia, squamous cell cancer, and basal cell cancer), 4 were lymphomas, and 7 were not specified. Mean durations of placebo exposure and active treatment exposure were 21 ± 9 weeks and 37 ± 22 weeks. Malignancies occurred in 12, 29, 27, and 7 patients with durations of active treatment exposure more than 52 weeks, 24–52 weeks, less than 24 weeks and less than 12 weeks, respectively.

The measurement of inconsistency between the RCTs (I (Lopez-Olivo et al., 2012)) was 0% (p = 0.85), which indicated that there was no statistical heterogeneity in these trials. Overall, there was an increased risk of malignancies in individuals with SpA using biological and targeted drugs vs. placebo patients (Peto OR 2.49, 95%CI 1.61–3.87, p < 0.001; **Figure 2**). The overall statistical significance did not change, while omitting any single trials, which indicated that the results were statistically robust (**Supplementary Figure S3**).

Subgroup analysis of trials was conducted by using biologics class. The risks of malignancy were higher than placebo for IL-17 inhibitors in patients with peripheral SpA (Peto OR 3.68, 95%CI 1.10–11.30, p = 0.023; I^2 = 0%, and p = 0.889) without evidence of publication bias (**Supplementary Figure S4**), small molecule targeted drugs in patients with peripheral SpA (Peto OR 3.08, 95%CI 1.37–6.90, p = 0.006; I^2 = 0%, and p = 0.923) without

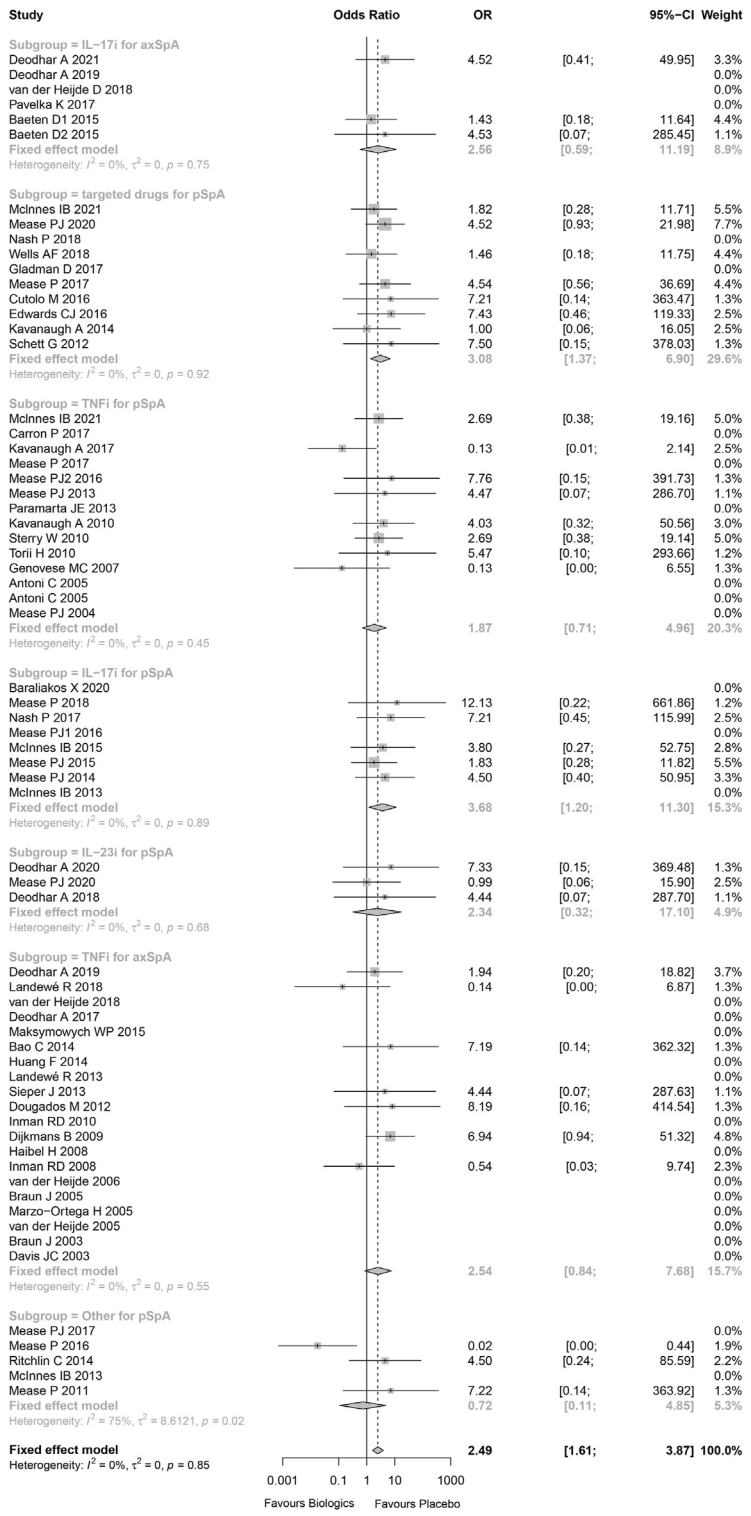

FIGURE 1 | Risk of bias graph.

evidence of publication bias (**Supplementary Figure S5**). In addition, while omitting these studies being high-risk bias, the results still indicated that the risks of malignancy were higher than placebo for IL-17 inhibitors in patients with peripheral SpA, small molecule targeted drugs in patients with peripheral SpA (**Supplementary Figure S6**).

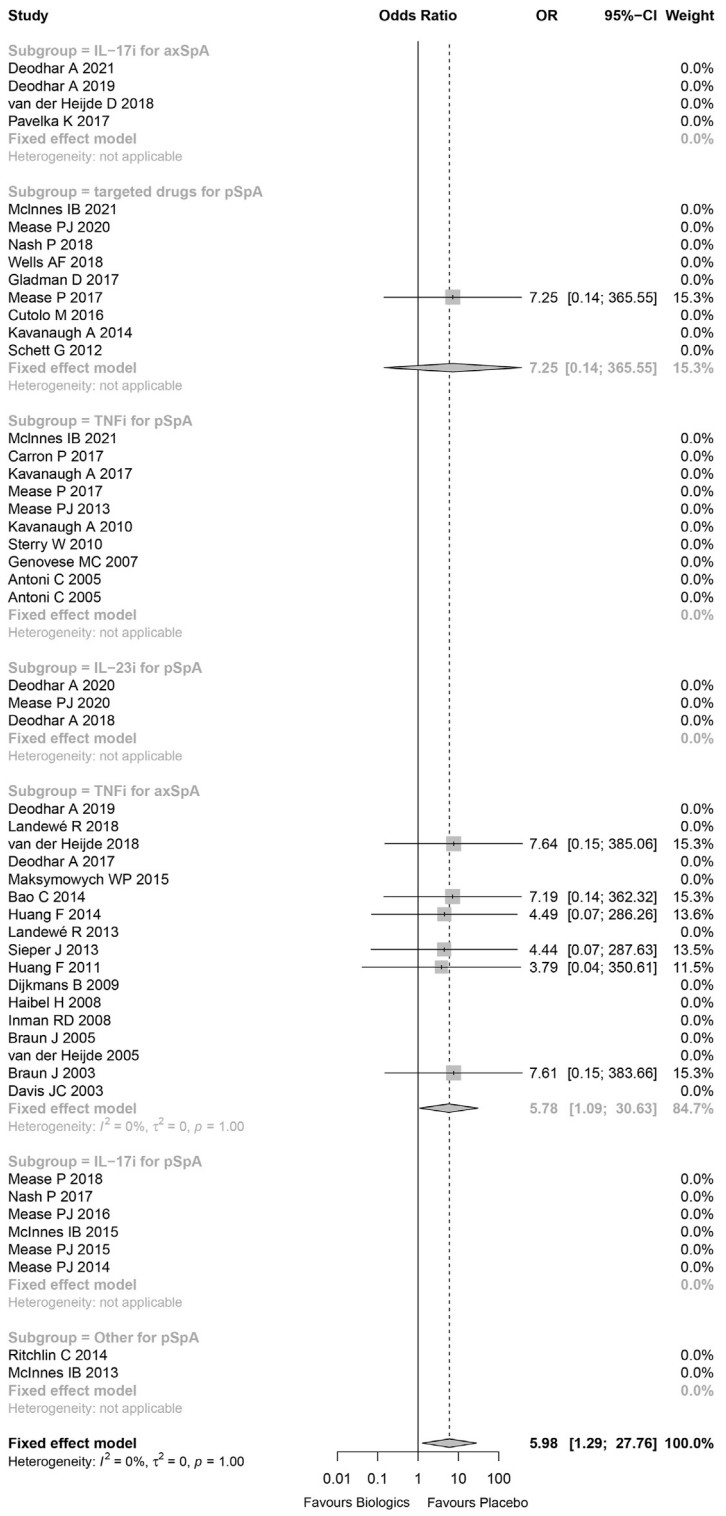

FIGURE 2 | Forest plot of meta-analyses comparing biologics versus placebo for risk of malignancy.

There was no significant difference between TNF-α inhibitors and placebo in patients with axial SpA (Peto OR 2.54, 95%CI 0.84–7.68, p = 0.099; I^2 = 0%, and p = 0.551) without evidence of publication bias (**Supplementary Figure S7**). A subgroup analysis was performed based on anti-TNFα antibody and TNF receptor-Fc fusion protein. The results

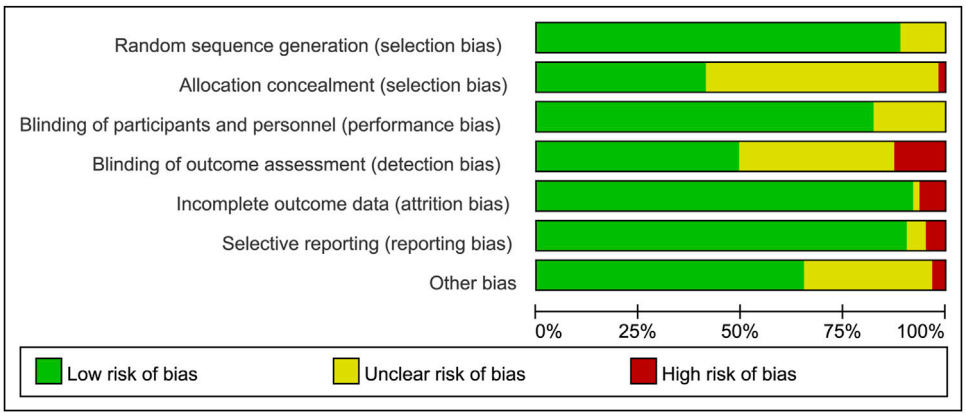

FIGURE 3 | Forest plot of meta-analyses comparing biologics versus placebo for risk of tuberculosis

showed that the risk of malignancy was higher than placebo for TNF receptor-Fc fusion protein in patients with axial SpA (Peto OR 7.18, 95%CI 1.21–42.69, p = 0.030; I^2 = 0%, and p = 0.940), while anti-TNFα antibody was not (Peto OR 1.32, 95%CI 0.32–5.43, p = 0.700) (**Supplementary Figure S8**).

Compared to placebo, there were slightly increased risk of malignancy for IL-23 inhibitors in patients with peripheral SpA (Peo OR 2.34, 95%CI 0.32–17.10, p = 0.403; I^2 = 0%, and p = 0.676), IL-17 inhibitors in patients with axial SpA (Peto OR 2.56, 95%CI 0.59–11.19, p = 0.212; I^2 = 0%, and p = 0.750) without evidence of publication bias (**Supplementary Figure S9**), and TNF-α inhibitors in patients with peripheral SpA (Peto OR 1.87, 95%CI 0.71–4.96, p = 0.206; I^2 = 0%, and p = 0.450) without evidence of publication bias (**Supplementary Figure S10–11**). Nevertheless, all Peto ORs had wide 95%CI with $p > 0.05$, that was, there were no statistical difference between the treatment groups and placebo.

Tuberculosis

Tuberculosis infections were reported in 7 patients in biological and targeted drugs therapy group, but no tuberculosis was reported in placebo group. Five of them occurred in areas with relatively higher background tuberculosis rates (China, Mexico, and Russia), and the other two in Germany. Two cases of tuberculosis occurred in the patients treated with adalimumab, two in patients treated with infliximab, one in patients treated with golimumab, and one in patients treated with tofacitinib.

The statistical heterogeneity was very low (I (Lopez-Olivo et al., 2012) = 0%) and not beyond variations that might be caused by chance (p = 1.00). Comparing to placebo, the risk of tuberculosis infection in patients treated with biologics or targeted drugs was increased (Peto OR 5.98, 95%CI 1.29–27.76, p = 0.022; **Figure 3**) without evidence of publication bias (**Supplementary Figure S12**). 71.4% of tuberculosis infections occurred in anti-TNFα antibody for axial SpA group, and the result demonstrated that there was significant increase in anti-TNFα antibody for axial SpA (Peto OR 6.17, 95%CI 1.03–37.13, p = 0.046; I^2 = 0%, p = 0.999) without evidence of publication bias (**Supplementary Figure S13**). No tuberculosis infectious was found in TNF receptor-Fc fusion protein (**Supplementary Figure S14**). In addition, no tuberculosis occurred in the studies being high-risk bias, so there was no effect on the above results while omitting these studies being high-risk bias (**Supplementary Figure S15**).

DISCUSSION

There has been concern regarding a putative increasing risk of malignancies and tuberculosis with either biologics and small molecular targeted drugs treatment because of the impacts of these therapies on the immune system. Our work shows that there is an elevated risk of malignancies and tuberculosis in patients with SpA receiving biologics or small molecular targeted drugs therapy, compared to placebo. While investigating the risk of malignancies in the treatment with biologics by different types of biological agents in each SpA type, we find a wide 95%CI and $p > 0.05$ in all pooled Peto ORs, except for IL-17 inhibitors and small molecular targeted drugs in peripheral SpA, compared to placebo. In addition, we only find an elevated risk of tuberculosis infection in individuals with axial SpA receiving TNF-α inhibitors therapy.

Malignancies

Malignancies in single RCTs of biological and targeted drugs treatment in patients with SpA were rare, and the observed differences were not statistically significant in their occurrence between groups. We investigated the risk of malignancies in treatment with biologics and small molecular targeted drugs in SpA including axial SpA and peripheral SpA by meta-analysis, overall as well as by biological agents' classes in each SpA type.

The comparison of IL-17 inhibitors to placebo was the only one biologic that achieved significant difference in patients with peripheral SpA rather than axial SpA. Nevertheless, all seven included RCTs investigated exclusively individuals with psoriatic

arthritis (a specific subtype of peripheral SpA). Interestingly, a comprehensive clinical trial safety dataset consisting of twenty-three RCTs of secukinumab in ankylosing spondylitis, psoriatic arthritis, and psoriasis indications indicated that the exposure-adjusted incident rates per 100 patient-years for malignancies in treatment with secukinumab were 0.8, 1.1, and 0.5 in the psoriasis, psoriatic arthritis, and ankylosing spondylitis studies, respectively (Deodhar et al., 2019). Because of the double-edged nature of IL-17, its exact role in tumorigenesis and metastasis is still unclear (Chang, 2019). On the one hand, IL-17 can play a tumor-promoting effect by inducing tumor angiogenesis, which increases the risk malignancies. On the other hand, IL-17 can mediate anti-immunity by enhancing the activity of natural killer cells and cytotoxic T lymphocytes, which decreases the risk of malignancies. There are reasons to believe that any increased risk for malignancy may be contributed in part by the nature of chronic inflammation on the disease with the involvement of IL-17. We recommend a long-term follow-up of these patients to investigate whether the tumor occurs or not and further research to elucidate the differential tumorigenic effects of IL-17 inhibitors in treatment of psoriatic arthritis and ankylosing spondylitis.

The comparison of TNF-α inhibitors to placebo in SpA including peripheral SpA and axial SpA did not achieve statistical significance. A collaborative study from the ARTIS and DANBIO registers showed that treatment with TNF-α inhibitors in patients (n = 8703) with SpA was not associated with increased risks of cancer, neither overall nor for the most cancer types, such as prostate cancer, lung cancer, colorectal cancer, lymphoma, breast cancer, and malignant melanoma (Hellgren et al., 2017). A subgroup analysis was performed based on anti-TNFα antibody and TNF receptor-Fc fusion protein in our study. The results showed that the risk of malignancy was higher than placebo for TNF receptor-Fc fusion protein in patients with axial SpA, while anti-TNFα antibody was not. However, only two studies reported the cases of malignancy, and pooled 95%CI was wide and imprecise, which may require more high-quality clinical trials to verify this result and also prompts us to pay attention to the effect of TNF receptor-Fc fusion protein on malignancy. As for anti-TNFα antibody, many studies indicated that it did not increase the risk of malignancy, which was consistent with our findings. A study by Burmester GR et al. analyzed the long-term safety of adalimumab treatment (Burmester et al., 2013). This analysis included 1,684 patients exposed to adalimumab in 4 clinical trials in ankylosing spondylitis, and results showed that standardized incidence rate for all malignancies including lymphoma and non-melanoma skin cancer was similar to the general population.

Additionally, in our study, there was a statistically significant increase in the risk of malignancies accompanying small molecule targeted drugs use, relative to placebo. In our study, two clinical trials on tofacitinib reported the occurrence of malignancy, one of which showed no tumorigenesis, and the other showed four patients with malignancy in the tofacitinib group. Besides, there were 8 patients with malignancy in the apremilast group, while only 2 patients with malignancy in placebo group. Due to the limitations of the shorter observation period in RCTs and the fact that these small molecule targeted drugs are newly used to treat SpA, our findings accentuate the necessity for long-term observational studies.

Tuberculosis

In our study, the main finding was that anti-TNFα antibody significantly increased the risk of tuberculosis infection in individuals with axial SpA. The increasing risk of tuberculosis is a main safety issue for anti-TNF-α therapy. Three widely used anti-TNF-α drugs with long-term safety profiles-infliximab and adalimumab-have been indicated to increase the risk of developing tuberculosis, which is similar to our finding. Anti-TNF-α therapies are well known to have a significant effect on immune cells, deactivating T cells and macrophages, and induction of apoptosis in key immune cells (Mitoma et al., 2008; Harris and Keane, 2010). Moreover, this has also been confirmed in the mouse models where TNF-α gene-deficient mice were infected with tuberculosis pathogens to study the role of TNF-α in tuberculosis infection (Kindler et al., 1989; Ehlers et al., 1999). The differences in the risk of tuberculosis infection associated with different anti-TNF-α drugs may be attributed to subtle differences in their mechanism of action. Monoclonal antibodies can increase the risk of tuberculosis infection by inhibiting the activation of T cells and the release of interferon-γ (Taylor, 2010). Due to different mechanisms of action, anti-TNF-α drugs, such as adalimumab, infliximab, and etanercept, are often studied and compared. In a national study in South Korea, it was found that the incidence of tuberculosis infection was highest among patients receiving infliximab therapy (IRR: 6.8), followed by adalimumab (IRR: 3.5) and etanercept (Jung et al., 2015). A study in the United States also showed that etanercept and infliximab were associated with tuberculosis infection, and the reporting rate of tuberculosis infection in patients using etanercept was lower than that of those using infliximab (10 cases vs. 41 cases per 100,000 patient-years of exposure) (Mohan et al., 2004). The standard of care in SpA adhere to the statement on the indications and safety monitoring for anti-TNF-α therapy, and advocate to assess patients for active and latent tuberculosis prior to treatment initiation (Kim et al., 2020). A study reported that the rate of active tuberculosis in adalimumab clinical trials has decreased from 15/1,000 patient-years to 2/1,000 patient-years, due to the implementation of screening and prophylaxis of latent tuberculosis infection in 1998 and 1999, respectively (Kim et al., 2020).

LIMITATIONS

There are several study limitations to consider. First, the short period of exposure in the included RCTs is a major solid limitation. Therefore, our results can only represent short- or medium-term risk assessment of malignancy and TB using biologics. Second, definitions of malignancies and TB may differ by individual studies, and many studies did not strictly follow the standard definition of tuberculosis and malignancies within the Medical Dictionary for Regulatory Activities (MeDRA) dictionary. These

cases were collected based on the original publication case description only with relevant differences between studies. These may result in a bias in collecting these cases. Third, there may be a bias for safety evaluation due to the patients previously exposed to biologics. Of the included studies, 24 studies explicitly excluded the patients previously treated with any biologics, 19 studies allowed the patients prior to biologics efficacy failures, but this was generally limited to ≤30% of enrolled patients, and the other studies did not report this information. Since the detailed information on individuals previously exposed to biologics were not reported, it is difficult to perform further subgroup analysis. Fourth, for *in situ* malignancies, in some countries, screening may be mandatory or highly recommended. However, in all the included studies, malignancies were only reported as an adverse event, and the details of the screening for *in situ* malignancies were not disclosed. It should also be noted that malignancies that occur within the short period of RCTs may not be new onset. It is necessary to perform in-depth screening while recruiting patients, due to the contraindications of biologics. Fifth, the doses of frequency of a biologic varied greatly among the included studies. It is difficult to performed a sensitivity analysis by dose. Therefore, this may be a bias for safety evaluation. Sixth, region of study performance and standard of care may differ by region on top of the local incidence of tuberculosis. However, it is difficult to compare the difference between studies conducted in regions where the background risk of tuberculosis may vary, as only six cases of tuberculosis were reported in the included studies. Seventh, screening and care for tuberculosis may change a lot over time (2003–2020). However, screening for tuberculosis were reported in only 11 of the 58 clinical trials, and were mostly based on PPD, chest radiographs, IGRA, or a combination, and the detailed information of screening, diagnosis and standard of care for tuberculosis were unavailable to us. This may result in a difference in the number of reported tuberculosis cases. Eighth, we only included these RCTs that have been published in peer-review journals since the results of these studies is more reliable. However, this may also lead to the potential publication bias. We have discussed them in the Limitation.

Above all, this systematic review and meta-analysis showed an increased risk of malignancies in individuals with peripheral SpA receiving biologics therapy, particularly for IL-17 inhibitors, and small molecule targeted drugs, a slightly increased risk of malignancy in TNF receptor-Fc fusion protein in axial SpA, and increased risk of tuberculosis in individuals with axial SpA treated with anti-TNFα antibody. Our findings may have direct implications in the management of a large number of patients treated currently with biologics and small molecule targeted drugs. In addition, due to limitations of very small number of events of malignancy and tuberculosis, our findings need to be validated by studies with larger population and longer follow-up.

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

FH conceived the study, participated in its design and coordination, and critically revised the manuscript. SM, LH, and XJ had access to the data collection, analysis, interpretation, and drafted the manuscript. YW, YM, LW, and JZ were study investigators and contributed to the process of data collection. 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.2021.705669/full#supplementary-material

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Real-World Data of Tigecycline-Associated Drug-Induced Liver Injury Among Patients in China: A 3-year Retrospective Study as Assessed by the Updated RUCAM

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Background: Tigecycline, a glycylcycline antibiotic, is increasingly used clinically for the treatment of severe infections caused by multidrug-resistant bacteria, but it is also associated with hepatotoxicity. However, the incidence and risk factors of tigecycline-associated drug-induced liver injury (DILI) are unclear. We conducted this study to investigate the incidence, characteristics and risk factors of tigecycline-associated DILI in the real-world clinic setting.

Patients and Methods: A retrospective analysis was conducted in inpatients who received tigecycline treatment from January 2018 to January 2020. Based on the biochemical criteria of DILI and the causality assessment by Roussel Uclaf Causality Assessment Method (RUCAM) using cases with a probable or highly probable causality grading, two clinical pharmacists and one clinician worked together to screen patients for tigecycline-associated DILI. Then patients with DILI were randomly matched by gender in a ratio of 1:2 to the remaining patients in the tigecycline cohort without biochemical abnormalities to identify risk factors.

Results: A total of 973 patients from 1,250 initial participants were included. The incidence of tigecycline-associated DILI was 5.7% (55/973). Among 55 DILI patients, 10 cases presented with the hepatocellular pattern, 4 cases belonged to the mixed pattern, and 41 presented with the cholestatic pattern. Most cases reached the severity of grade 1 and 2. The rate of recovery in hepatocellular pattern, mixed pattern, and cholestatic pattern was 70.0, 50.0, and 41.5%, respectively. The proportion of the DILI cases treated with high dose (100 mg) and prolonged duration (>14 days) was significantly higher than standard dose and routine duration (100.0% *vs.* 18.1%, $p < 0.05$). Logistic regression analysis showed that high maintenance dose (OR = 1.028, $p = 0.002$), prolonged duration (OR = 1.208, $p = 0.000$), and number of hepatotoxic drugs (OR = 2.232, $p = 0.000$) were independent factors of tigecycline-associated DILI.

Conclusion: Tigecycline was associated with liver injury, with a slightly higher incidence (5.7%) than the frequency of "frequent" (5%) defined by the Medical Dictionary for Regulatory Activities. Patients with a high maintenance dose and prolonged tigecycline regimen, as well as concomitant use of multiple hepatotoxic drugs should be paid more attention.

Keywords: tigecycline, liver injury, hepatotoxicity, causality assessment, RUCAM, roussel uclaf causality assessment method

INTRODUCTION

Tigecycline is the first clinically available glycylcycline antibiotic approved for the treatment of complicated intra-abdominal infection, complicated skin and soft-tissue infection, and community-acquired pneumonia, with a loading dose of 100 mg followed by 50 mg twice daily. It not only has good activity against Gram-positive and Gram-negative bacteria but also keeps highly sensitive to multidrug-resistant bacteria (Brink et al., 2010). Data from China Antimicrobial Surveillance Network (CHINET) showed that the resistance rates of *Acinetobacter* and *Enterobacter* to tigecycline were only 2.9% (http://www.chinets.com/). The expert consensus recommended tigecycline-based regimen as one of the options for the treatment of multidrug-resistant even pan-resistant Gram-negative bacteria (Bassetti et al., 2016; Guan et al., 2016), increasing its clinical application.

The most common adverse reactions of tigecycline are nausea and vomiting, but clinical trials in phase 2 and phase 3 found that tigecycline could cause elevated serum aminotransferase in 2–5% of patients (Ellis-Grosse et al., 2005; Oliva et al., 2005; Sacchidanand et al., 2005). The label of tigecycline provided by the manufacturer noted that isolated cases of severe liver dysfunction, cholestasis, and jaundice have been reported during post-marketing. Admittedly, both clinical trials and case reports have limitations. In particular, clinical trials are characterized by milder comorbidity, short drug duration, and relative homogeneity. It would compromise the understanding of the safety profile of tigecycline and could lead to insufficient attention to the potential hazards.

On the other hand, due to increasing bacterial resistance and limited available effective drug options, tigecycline was often prescribed off-label (Curcio et al., 2011; Moghnieh et al., 2017). The common off-label indications included ventilator-associated pneumonia, hospital-acquired pneumonia, and bacteremia etcetera. (Gardiner et al., 2010; Curcio et al., 2011; Moghnieh et al., 2017). In addition, based on the population pharmacokinetic and pharmacodynamic studies, the approved dose was often considered unable to achieve the optimal exposure in critically ill patients, thus the dosage of tigecycline was also off-label use (De Pascale et al., 2014; Xie et al., 2017). The prevalence of off-label use means that the adverse reactions might become more frequent and severe than those described in the label (Eguale et al., 2016).

So far, most current reports of tigecycline-associated liver injury were case reports or retrospective studies of small samples, and none had used a standardized, reliable method for assessing causality (Sabanis et al., 2015; Chen and Shi 2018; Geng et al., 2018; Xia and Jiang 2020). The purpose of this study was to investigate the incidence of tigecycline-associated drug-induced liver injury (DILI) in the real-world clinic setting by Roussel Uclaf Causality Assessment Method (RUCAM), describing the characteristics, management and outcomes, and exploring the risk factors of tigecycline-associated DILI.

METHODS

Study Design and Patient Population

We conducted a single-center, retrospective study to investigate the incidence, characteristics, risk factors and outcomes of DILI in tigecycline-treated patients in the Zhongshan Hospital, Fudan University, with approximately 2,000 beds. Medical Ethics Committee of Zhongshan Hospital approved this study and waived the requirement for informed consent because this retrospective analysis was limited to preexisting data from medical records and collected as a part of the routine treatment by clinicians. Data extracted from electronic medical records were coded to ensure privacy issues confidentiality.

All adult inpatients (age ≥18 years) medicated with tigecycline between January 1, 2018 and January 1, 2020 were identified from the electronic medical records. The index date was the day of initiation of tigecycline during hospitalization. Patients were excluded if they met any of the following criteria: 1) incomplete laboratory data (lack of data obtained within 7 days prior to the index date or lack of follow-up liver function tests); 2) duration <72 h; 3) patients with Child C liver function.

First, after the causality assessment by RUCAM (Danan and Teschke 2016), patients in DILI group were screened from eligible enrolled individuals. A flow chart summarizing the process of DILI case identification is presented in **Figure 1**. Second, in order to identify risk factors of tigecycline-associated liver injury, patients with DILI were randomly matched by gender in a ratio of 1:2 to the remaining patients in the tigecycline cohort without biochemical abnormalities.

Data Collection

Demographic details and clinical information obtained from the electronic medical records were reviewed to identify the suspected DILI following tigecycline medication. Baseline values were defined as those obtained on the onset day of tigecycline or within 7 days prior to the onset date. The occurrence date of DILI was defined as the date after the initiation of tigecycline and

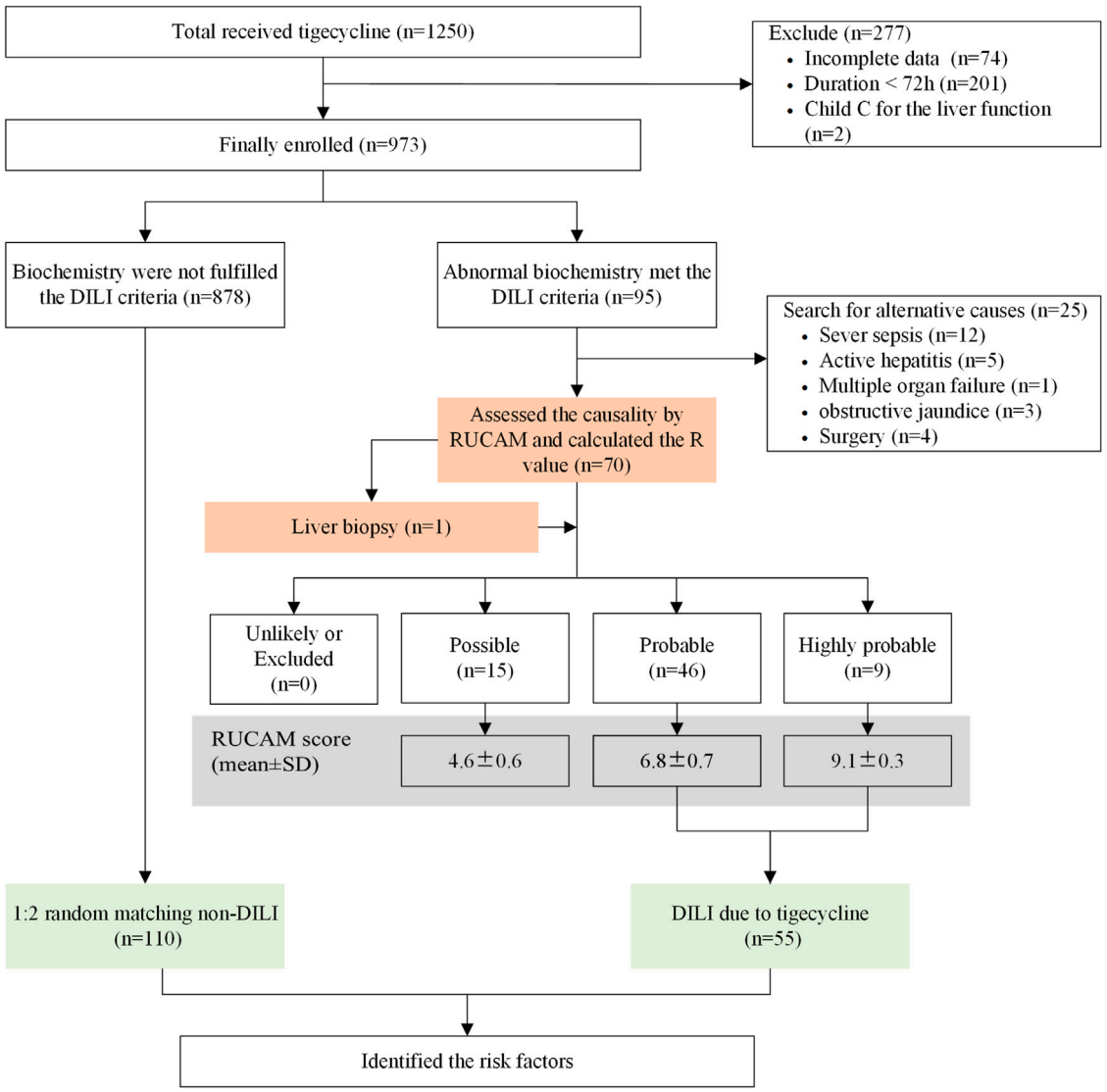

FIGURE 1 | Study design. Abbreviation: DILI, drug-induced liver injury.

within 3 days following discontinuation, in which the biochemical criteria of the DILI was reached. Patients were divided into two groups based on the results whether the level of liver enzyme elevations met the biochemical diagnostic criteria of DILI.

For patients whose elevated values of liver function fulfilling the biochemical diagnostic criteria of DILI, each record was reviewed in detail by two clinical pharmacists independently to determine the presence of suspected DILI. A standardized data collection form was applied for all eligible patients, including information regarding history of smoking or alcohol consumption, index date, diagnosis on admission, location of ward, history of liver disease, comorbidities, mechanical ventilation, tigecycline dose, duration of tigecycline therapy in days, baseline liver function and coagulation function, subsequently determined liver function and coagulation function, concomitant hepatotoxic medications, examinations for excluding other causes of liver injury (including hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes virus, Wilson's disease, and autoimmune hepatitis) and clinical outcome. Indicators of liver function included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bilirubin (TB), albumin (ALB). Indicators of coagulation function included prothrombin time (PT) and international normalized ratio (INR). If a liver-associated adverse event was listed as common one (incidence ≥1%) in the drug label of a suspected case concomitant drug, the drug was defined as a concomitant hepatotoxic medication.

For each DILI case, follow-up information on characteristics of DILI, treatment options, response of re-exposure, clinical

outcomes and liver biopsy were collected if applicable. Characteristics of DILI included liver injury pattern and grade of severity, latency time of liver injury. Treatment options included withdrawal of tigecycline after liver injury, reduction of tigecycline dosage or continuous treatment without adjustment, and medical therapy for DILI. Outcomes of DILI included liver outcome categorized by recovery, improvement, no improvement, and aggravation, 30-day all-cause mortality, and length of stay in hospital.

Biochemical Diagnostic Criteria of Suspected DILI Cases

Cases whose elevated liver enzymes met the following criteria were defined as the suspected DILI cases (Aithal et al., 2011; The Study of Drug Induced Liver Disease of Chinese 2015): 1) ALT ≥5×upper limit of normal (ULN), 2) ALT ≥3×ULN and TB ≥ 2×ULN, or 3) ALP ≥2×ULN, particularly with accompanying elevations in concentrations of GGT in the absence of known bone pathology driving the rise in ALP level. In patients with abnormal liver tests prior to starting treatment with tigecycline, ULN was replaced by the mean baseline values obtained before the index date of tigecycline and elevations should be calculated proportionate to this modified baseline.

Causality Assessment of Suspected DILI

First, suspected DILI cases with alternative causes for the liver injury were excluded, including severe sepsis, active hepatitis, multiple organ failure, obstructive jaundice, and surgery. Then causality assessment of suspected DILI was conducted based on updated RUCAM (Danan and Teschke, 2016). The final score calculated from RUCAM led to the causality levels as follows: 0 points, excluded causality; 1-2, unlikely; 3-5, possible; 6-8, probable; and 9, highly probable. After the independent causality assessments, all suspected DILI cases were reviewed in detail by consensus of two clinical pharmacists and one clinician to ensure agreement on all assessments.

After ruling out alternative explanations for abnormal liver biochemical indicators and assessment by RUCAM, only patients with a RUCAM score ≥6 were stratified into the DILI group. Cases with a "possible" grading were excluded to minimize the influence on the evaluation of DILI, as this group showed a relatively weaker causal relationship between tigecycline and liver injury.

Clinical Classification of DILI Patterns

Based on the Chinese guideline for the management of DILI (The Study of Drug Induced Liver Disease of Chinese 2015), we categorized the patients with DILI into 3 patterns on the basis of the ratio (R): 1) hepatocellular injury, ALT ≥3×ULN and R ≥ 5; 2) cholestatic injury, ALP ≥2×ULN and R ≤ 2; 3) mixed injury, ALT ≥3×ULN, ALP ≥2×ULN and 2 < R < 5. The R value was calculated for each DILI case on the day of the peak elevation of biochemical value which met the criteria of DILI. R was calculated as follows $[(ALT_{current}/ALT_{baseline})/(ALP_{current}/ALP_{baseline})]$.

Classification Criteria for the Severity of DILI

The severity of DILI was graded into five levels (The Study of Drug Induced Liver Disease of Chinese 2015):

Grade 1 (mild liver injury): The patients' serum level of ALT or ALP is elevated, but total bilirubin (TB) < 2.5×ULN (42.75 μmol/L), and with INR <1.5;

Grade 2 (moderate liver injury): Patients with elevated serum levels of ALT or ALP, and TB ≥ 2.5×ULN or INR ≥1.5;

Grade 3 (severe liver injury): Patients with elevated serum levels of ALT or ALP, TB ≥ 5×ULN (85.5 μmol/L), with or without INR ≥1.5;

Grade 4 (acute liver failure): Evidence of coagulation abnormality indicated by INR ≥2 or PTA (prothrombin activity) < 40%, and TB ≥ 10×ULN (171 mmol/L) or daily increase ≥17.1 mmol/L;

Grade 5 (fatal): Death due to DILI or necessitates a liver transplant for survival.

Definition on the Prognosis of DILI

Definition of the prognosis of DILI varied among different injury patterns. For hepatocellular injury, recovery was defined as ALT decreasing to below the ULN or baseline value; improvement was defined as ALT decreasing to below 3×ULN or baseline value; no improvement was defined as ALT not decreasing to below 3×ULN or baseline value; and aggravation was defined as ALT beyond the peak value.

For mixed injury, recovery was defined as both ALT and TB decreasing to below the ULN or baseline value; improvement was defined as ALT decreasing to below 3×ULN or baseline value, as well as TB decreasing to below; 2×ULN or baseline value; no improvement was defined as ALT not decreasing to below 3×ULN or baseline value, or TB not decreasing to below 2×ULN or baseline value; and aggravation was defined as either ALT or TB beyond the peak value.

For cholestatic injury, recovery was defined as ALP decreasing to below the ULN or baseline value; improvement was defined as ALP decreasing to below 2×ULN or baseline value; no improvement was defined as ALP not decreasing to below 3×ULN or baseline value; and aggravation was defined as ALP beyond the peak value. Both recovery and improvement of liver enzyme values can be regarded as effective outcomes.

Statistical Analysis

Statistical analysis was performed through SPSS Statistics v.22.0 (IBM Corp., Armonk, NY, United States). Normally and non-normally distributed continuous variables were presented as mean ± standard deviations (SD) or median (interquartile ranges, IQR) and were compared by independent sample t-test and Mann-Whitney U test, respectively. Categorical variables were compared by Chi-square test or Fisher's exact test. Variables with p value less than 0.1 in the univariate analysis were analyzed in Logistic regression model. A backward logistic regression model was adopted to analyze the independent risk factors of tigecycline-associated DILI. A p value <0.05 was considered statistically significant.

TABLE 1 | Characteristics and outcomes of DILI caused by tigecycline.

Variables	Pattern of liver injury		
	Hep (*n* = 10)	Mix (*n* = 4)	Chol (*n* = 41)
Causality assessment			
Highly probable, *n* (%)	3 (30.0%)	0 (0.0%)	6 (14.6%)
Probable, *n* (%)	7 (70.0%)	4 (100.0%)	35 (85.4%)
Liver injury during tigecycline treatment, *n* (%)	7 (70.0%)	3 (75.0%)	32 (78.0%)
Withdrawal tigecycline after liver injury, *n* (%)	3 (30.0%)	2 (50.0%)	20 (48.8%)
Reduction tigecycline dosage after liver injury, *n* (%)	0 (0.0%)	0 (0.0%)	3 (7.3%)
Continuous treatment without adjustment, *n* (%)	4 (40.0%)	1 (25.0%)	9 (22.0%)
Liver injury within 3 days of discontinuation of tigecycline, *n* (%)	3 (30.0%)	1 (25.0%)	9 (22.0%)
Latency time of liver injury, median (IQR), days	4.5 (2.0–7.4)	6.5 (1.8–12.0)	12.0 (9.0–16.0)
Re-exposure to tigecycline and recurrent ALT or ALP increase, *n* (%)	0 (0.0%)	0 (0.0%)	2 (4.9%)
Drugs for treatment			
Anti-inflammation, *n* (%)	5 (50.0%)	2 (50.0%)	16 (39.0%)
Antioxidants, *n* (%)	8 (80.0%)	3 (75.0%)	31 (75.6%)
Phospholipids, *n* (%)	3 (30.0%)	2 (50.0%)	4 (9.8%)
Cholagogue, *n* (%)	3 (30.0%)	2 (50.0%)	19 (46.3%)
Outcome of liver injury*			
Recovery, *n* (%)	7 (70.0%)	2 (50.0%)	17 (41.5%)
Improvement, *n* (%)	1 (10.0%)	1 (25.0%)	5 (12.2%)
No improvement, *n* (%)	1 (10.0%)	0 (0.0%)	15 (36.6%)
Aggravation, *n* (%)	0 (0.0%)	1 (25.0%)	0 (0.0%)
Time to recovery, median (range, min-max), days	11.0 (4.0–37.0)	13.5 (2.0–25.0)	24.0 (8.0–66.0)
30-day all-cause mortality, *n* (%)	2 (20.0%)	1 (25.0%)	3 (7.3%)
Length of stay in hospital, median (IQR), days	42.0 (20.3–59.3)	33.5 (24.0–62.5)	46.0 (38.0–63.5)

** One of patients with hepatocellular injury pattern lack of outcome data, while eight of patients with cholestatic injury pattern. DILI, drug-induced liver injury; Hep, hepatocellular injury pattern; Mix, mixed injury pattern; Chol, cholestatic injury pattern; IQR, interquartile ranges; ALT, alanin aminotransferase; ALP, alkalinephosphatase; min, minimal; max, maximal.*

RESULTS

Enrollment Process

A total of 1,250 patients treated-tigecycline were identified from the electronic medical records. 277 patients were excluded due to incomplete laboratory data (*n* = 74, 26.7%), duration of tigecycline less than 72 h (*n* = 201, 72.5%), and patients with Child C liver function (*n* = 2, 0.7%). Then, 95 of the assessed 973 patients had abnormal biochemical values. Among whom, 25 patients' abnormal liver function could be explained by alternative causes, including severe sepsis (*n* = 12), active hepatitis (*n* = 5), multiple organ failure (*n* = 1), obstructive jaundice (*n* = 3), surgery (*n* = 4). Therefore, 70 patients were finally assessed by RUCAM and the R value (**Figure 1**).

Clinical Outcome of DILI Pattern, Severity, and Treatment

Characteristics and outcomes of DILI caused by tigecycline were listed in **Table1**. Only one patient had performed liver biopsy. Based on the causality assessment by RUCAM, 15 cases with RUCAM scores between 3-5 were considered as possible, 46 cases whose RUCAM scores between 6-8 were considered as probable, and 9 cases with RUCAM scores equal to or beyond nine were considered as highly probable. After ruling out 15 cases with possible causality grade, 55 patients had confirmed tigecycline-associated DILI, with an incidence of 5.7% (55/973).

According to the R value at initial valuation, 10 out of 55 DILI patients presented with the hepatocellular pattern, 4 cases belonged to the mixed pattern, and 41 presented with the cholestatic pattern (**Table 1**). When comes to initial evaluation on the grade of severity (**Table 2**), most cases among three patterns reached grade 1 and 2. It should be noticed that 5 cases in the cholestatic and 1 case in the mixed group reached grade 3, while 1 case in the cholestatic pattern was observed achieving grade 4.

Only cases with continued deterioration of liver enzyme values were re-evaluated (**Table 2**). In one of the re-evaluated patients, the pattern of DILI changed from hepatocellular to mixed type on day 2 after discontinuation of tigecycline, and the severity grade increased from 1 to 2. Two patients changed from hepatocellular injury to cholestatic injury, one of them increased from level 2 to 3 on day 3 after withdrawal, and the other remained level 1 during the course. For mixed injury, no change in a liver injury pattern and severity level was observed. In addition, cases with cholestatic patterns changed only in the grade of severity. Specifically, severity developed from level 1 to level 2 was found in 1 case with cholestatic injury on the second day after withdrawal, 2 cases progressed from level 1 to level 3 on the last day of tigecycline treatment or the third day after withdrawal, 1 case progressed from level 1 to level 4 on the second day after withdrawal, 2 cases progressed from level 2 to level 3 during the course or the third day after withdrawal, 1 case progressed from level 2 to level 4 during the course, and 1 case progressed from level 3 to level 4 on the day after withdrawal.

For hepatocellular injury, mixed injury and cholestatic injury, the median latency of liver injury was 4.5 days (range, 2.0–7.4 days), 6.5 days (range, 1.8–12.0 days), and 12.0 days (range, 9.0–16.0 days), respectively (**Table 1**). Most patients

TABLE 2 | Characteristics and outcomes in DILI patients and non-DILI patients.

Severity of DILI	Hep		Mix		Chol	
	Initial valuation (*n* = 10)	Revaluation (*n* = 2)	Initial valuation (*n* = 4)	Revaluation (*n* = 2)	Initial valuation (*n* = 41)	Revaluation (*n* = 21)
Stage 1	5 (50.0%)	1 (50.0%)	2 (50.0%)	1 (50.0%)	25 (61.0%)	11 (52.4%)
Stage 2	5 (50.0%)	1 (50.0%)	1 (25.0%)	1 (50.0%)	10 (24.4%)	2 (9.5%)
Stage 3	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	5 (12.2%)	5 (23.8%)
Stage 4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.4%)	3 (14.3%)

DILI, drug-induced liver injury; Hep, hepatocellular injury pattern; Mix, mixed injury pattern; Chol, cholestatic injury pattern.

developed DILI during tigecycline treatment, but 3 (30.0%) patients in the hepatocellular group, 1 (25.0%) in the mixed group and 9 (22.2%) in the cholestatic group developed DILI within 3 days of discontinuation of tigecycline. Recurrent double ALP increase as diagnostic criterion (Danan and Teschke 2016) was observed in two cholestatic cases when they were unintentionally re-challenged with tigecycline. No patient with other injury patterns underwent re-exposure to tigecycline in the study.

For patients with DILI during tigecycline treatment, approximately half of the patients classified in mixed (50.0%) and cholestatic pattern (48.8%) withdrew tigecycline after DILI onset, while 40.0% of patients in the hepatocellular group continued treatment without adjustment. Of the 55 patients with DILI, 50 cases received hepatoprotective drugs, antioxidants were most frequently administrated to treat DILI, followed by anti-inflammation agents, cholagogue, and polyene phosphatidylcholine (**Table 1**).

Notably, one of the patients with hepatocellular injury pattern and eight of patients with cholestatic injury pattern were lacking of data on DILI outcome. For the remaining parts (**Table 1**), patients with hepatocellular pattern had the highest rate of recovery (70.0%) and improvement (10.0%), followed by mixed pattern (50.0% for recovery and 25.0% for improvement), and then cholestatic pattern (41.5% for recovery and 12.2% for improvement). The median time to recovery for hepatocellular injury pattern was 11.0 days, 13.5 days for mixed pattern and 24.0 days for cholestatic pattern, respectively. Cholestatic cases seemed to have the longest median hospitalization length (46.0 days, IQR: 38.0–63.5d), followed by hepatocellular cases (42.0 days, IQR: 20.3–59.3d) and mixed cases (33.5 days, IQR:24.0–62.5d).

After excluding auto-discharged patients, 24 patients died within 30-day after being treated with tigecycline in the whole cohort. The 30-day all-cause mortality rate in the DILI group was 10.9% (6/55), with 2 cases in the hepatocellular type, 1 case in the mixed type and 3 cases in the cholestatic type. Among all types, three patients died from multiple systemic organ failure, two patients died from severe pneumonia, and the remaining patient died of malignant arrhythmia. As for the non-DILI group, the 30-day all-cause mortality rate was 16.4% (18/110), with seven patients died due to multiple systemic organ failure, five patients due to severe pneumonia, and the remaining six patients due to primary diseases or complications, including acute respiratory distress syndrome, gastrointestinal hemorrhage, heart failure, or NK/T-cell Lymphoma.

Comparison of Biochemical Tests Between Baseline and After Tigecycline Treatment

As shown in **Figure 2**, we analyzed changes of biochemical tests of 55 patients with DILI before and after tigecycline treatment, to investigate whether tigecycline affected other liver function parameters to some extent. Our study found that not only ALT and ALP, but also median values of TB, AST, and PT were significantly increased after tigecycline treatment compared to baseline values in DILI cases ($p < 0.0001$).

Analysis of Risk Factor Between DILI Group and Non-DILI Group

Characteristics of DILI group and non-DILI group were depicted in **Table 3**. 55 DILI patients were matched with 110 non-DILI patients. By univariate analysis, there was significant difference regarding mechanical ventilation, maintenance dose of tigecycline, duration of tigecycline and number of concomitant hepatotoxic medications between DILI group and non-DILI group. DILI patients with mechanical ventilation (58.2% *vs.* 29.1%, $p = 0.000$) were more likely to develop DILI. Meanwhile, proportion of patients with DILI among various maintenance doses or duration was summarized in **Figure 3** and **Table 3**. Both high maintenance dose (100 mg) and prolonged duration (>14 days) of tigecycline were positively related to DILI risk. Proportion of the DILI cases treated with standard dose/prolonged duration was significantly higher than those treated with standard dose/routine duration (64.7% [11/17] *vs.* 18.1% [17/94], $p < 0.05$). Similar trends could be observed between high dose/routine duration and standard dose/routine duration (40.0% [18/45] *vs.* 18.1% [17/94], $p < 0.05$), high dose/prolonged duration and high dose/routine duration (100.0% [9/9] *vs.* 40.0% [18/45], $p < 0.05$), high dose/prolonged duration and standard dose/routine duration 100.0% [9/9] *vs.* 18.1% [17/94], $p < 0.05$), respectively (**Figure 3**). However, there showed no significant difference in terms of age, gender, weight, BMI, history of alcohol and smoke consumption, department of ward, history of liver disease, payments method, comorbidities, baseline laboratory variables, baseline liver function, loading dose of tigecycline, and 30-day all-cause mortality between two

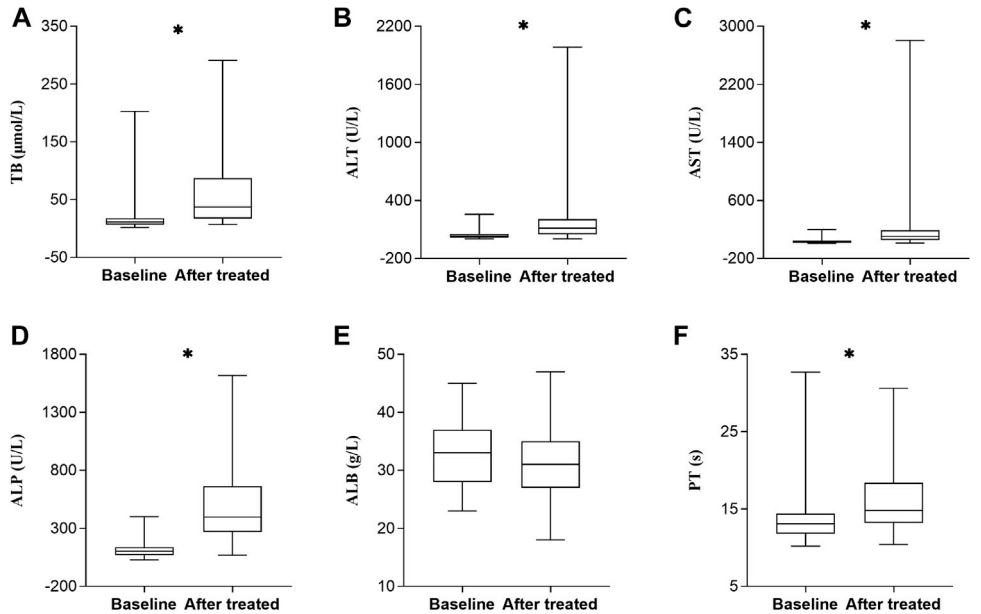

FIGURE 2 | Changes of laboratory test values before and after tigecycline treatment in DILI patients (n = 55). Horizontal bars represent the median value, boxes represent the interquartile range and whiskers indicate the minimum and maximum value. Wilcoxon's test was used to compared baseline and after tigecycline treatment's biochemical test values. *p < 0.0001. Abbreviation: TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALB, albumin; PT, prothrombin time; DILI, drug-induced liver injury.

groups. Five variables (p < 0.1) were included in multivariate logistic regression analysis, including chronic liver disease, mechanical ventilation, maintenance dose, duration and number of concomitant hepatotoxic drugs. High maintenance dose (OR = 1.028, p = 0.002), prolonged duration (OR = 1.208, p = 0.000), and number of hepatotoxic drugs (OR = 2.232, p = 0.000) were found to be independent factors of tigecycline-associated DILI (**Table 4**).

DISCUSSION

To the best of our knowledge, this single-center retrospective study involving 973 patients treated with tigecycline is the first to elucidate the incidence, characteristics, prognosis, and risk factors of tigecycline-associated DILI in China. The study provides real-world evidence for the hepatic safety profile in patients treated with tigecycline and has a relatively large sample size.

Off-label use (including high dosage) of tigecycline had been widely applied for critically ill patients, including patients with sepsis or septic shock (Curcio 2009; Conde-Estévez et al., 2010; Borsuk-De Moor et al., 2018). An early review displayed that high doses of tigecycline for patients were safe and tolerable (Peterson 2008). Regardless of some treatment-emergent adverse events, it seemed to be pretty safe.

Diagnosis of DILI is always considered a challenging issue due to non-specific symptoms and a lack of a valid diagnostic biomarker, often confounded by alternative causes. Therefore, identification of DILI requires exclusive diagnosis, and causality assessment by RUCAM might contribute to clarify the causality of the suspected DILI cases (Teschke and Danan 2016). At present, the diagnosis of DILI caused by tigecycline was very rare, and there was no literature on tigecycline-associated DILI (LiverTox, 2012). Some published studies on liver toxicity caused by tigecycline only mentioned the elevation of some liver enzyme levels, not classified by the biochemical criteria of DILI (Yahav et al., 2011; Kadoyama et al., 2012). Moreover, many DILI cases presented in LiverTox database were insufficiently documented without using RUCAM (Teschke and Danan 2021). There were gaps in the knowledge regarding the incidence, duration, pattern, and prognosis of tigecycline-associated DILI.

Herein, one of our key findings was that the incidence of tigecycline-associated DILI was 5.7% (55/973) in the real-world clinic setting, which appeared to be a little higher than the reported rate of elevated ALT/AST (approximately around 2–5%) in some phase 2 and phase 3 clinical trials (Ellis-Grosse et al., 2005; Oliva et al., 2005; Sacchidanand et al., 2005). The difference might be due to the higher proportion of high-maintenance dose regimes and prolonged duration in this study, compared with these clinical trials. Meanwhile, we noted that the incidence of DILI was lower than the rate of reported-hepatoxicity events in some post-marketing retrospective studies (De Pascale et al., 2014; Chen and Shi 2018; Geng et al., 2018; Xia and Jiang 2020). This could be related to our strict screening criteria and assessment using a standard causality assessment scale, whereas other retrospective studies only considered some abnormal liver enzyme values as adverse events in the liver.

TABLE 3 | Characteristics and outcomes in DILI patients and non-DILI patients.

Variable	DILI (*n* = 55)	Non-DILI (*n* = 110)	*p*-value
Age, median (IQR), y	68 (55–75)	66 (57–77)	0.700
≤65 years, *n* (%)	23 (41.8%)	51 (46.4%)	0.580
>65 years, *n* (%)	32 (58.2%)	59 (53.6%)	
Male sex, *n* (%)	34 (61.8%)	68 (61.8%)	1.000
Weight, median (IQR), kg	60.0 (55.0–70.0)	62.0 (54.8–70.0)	0.974
BMI, median (IQR), kg/m^2	22.7 (20.3–24.8)	22.7 (20.0–24.5)	0.753
<18.5 kg/m^2, *n* (%)	5 (9.1%)	16 (14.6%)	0.589
18.5–24 kg/m^2, *n* (%)	32 (58.2%)	58 (52.7%)	
>24 kg/m^2, *n* (%)	18 (32.7%)	36 (32.7%)	
Hospital admission, *n* (%)			0.318
Medical ward	34 (61.8%)	59 (53.6%)	
Surgical ward	21 (38.2%)	51 (46.4%)	
Payment methods, *n* (%)			0.420
Medical insurance	38 (69.1%)	69 (62.7%)	
Self-paying	17 (30.9%)	41 (37.3%)	
Underlying disease, *n* (%)			
Chronic liver disease	6 (10.9%)	24 (21.8%)	0.087
Diabetes mellitus	13 (23.6%)	34 (30.9%)	0.329
Solid organ cancer	15 (27.3%)	36 (32.7%)	0.475
Hematologic malignancy tumor	0 (0.0%)	8 (7.3%)	0.096*
Heart disease	13 (23.6%)	38 (34.5%)	0.153
Smoking, *n* (%)	8 (14.5%)	18 (16.4%)	0.763
Alcohol use, *n* (%)	6 (10.9%)	9 (8.2%)	0.566
Mechanical ventilation, *n* (%)	32 (58.2%)	32 (29.1%)	**0.000**
Baseline laboratory variables			
ALB [40–55 g/L], median (IQR), g/L	33.0 (28.0–37.0)	31.5 (27.0–34.3)	0.108
PT [10.0–13.0 s], median (IQR), s	13.1 (11.8–14.4)	13.4 (12.0–14.5)	0.647
TB [3.4–20.4 μmol/L], median (IQR), μmol/L	11.5 (6.8–17.7)	12.2 (8.7–17.3)	0.295
ALT [Male: 9–50 U/L; Female: 7–40 U/L], median (IQR), U/L	33.0 (14.0–52.0)	22.0 (14.0–45.5)	0.219
AST [Male: 15–40 U/L; Female: 13–35 U/L], median (IQR), U/L	28.0 (17.0–44.0)	26.0 (17.0–43.5)	0.912
ALP [Male: 45–125 U/L; Female: age <50 years 35–100 U/L, age ≥50 years 50–135 U/L], median (IQR), U/L	102.0 (68.0–137.0)	91.0 (65.8–128.5)	0.674
GGT [Male 1060 U/L; Female 745 U/L], median (IQR), U/L	57.0 (27.0–119.0)	50.5 (27.8–107.8)	0.667
Baseline liver function			0.911
Normal, *n* (%)	22 (40.0%)	45 (40.9%)	
Abnormal, *n* (%)	33 (60.0%)	65 (59.1%)	
Tigecycline therapy			
Loading dose, *n* (%)	29 (52.7%)	64 (58.2%)	0.505
Maintaining dose, *n* (%)			**0.002**
Standard dose (50 mg)	28 (50.9%)	83 (75.5%)	
High dose (100 mg)	27 (49.1%)	27 (24.5%)	
Duration, median (IQR), days	13.0 (7.5–19.5)	7.0 (4.5–9.6)	**0.000**
≤14d, *n* (%)	35 (63.6%)	104 (94.5%)	
>14d, *n* (%)	20 (36.4%)	6 (5.5%)	
Number of concomitant hepatotoxic medications, median (IQR)	2.0 (1.0–4.0)	1.0 (1.0–2.0)	**0.000**
30-day all-cause mortality, *n* (%)	6 (10.9%)	18 (16.4%)	0.349

** Continuity correction. DILI, drug-induced liver injury; BMI, Body Mass Index; ALB: albumin; PT, prothrombin time; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; IQR, interquartile ranges. The bold vaules provided in Table refers to* $p<0.05$.

Another key finding was that the most common DILI pattern in this study was cholestatic type, followed by mixed type and hepatocellular type. This finding differed from previous literature involving DILI caused by other medications (Aiso et al., 2019), as tigecycline was the only indicated drug involved in our study. Notably, case series of tetracycline-induced bile duct paucity and prolonged cholestasis with liver histological changes of microvesicular steatosis had been reported previously (Hunt and Washington 1994). Tigecycline-associated DILI presented with cholestatic injury pattern most frequently, which might be explained by its structure derivation from tetracycline, similar with tetracycline in biochemical properties to some extent.

The majority of DILI patients presented with mild liver injury, with the highest proportion of grade 1 (58.2%, 32/55), which may be attributed to the early detection and intervention. Generally, their liver function could be recovered or improved after the withdrawal of the drug. Noticeably, patients in cholestatic injury pattern seemed to be more prone to deteriorate during the course of the disease in our study. Similarly, published papers (Chalasani et al., 2015; Alhaddad et al., 2020; Liu et al., 2020) also revealed that fatal cases and prolonged disease course occurred more frequently among patients with cholestatic injury.

Our study observed that the length of recovery time for all tigecycline-associated DILI patterns ranged from 2 to 66 days after the onset of DILI (**Table 1**). Previous papers (Andrade et al.,

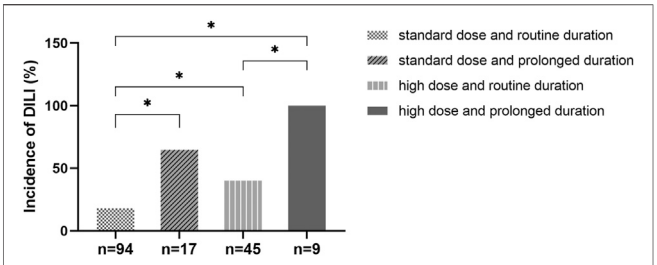

FIGURE 3 | Effects of tigecycline treatment dose and duration on DILI. The proportion of the DILI cases treated with standard dose and routine duration, standard dose and prolonged duration, high dose and routine duration, high dose and prolonged duration was 18.1, 64.7, 40.0, and 100.0%, respectively. Abbreviation: DILI, drug-induced liver injury. *$p < 0.05$.

coagulation function have been noted, but limited data are available to assess effects on liver function (Campany-Herrero et al., 2020). In spite of a meta-analysis showed no significant difference in the incidence of liver injury between the standard-dose group and high-dose group (Zha et al., 2020), the result needed to be interpreted with caution. Because the data used to analyze hepatoxicity came from four single-center, retrospective studies (only 294 patients in total), even the definition of hepatoxicity was not described in two of the studies. To better understand the effects of dose and duration of therapy on tigecycline hepatoxicity, we further performed a stratified analysis. Based on the results, we appealed to clinicians for balancing the relationship between efficacy and safety in caution when choosing a treatment regimen.

TABLE 4 | Risk factors for DILI caused by tigecycline.

	β	S.E.	Wald χ^2	Or (95%CI)	*P*
Chronic liver disease	0.594	0.616	0.928	1.811 (0.541–6.058)	0.335
Mechanical ventilation	−0.820	0.435	3.550	0.440 (0.188–1.033)	0.060
Maintaining dose	0.027	0.009	9.455	1.028 (1.010–1.046)	**0.002**
Duration	0.189	0.045	17.718	1.208 (1.106–1.319)	**0.000**
Number of concomitant hepatotoxic	0.803	0.216	13.771	2.232 (1.461–3.411)	**0.000**

DILI, drug-induced liver injury. The bold vaules provided in Table refers to p*<0.05.*

2005; Chalasani et al., 2015) reported that recovery days of the majority of cases exceeded 60 days and the rate of chronic DILI (recovery time after the onset of DILI longer than 180 days) accounted over 10%, regardless of DILI patterns. On the one hand, this could be due to different characteristics between tigecycline and other implicated drugs associated with DILI. On the other hand, the difference in results from other studies could be attributed to the limited follow-up period and undetermined DILI outcome for some cases in the present study. Furthermore, the 26 mentioned patients recovered more quickly than reported mainly due to withdrawal or reduction of tigecycline and provision of hepatoprotective medication timely.

Generally, liver injury patterns could relate to prognosis. Pang et al. (2018) from China observed that patients with cholestatic and mixed injury patterns were more prone to develop chronic liver injury. Previous literature from America (Food and Drug Administration USD of H and HS) revealed that the hepatocellular type was more frequent and predominantly leads to severe DILI. Moreover, other studies showed hepatocellular type and cholestatic liver injury led to poor outcomes and significant mortality (Andrade et al., 2005; Björnsson and Olsson 2005; Chalasani et al., 2008). Regardless of various patterns, it is important to identify DILI and take interventions as early as possible. Our study found that tigecycline-associated DILI seemed not to be the contributory factor for the causes for death within 30 days of treatment after comparing these causes between DILI group and non-DILI group.

Additionally, the present study was the first to report high maintenance dose, prolonged duration, and number of concomitant hepatotoxic drugs as independent risk factors for tigecycline-associated DILI. The effects of off-label use on

The present study has several limitations. First, this was a single-center, retrospective study. The findings relied on the accuracy of medical records and were subject to confound by the propensity to prescribe tigecycline and the bias of the population attended at the department. Second, although all suspected DILI cases were reviewed by consensus of two clinical pharmacists and one clinician, the diagnosis could still be confounded by alternative causes. Third, we acknowledged the limitation of merely calculating a RUCAM score in the DILI assessment. Thus, a prospective study design is recommended in the future to allow for complete data sets and for avoiding possible causality gradings, resulting in a more acceptable and convincing outcome.

Despite as mentioned above, this study also has valuable implications for clinical practice. A strict DILI definition, as well as a standardized, validated causality assessment approach (RUCAM) was applied to objectively and accurately evaluate the occurrence and causality of tigecycline-associated liver injury. Moreover, the study described the main characteristic, management, risk factors and outcome dynamically assessed the evolution of the pattern and severity of tigecycline-associated DILI, which could assist clinicians in managing cases of the elevated liver enzymes after prescribing tigecycline.

CONCLUSION

Our study revealed that tigecycline was associated with liver injury, with a slightly higher incidence (5.7%) than the frequency of "frequent" (5%) defined by the Medical Dictionary for Regulatory Activities. In addition, tigecycline-associated DILI is related to high maintenance

dose, prolonged duration, and number of concomitant hepatotoxic drugs. Therefore, maintaining diligent monitoring and keen insight is required. Cognizant of this, clinicians should pay particular attention to high maintenance dose and prolonged tigecycline regimen, as well as concomitant use of multiple hepatotoxic drugs.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Zhongshan Hospital (approval number B2021-077R). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XS, CZ, QX, and QL conceived the study. XS and CZ contributed to the data analysis and prepared the manuscript. LY, DL, and QX enrolled the patients and collected information. XS, CZ, and LY assessed and reviewed suspected DILI cases. XL and QL supervised the study.

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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.2021.761167/full#supplementary-material

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MINI REVIEW
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Factors that Facilitate and Hinder the Comprehension of Patient Information Leaflets (PILs): A Brief Scoping Review

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Introduction: Patient information leaflets (PILs) of medicinal products are informative documents that accompany medicines and explain their components, modes of use, interactions with other medicines, and other relevant issues. When patients do not adequately understand the information in the leaflets, they may engage in behaviors that affect their health (e.g., self-medication).

Objective: To identify patient-related factors and characteristics of PILs that can promote cognitive, emotional, and behavioral changes that lead to appropriate drug use practices. Additionally, we aimed to determine strategies that could be implemented to design leaflets that convey adequate information and are easier to understand.

Method and Results: We evaluated scientific articles published in databases and containing information on PILs suitability to be used in a patient population. A total of 51 articles were selected as the sample. Certain leaflet factors that favored or hindered understanding were identified (e.g., format in which the leaflets are presented, their structure, their adaptation to the sociodemographic and linguistic characteristics of the population, their wording. . .). Similarly, we also identified patient factors, such as previous experience taking the drugs referred to in the leaflet; the type of emotions experienced when reading the leaflets; the emphasis on the adverse effects of the medications; sociodemographic variables (i.e., age or educational level); and degree of interest in their own healthcare.

Conclusion: Patient and leaflet factors influence the comprehension of information in the PIL; hence, emphasis should be placed on these factors to increase treatment and medication adherence and to reduce health-risk behaviors.

Keywords: comprehension, drug labeling, drug package insert, medicine package insert, patient information leaflet (PIL), readability, self medication, understanding

INTRODUCTION

There are numerous definitions of patient information leaflets (PILs); one refers to the documents provided to study participants, or their corresponding representatives, in clinical trials. As in other studies (Herber et al., 2014), in this study, PILs will be considered as the technical documents that contain written information about a drug and accompany it. In a PIL, the composition and conditions for usage of a drug are specified with the aim that patients can consume the drugs

responsibly without incurring risks to their health (Vinker et al., 2007). PILs also include information on what precautions should be followed by the individual taking the drug, and the possible side effects that the drug may have. As a DeCS (Health Science Descriptors) term, PILs or Medicine Package Inserts are defined as "legal documents containing technical and scientific information and guidelines about medicines", a definition that was set forth in some of the articles included in our study (Pizzol et al., 2019).

Given the importance of the PIL, it needs to be easy to comprehend and accessible so that anyone can understand it without difficulties. For this reason, PILs adapted to the characteristics of the target population have been developed. These documents facilitate understanding through the use of non-technical terminology, pictograms, and brief sections to try to answer any doubts that may arise when consuming the medication (Miquel et al., 2000). Additionally, although technological advances have increased considerably in recent years, users tend to consider the leaflet the primary source of information, even if it is confusing and hard to understand (Pizzol et al., 2019). Therefore, there is a need to review the practicality of these documents, ensure that they are relevant and frequently used, and use a psychological approach to explain consumer behavior.

When patients do not fully understand the information in the leaflets, they may engage in self-medicating behaviors (to see other factors that may lead to self-medication, see Bennadi, 2014). Some of these behaviors may be motivated or reinforced by the variability of the information provided by health institutions at the time of administering a drug (Clausen et al., 2016). This phenomenon shows the need to unify instructions for medication use between countries and/or regions. Therefore, it is necessary and relevant to study the characteristics of PILs to understand what makes them clear and effective when patients read them (Pizzol et al., 2019). When patients do not correctly understand or follow the instructions given in the PILs, there is a health risk that is not caused directly by the composition or the active principle of the drug itself but by its incorrect consumption. This, together with the lack of understandable information in the leaflets, has become a large-scale problem that affects the health of many, generates unforeseen expenses in the health system (Bologna, 2009), and affects the decision-making process regarding medication consumption (Clausen et al., 2016). Thus, there is a need to develop PILs that provide clear and precise information to protect people's health.

This paper presents an overview of the recent literature (latest 15 years) that have identified factors facilitating and hindering the comprehension of PILs. Particularly, this mini scoping review primarily aims to describe the characteristics of a PIL that can promote cognitive, emotional, and behavioral change that leads to proper drug use practices. Additionally, we aim to identify the characteristics of the PIL that do not favor responsible and informed consumption of drugs. This way, it would be possible to suggest strategies that could be implemented in the future to design adequate and easily understandable leaflets. As stated before, the incorrect consumption of drugs is often influenced by variables that appear in the subject-leaflet interaction. Hence, this research focuses on studying higher psychological processes, such as memory, learning, understanding, and reasoning, as these components act as mediators between the PIL and the subsequent drug consumption behavior.

METHOD

Search Strategy

The research question that guided this brief scoping review was addressed using a PIO (Population, Intervention and Outcome) format. Specifically, the population was anyone who was a user of medication, the intervention was the exposure to a PIL, and the outcome was the psychological interaction that occurred between the population and the intervention. We operationalized this psychological interaction in terms of the psychological factors that appear when people read PILs (i.e., readability, comprehension, learning, memory, reasoning, and impact, which referred to the consequences that reading the leaflet had on people's decisions regarding medication). Thus, the outcomes of interest were the key mediating factors between the contact with the leaflet and the subsequent consumption behavior. Scopus, Pubmed, and Scielo were searched, and a total of 383 articles with the following key terms were found: *patient AND patient information leaflet AND impact OR readability OR comprehen* OR learning OR memory OR reasoning*. The information from the articles was organized in a database designed according to PRISMA specifications in order to apply the inclusion and exclusion criteria.

Selection Criteria

We defined the following inclusion criteria: (I) Studies in which PILs were defined as "legal documents containing technical and scientific information and guidelines on drugs." (II) Studies describing a specific target population of any age or nationality that has read the leaflets. (III) Studies in which the information in the leaflet was related to psychological variables that influenced people's self-medicating behavior. (IV) Studies that included the terms memory, impact, readability, comprehension, learning, and/or reasoning. (V) Research written in English, Portuguese, French, or Spanish. The exclusion criteria were: (a) Studies describing other types of non-pharmacological inserts. (b) Studies written in languages other than those mentioned in the inclusion criteria, even if the abstract was translated into English. (c) Research published before 2005 (more than fifteen years prior to the beginning of the review). (d) Studies on pharmacological information strategies that included informative documents but not medical leaflets (e.g., documents provided before and after surgery or delivery).

Selection Process

We obtained a total of 383 articles from the databases. These were organized according to a PRISMA-type classification. Two or three reviewers screened each record (title/abstract), leaving 155 publications preliminarily selected. Then, each report was

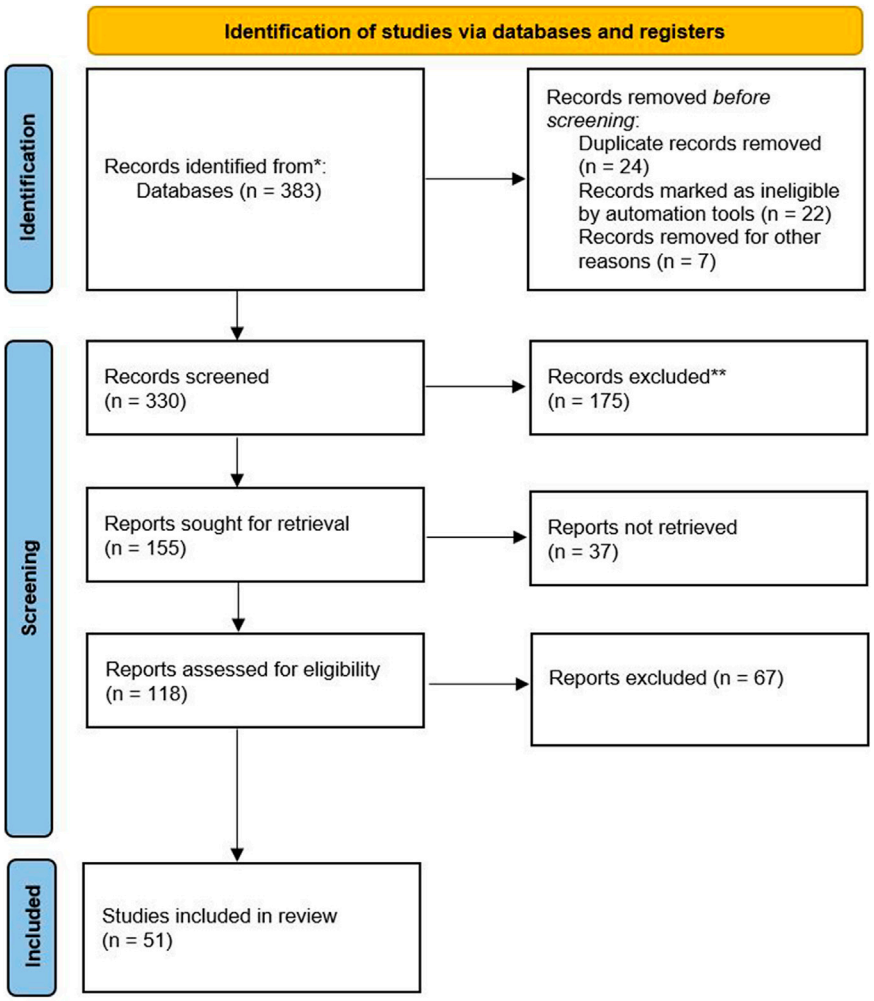

FIGURE 1 | PRISMA 2020 flow diagram to represent the article selection process. Figure with yellow (identification of studies via databases and registers), blue (identification, screening and included) and white.

screened by two reviewers, who identified 118 articles of interest. Disagreements between reviewers were solved by a discussion with the other two researchers. Then, scoping reviews, systematic reviews, briefing notes, and book chapters were excluded. Using the inclusion and exclusion criteria, the abstracts were reread and the sample size was narrowed down to 54 articles. Subsequently, the articles were reread, applying the selection criteria again and rechecking for duplicates, leaving 51 articles as the final number (see **Figure 1**). Since scoping reviews aim to provide an overview of the existing evidence regardless of the risk of bias, we did not assess the risk of bias of the articles included in this review (Tricco et al., 2018).

RESULTS

Results were obtained from 51 selected articles, whose countries of origin were Australia, Austria, Belgium, Brazil, Denmark, France, Germany, Ghana, India, Iran, Ireland, Malaysia, Netherlands, New Zealand, Norway, Pakistan, Qatar, Saudi Arabia, Slovenia, South Africa, Spain, Sweden, Tanzania, United Kingdom, and United States. Forty-nine articles were published in English, one in Spanish, and one in French. As the study's main objective was to find the characteristics of the leaflets and users that aided or hindered the comprehension of the leaflet, the variables were divided into two categories: leaflet and user variables (see **Figure 2**). More information regarding each one of the articles included in the review can be found in the **Supplementary Material** of this paper.

Leaflet variables

Some of the articles indicated the components of the PILs required to provide adequate information to consumers. These components included the names of the medicinal products, expected actions, appropriate forms of use, dosage, contraindications, external

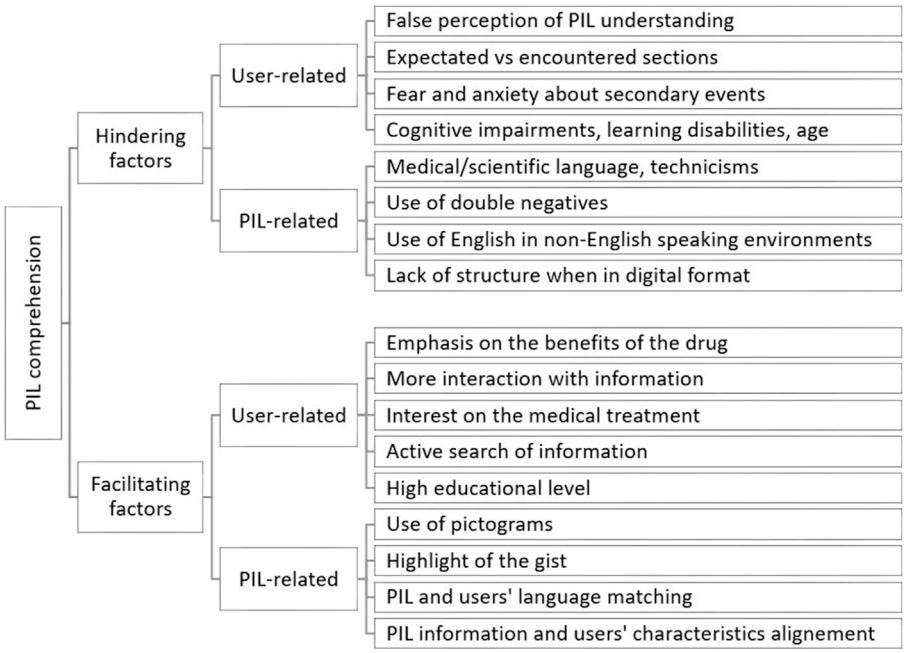

FIGURE 2 | Aspects that facilitate and hinder PILs comprehension. Diagram where it can be read "PIL comprehension," "Hindering fctors," "Facilitating factors," etc.

effects, special use for athletes, and storage conditions (Mwingira and Dowse, 2007). In addition, information regarding the possible side effects and interactions with other natural products and vitamins and the shelf-life of the active component is required in the leaflet (Ahmadi et al., 2019), and some leaflets have been reported as having information on the implications of its use and effects that the drugs would have in the elderly (Liu et al., 2014), infant-juvenile (Zidarič and Kreft, 2019), pregnant, and lactating population (Azari et al., 2018). Although there may be several sections within each package leaflet, patients consider that information regarding the usage instructions and side effects are more important than that on the composition and appearance of the medicinal products (Burgers et al., 2015).

The leaflets must be clear and concise in their explanations and must be available in the user's language and adapted to their understanding (Clausen et al., 2016; Khodambashi et al., 2017). However, a considerable proportion of the leaflets evaluated in these articles did not meet these characteristics as they were not understood by the general public (Rajasundaram et al., 2006; Symonds et al., 2011; Brooke et al., 2013; Spinillo, 2014; Bennin and Rother, 2015; Alaqeel and al Obaidi, 2017; Haller et al., 2019), nor did they meet the parameters of utility (Sukkari et al., 2012), readability (Kasesnik and Kline, 2011), adaptation to the educational level of the target population (Cronin et al., 2011), or use of non-medical terminology (Hirsh et al., 2009; Bennin and Rother, 2015). Participants in other studies confirmed that the leaflets were clear, legible (Gustafsson et al., 2005; Williamson and Martin, 2010; Potter et al., 2014), and useful (Edwards et al., 2013), even though the participants showed low scores in a test on knowledge acquired from the PILs (Alaqeel and al Obaidi, 2017). Additionally, there is also evidence that only some sections were difficult to understand (Gustafsson et al., 2005). Thus, studies examining the PILs' clarity showed mixed results.

The information format presentation was found to play a relevant role to promote appropriate PIL usage. In five out of the 51 articles included in this review, researchers explored the effect of pictograms on PIL comprehension and found that pictograms facilitated the PIL understanding (Mansoor and Dowse, 2006; Mwingira and Dowse, 2007; Dowse et al., 2014; Spinillo, 2014; Spinillo, 2016). The combined format of the text and pictograms produces robust and useful representations of the information, which leads to easier decision-making for both professionals (Arsalan et al., 2015) and patients (Mansoor and Dowse, 2006; Dowse et al., 2014; Hammar et al., 2016). The use of section titles is another format issue that seems useful for a faster and more comprehensible search of the information in the PIL (Dickinson et al., 2016), as sometimes patients report having problems finding the information (Pander Maat and Lentz, 2010; Pander Maat et al., 2015). Also, PILs on a physical format have shown to be more structured in their content and explanation than PILs on a digital format (Afreh et al., 2017). In other study, the PILs approved by the national health authorities proved to be more readable and understandable than the information available on the internet (Mira et al., 2013), even though the PILs did not meet the expected quality criteria. The studies examining patients' adherence to treatment related to PILs' characteristics showed that the use of grammatical negatives hindered comprehension (Burgers et al., 2015). Poor readability and comprehensibility affected the patients' behavior, leading to a lower degree of medication adherence (Burgers et al., 2015; van Beusekom

et al., 2016; Munsour et al., 2017). In contrast, pictograms in PILs seemed to be beneficial to increase patients' adherence (Mansoor and Dowse, 2006). Adherence also increased in a study where PILs were administered together with clinical pharmacists patient-education interventions (Ashok et al., 2017).

User variables

Several of the reviewed articles indicated that the participants' perception of understanding was higher than their real comprehension. For instance, participants could not reproduce what they read in the PIL in their own words (Mwingira and Dowse, 2007). Additionally, participants also did not perform well in questionnaires regarding their knowledge about PILs, especially in sections on contraindications (Gustafsson et al., 2005; Alaqeel and al Obaidi, 2017) and risks of interactions (Gustafsson et al., 2005). Likewise, the findings highlight that the sections of the PILs do not match patients' expectations regarding the importance of the contents. For example, patients would rather know the benefits and risks of taking the drug than know its composition (Maat and Lentz, 2011). To know how they can feel better, users also prefer to know the benefits of the drug rather than their side effects (Hirsh et al., 2009).

Although it is difficult to understand the leaflets, patients showed a great interest in learning about medicinal products (Hirsh et al., 2009), so they tended to search the internet for some prototypes of virtual leaflets that provided them with information easily and quickly (Afreh et al., 2017; Dickinson et al., 2017; Ahmadi et al., 2019). Further, the advice of a health professional that complements the information in the leaflets increases adherence to treatment (Ashok et al., 2017) and the active search for information (Symonds et al., 2011; Potter et al., 2014). In addition, people's opinion of the leaflets are based, above all, on the healthcare professionals' recommendations on the use of medicinal products and their benefits, main characteristics (Kohler et al., 2009), and side effects (Schmitz et al., 2017).

Previous experience plays an important role in how the PIL is used. In fact, the main reason parents medicate their children seems to be their own experience with the symptoms that the minors present with (Afreh et al., 2017). However, reading PILs significantly increased knowledge about the medicinal product despite not having much background information before reading it (Dowse et al., 2014). Also, when people read PILs, the information they store is combined with or framed by preexisting mental representations that people have previously formed about medicines (Kohler et al., 2009). People who present with side effects after taking the drugs tend to reduce the perception of causality between drug use and said symptom if the leaflet presents the information through affirmative sentences rather than negative ones (Webster et al., 2018). In addition, side-effects expectations prior to ingestion lead to the belief that the common or very common side effects and adverse effects proposed in the leaflets have a higher incidence than they actually do (Webster et al., 2017a). This may be related to an overestimation of side or adverse effects, making people less willing to consume the medicinal products (Webster et al., 2017b). Thus, some people sometimes understand medication instructions differently than the average population. This could happen when the information is ambiguous (Spinillo, 2016) and is expressed using scientific terms instead of plain language (Rajasundaram et al., 2006; Hirsh et al., 2009; Bennin and Rother, 2015). Additionally, it has been found that the greater the variety of medications ingested by a patient, the less is the understanding of the PILs (Gupta et al., 2005).

Unpleasant emotions play an important role in the use of PIL, both for direct users and for minors who are administered the medicinal product by their parents, as anxiety precipitates the decision to ingest or administer the drug without prior consultation with a professional or reading of the PIL (Afreh et al., 2017). The information presented in the leaflets can generate emotions, such as anxiety about ingestion, which can cause a change in the way of taking the drugs (i.e., increasing or decreasing doses, or discontinuing medication, or taking medications that are at home or that have worked for another person in the past without consulting professionals) (Thomas et al., 2018). Reading the leaflet can reduce the drug intake due to increased knowledge regarding side effects (Schmitz et al., 2017). PILs reading can trigger anxiety and fear, although no quantitatively measurable significant variation has been found in terms of these emotional reactions (Herber et al., 2014). Additionally, patients may resort to reading the leaflet driven by the need to know if something new or different will happen to them after the intake (Krska and Morecroft, 2013).

In terms of sociodemographic variables, information-seeking behavior differs between sexes as women, compared with men, tend to search for more information (Dickinson et al., 2017). Also, natural aging appears to increase cognitive storage and processing of the leaflet's general idea, rather than the specific details. Therefore, adults find it easy to understand the leaflets that present the information in a combined textual–pictographic format (Dowse et al., 2014). In fact, evidence was found regarding the existence of certain groups of the population that would face difficulties in reading and understanding the PIL, such as people with learning difficulties (Young et al., 2018) and the population with some type of cognitive (Pertl et al., 2014) or visual impairment, like older adults (Geest et al., 2005). In contrast, people with a higher degree of literacy tend to better understand the importance of images in leaflets (van Beusekom et al., 2016) and the information in the leaflet itself as the leaflets are developed for people with a medium educational level, such as those who have passed grades six through ten (Williamson and Martin, 2010; Brosnan et al., 2012; Arsalan et al., 2015). However, people with a higher educational level, younger age, and higher socioeconomic status are most likely to self-administer the medicinal product (Pander Maat et al., 2015).

DISCUSSION

This study aimed to identify the cognitive, behavioral, and emotional factors that facilitate or hinder the acquisition of information from PILs by patients who buy the drugs. The findings show that patients are aware that PILs are necessary for understanding the drug. However, in terms of PILs' general

public acceptance, results are not homogeneous. PILs are perceived as useful, but they can also generate adverse emotional reactions (Herber et al., 2014; Afreh et al., 2017; Thomas et al., 2018). In addition, the PILs in the current market are considerably illegible (Rajasundaram et al., 2006; Symonds et al., 2011; Brooke et al., 2013; Spinillo, 2014; Bennin and Rother, 2015; Clausen et al., 2016; Alaqeel and al Obaidi, 2017; Haller et al., 2019; Zidarič and Kreft, 2019), impractical to use (Sukkari et al., 2012), and can generate emotional discomfort and confusion due to their format. Therefore, several factors that facilitate the PIL understanding need to be consolidated in a future proposal to improve the PILs.

First of all, it would be important to organize PIL sections according to the most immediate needs of the users. This way, patients' expectations about the information location and the place where they look for information in the PIL would match. Specifically, patients expect to find information in the leaflets in the following order: benefits, side effects, and contraindications (Hirsh et al., 2009; Burgers et al., 2015). In addition, they should be presented in a typographic–pictorial format with images that allow a better understanding of the PIL. If possible, PILs should allow a certain degree of active interaction with the information to create a more lasting memory footprint (Dowse et al., 2014; Spinillo, 2014; van Beusekom et al., 2016). Likewise, although physical formats are recommended, as already described, virtual formats could be useful to complement the information and create a broader didactic spectrum.

Secondly, according to the results presented (Hirsh et al., 2009; Cronin et al., 2011; Bennin and Rother, 2015), the PIL should be written in a simple language that uses general terms that are as non-scientific as possible as many of the consumers could have a low educational level. In addition, the PIL should be adapted to the target population using a larger typographic style and more straightforward terms with a focus on the population that finds it difficult to read the PILs, such as the elderly population and those with cognitive and/or learning difficulties (Geest et al., 2005; Pertl et al., 2014; Young et al., 2018).

Finally, a trained healthcare professional could guide patients after a detailed reading of the PIL to solve any doubts that may arise (Ashok et al., 2017). This guidance could be an effective strategy to minimize the overestimation of the risk associated with the intake, reduce users' overestimation of their own understanding of the leaflet, emphasize the benefits of treatment, and answer personalized questions about the interactions between various medications or other substances and the drug in question. Thus, patient adherence to the treatment would be increased, and the risk of inappropriate self-medication leading to poor health could be reduced.

In sum, we presented a first approach to studying an overriding subject in the healthcare context. We hope that our work help to raise researchers' interest in this particular area, which in turn could lead to an increase in the number of studies focused on improving PILs as defined in the introduction (technical documents that contain written information about a drug and accompany it). Research has found some PIL-related variables that could be easily implemented to reduce risks associated with medication consumption errors. Therefore, PILs designers are called to explore further and use these variables to protect people's health.

As limitations of our work, we identified that the studies are difficult to compare with each other due to the great diversity of methods used to carry out the research. Research could also be extended to other types of patient information leaflets, such as those found in clinical trials or before a surgical procedure. In this sense, our review would constitute a first step towards identifying factors that are decisive for the improvement of PILs. Much more research is needed, and further systematic reviews or meta-analyses on the subject would be warranted.

AUTHOR CONTRIBUTIONS

MM-C, SC, and AMP-A conceived and planned the study. MM-C, VA-G, SC, and AMP-A read and selected the articles included in the review. MMC wrote the original draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Microbiome Analysis and Pharmacovigilance After Inhaled Glucocorticoid: Oral Dysbiosis With the Isolation of Three *Rothia* Species and Subsequent Sjögren's Syndrome

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Background: Treatment of respiratory tract diseases with inhaled glucocorticoids is a form of therapy that has been used for many years. It shows lower potency of side effects; nevertheless, microbiome change, sinopulmonary dysbiosis, secondary immunodeficiency, and immunomodulatory effects are underestimated. The latest guideline recommendations introduce the use of empirical antibiotic and/or multiplying inhaled glucocorticoids in therapeutic intervention of asthma and chronic pulmonary obstructive disease.

Aims and objectives: The aim of the study was to describe a simple, universal, and cost-effective method of microbiome analysis for clinical trials. Such a general method for monitoring pharmacovigilance should be widely available and reliable.

Methods: The study material included two kinds of swabs, taken from the same mouth ulcerations of patients with asthma treated with a temporary quadruple dose of fluticasone. The microbiological investigation was performed, and identification of the isolates was carried out using the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF-MS) Biotyper.

Results: The analysis of dry swab demonstrated the presence of typical oral bacteria (*Neisseria* spp. and *Streptococcus* spp.), alongside with the potentially pathogenic *Actinomyces* spp. and three different *Rothia* species, identified simultaneously: *R. aeria*, *R. dentocariosa*, and *R. mucilaginosa*. Although quadrupled dose of corticoids was discontinued and ulcer healing was observed, the patients required topical therapy for maintained xerostomia. Progressive systemic autoimmunity (seronegative Sjögren's syndrome with major organ involvement) was observed later.

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Abbreviations: ACRH, adrenocorticotropic hormone; ADRs, adverse drug reactions; AE, adverse event; COPD, chronic obstructive pulmonary disease; CTCAE, Common Terminology Criteria for Adverse Events; ICS, inhaled glucocorticoids; LABA, long-acting beta2-agosts; MALDI-TOF-MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry; MALT, mucosa-associated lymphoid tissue; SS, Sjögren's syndrome; TCR, T cell receptor; URTI, upper respiratory tract infection.

Conclusion: Topical steroids (especially in quadruple dose) require attention to safety, immunomodulation, and microbiological outcome. They showed systemic side effects: microbiome alteration, humoral (IgG) immunodeficiency, and systemic autoimmunity. Isolation of three species of *Rothia* from a patient with mouth ulcers after steroid therapy suggests their participation in infectious and inflammatory processes. The proposed a methodology using MALDI-TOF-MS may be a prototype approach for microbial diagnostics in clinical trials of immunomodulatory drugs.

Keywords: inhaled glucocorticoids, *Rothia*, adverse drug reaction, microbiome and dysbiosis, Sjögren's syndrome, macroglossia, epithelitis, autoimmunity

1 INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) have been treated with steroids for many years. Inhaled glucocorticoids (ICS) were introduced later, initially as a topical application of hydrocortisone (London and Alexander, 1951), with higher efficacy than antihistamines or allergen-immunotherapy (Bagratuni, 1960). ICS have become the first-line treatment for asthma and sometimes COPD, because of beneficial effects in many inflammatory respiratory system diseases. On the other hand, bacterial infection of the lower respiratory tract contributes to approximately 50% of COPD exacerbations. Lung microbiome may reflect micro-aspiration of oral microbiota, but the strict role of the lung microbiome remains unidentified (Pragman et al., 2012; Zdziarski, 2020). For example, bacteria-associated exacerbation was defined as colony-forming units greater than 10^7/ml sputum or a positive culture result (Ghebre et al., 2018), but collected microorganisms in the respiratory tract may be micro-aspiration-derived or through carryover (e.g., bronchoscopic) (Charlson et al., 2011). Although recently in pediatric (Cazeiro et al., 2017) and adult practice (Chen et al., 2020) there are ample data and meta-analyses showing the increased incidence of ICS-induced infections, they raise serious doubts. Many studies of ICS safety had reporting bias: infectious and inflammatory complications are insufficiently described with non-adequate terminology such as pneumonia, upper respiratory tract infection, etc. (Zdziarski et al., 2017a). Confirmation of infectious process with laboratory and microbiological testing was not carried out. Furthermore, in a meta-analysis, cases of serious pneumonia were defined as for hospitalization or as death from pneumonia (Yang et al., 2017). In clinical trials, drug-induced dysbiosis, secondary immunodeficiency, and opportunistic infection profile are not reported. Although the changes in the microbiome in asthmatic and COPD patients are well described, the effects of ICS have not been evaluated (Charlson et al., 2011; Erb-Downward et al., 2011; Ghebre et al., 2018). Only one study shows data with increasing caries and dental plaque in asthmatic adolescents using ICS but without strict microbiological analysis (Santos et al., 2012). Sample collection and microbiological analysis are crucial for further interpretation and conclusions. Only one recent study revealed an increased risk of oropharyngeal colonization by *Streptococcus pneumoniae* (Zhang et al., 2013), but it is not known whether such colonization should be considered as a preclinical phase of the disease or a change in the natural microbiome. However, one meta-analysis revealed a protective effect of ICS against pneumonia in patients with asthma (Bansal et al., 2015). The anti-proliferative and immunosuppressive effects of ICS, the direct effect on the respiratory epithelium, and the disruption of lung microbiome are most likely to be implicated.

The aim of the study was to describe a simple, universal, and cost-effective method of microbiome analysis for clinical trials. Such a general method for monitoring pharmacovigilance should be widely available and reliable. We were looking for the optimal method of sampling, culturing, and microbiological analysis. By trying the method in one of the patients treated with high doses of ICS, we made an unexpected finding: severe dysbiosis during induction phase of autoimmune lymphoproliferative disease, i.e., Sjögren's Syndrome (SS).

2 MATERIALS AND METHODS

2.1 Material (Case Presentation)

A 32-year-old, non-smoking male patient was admitted to the Department of Clinical Immunology of Lower Silesian Oncology Center in Wroclaw, Poland, with suspicion of Sjögren's syndrome (difficulty in swallowing dry food, xerostomia, and ocular discomfort). Previously, he has been treated with a low dose of ICS (i.e., fluticasone propionate 150–250 μg/day) and long-acting beta$_2$ agonists (LABA, i.e., formoterol) due to atopic asthma (**Figure 1**). On exertion, he was treated with high doses, up to 1,000 mcg, of fluticasone daily (quadrupled dose) and was asked to rinse his mouth with water after using the inhaler, but without the use of a spacer to reduce side effects in the mouth and throat. The patient was neither on an extreme diet, disease-modifying antirheumatic drugs, retinoids, antibiotics nor had mucositis/gastroenteritis or dental intervention for at least 2 months prior to sampling.

2.2 Adverse Drug Reaction Causality Assessment

The clinic-based World Health Organization–Uppsala Monitoring Center system (WHO–UMC) or Naranjo's algorithm was used for causality assessment in type B (unpredictable) and type A (predictable) adverse drug

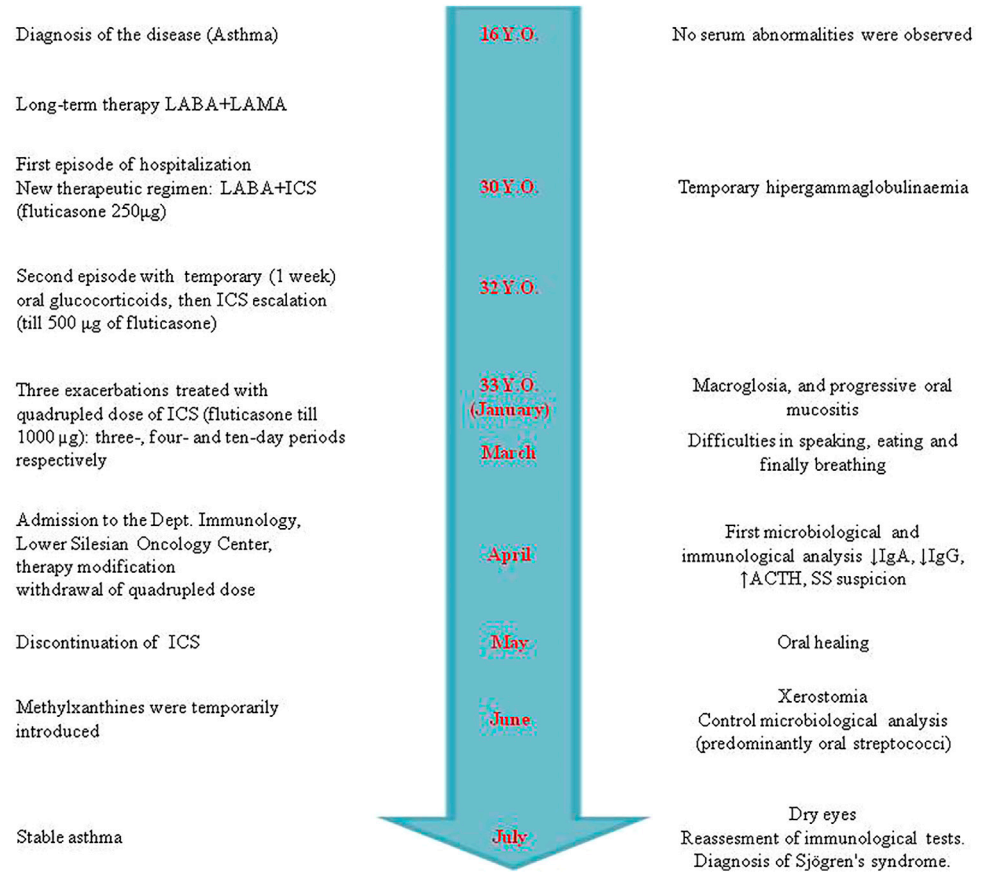

FIGURE 1 | Timeline of clinical presentation, therapeutic regimen of asthma, and Sjögren's syndrome development. The course and therapeutic interventions of asthma are presented on the left. The right side shows the sequence of symptoms and immune parameters during the development of Sjögren's syndrome. In contrast to the similar adverse drug reactions (ADRs), i.e., drug-induced lupus (DIL), disappearance of clinical manifestation was not observed. The patient has Sjögren's syndrome (SS) symptoms even now [more than a year and a half after the inhaled corticoid (ICS) discontinuation].

reactions (ADRs) (Pande, 2018). The WHO–UMC scale was used, intended mainly as a convenient tool for assessing individual case. In Naranjo's algorithm, ADR was categorized into the following four categories: ≥9 = definite ADR, 5–8 = probable ADR, 1–4 = possible ADR, and 0 = doubtful ADR.

2.3 Sample Collection and Growth Condition

Oral dry swab was taken with simple Viscose Swabs Applicator (Equimed, ELATALA®, DELTALAB S.L. SP) or with transport Amies medium without charcoal (Equimed®, DB S.L. SP) in the laboratory, seeded within 20 min and 6 h, respectively. In this way, it was compared whether the transport and the use of the transport medium can positively or negatively affect the microbiological result. This pre-analytical element influences the results and reporting of clinical trials, usually with the submission of materials to a central laboratory. Both swabs were cultured on solid media: blood agar, nutrient agar, tryptic soy-thioglycolate agar (Paściak et al., 2003), and brain–heart infusion (BHI) agar in duplicate to investigate different growth conditions, i.e., aerobic and anaerobic. Aerobic culture takes from 24 to 48 h at 37°C. Anaerobic conditions were obtained with the use of *GasPak EZ Anaerobe Pouch System*® (Pouchoxygen Becton, Dickinson and Company) and 5–7 days of incubation at 37°C. All different colonies were selected by two experienced microbiologists and subjected to matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF-MS) analysis.

2.4 Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Analysis

After excising the separate colony, the standard ethanol-formic acid protein extraction method was used according to the procedure recommended by the spectrometer manufacturer (Pasciak et al., 2015). Alpha-cyano-4-hydroxycinnamic acid was used as a matrix, and MALDI-TOF-MS analysis was performed on the Ultraflex mass spectrometer (Bruker Daltonics, Germany). Spectra were recorded in the positive linear mode for a mass range of 2,000–20,000 Da and were

TABLE 1 | MALDI-TOF-MS Biotyper identification of microorganisms.

Cultivation conditions[a]	Dry swab[b]	Transport amies medium[c]
Aerobic	*Staphylococcus epidermidis* (2.270)	*Streptococcus sanguinis* (2.127)
	Streptococcus salivarius (2.23)	*Streptococcus salivarius* (2.268)
		Streptococcus oralis (2.133)
	Neisseria macacae (2.048)	
	Neisseria perflava (2.384)	
	Neisseria flavescens (2.368)	*Neisseria flavescens* (2.277)
	Neisseria mucosa (1.835)	*Neisseria mucosa* (2.518)
	Rothia aeria (2.165)	*Rothia aeria* (2.176)
	Rothia dentocariosa (1.969)	*Rothia dentocariosa* (2.518)
	Rothia mucilaginosa (2.473)	
Anaerobic	*Staphylococcus epidermidis* (2.24)	
		Streptococcus mitis (2.300)
	Streptococcus pneumoniae (2.294)	*Streptococcus pneumoniae* (2.294)
	Streptococcus salivarius (2.349)	
	Streptococcus sanguinis (2.263)	
	Streptococcus parasangunis (2.107)	
	Streptococcus australis (1.747)	
	Propionibacterium sp (2.026)	
	Propionibacterium acidifaciens (1.776)	
	Propionibacterium acnes (1.991)	
	Actinomyces odontolyticus (2.126)	
	Actinomyces oris (2.29)	

Microbiota from the patient were cultivated in aerobic and anaerobic conditions (the same clinical sample) taken parallel on a dry swab and transport medium, respectively. Score values are presented in brackets.

[a]*Cultivate conditions: aerobic—24–48-h incubation on solid medium—blood agar, BHI agar, or nutrient agar at 37°C; anaerobic—5-day incubation on solid medium blood agar, brain–heart infusion (BHI) agar, or tryptic soy thioglycollate agar at 37°C in jars with use of Gas-Pack system (GasPak EZ Anaerobe Pouchoxygen Becton, Dickinson and Company© with* O_2 *≤1% and ≥10%* CO_2 *as described in product details.*

[b]*Sample from dry swabs were collected with simple Viscose Swabs (Equimed®, DELTALAB S.L. SP)*

[c]*With transport Amies medium Equimed® (DELTALAB S.L. SP).*

obtained by at least 2,800 laser shots acquired from four spot positions under control of Flex Control software 3.1 (Bruker Daltonics). The spectra were externally calibrated using the *E. coli* DH5-alpha standard (Bruker Daltonics) consisting of six ribosomal *E. coli* proteins, RNase A, and myoglobin. The Biotyper 3.1 software (Bruker Daltonics) with a database containing 4,613 entries was used for strain identification. Criteria used in identification, according to the manufacturer, were as follows: a score value below 1.699 meant that the identification was unreliable, 1.7–1.999 probable genus identification, 2.0–2.299 reliable genus identification, and 2.3–3.0 highly probable species identification.

3 RESULTS

3.1 Microorganism Identification

The clinical material was taken by the application of dry swabs with simple Viscose Swabs Applicator and transport Amies medium without charcoal. Identification of the isolates after cultivation in appropriate conditions was carried out using the MALDI-TOF Biotyper (**Table 1**). Comparing the aerobic and anaerobic cultures, only two species taken from dry swab were repeated (i.e., *Staphylococcus epidermidis* and *Streptococcus salivarius*), but none from the transport Amies medium. Surprisingly, we found three different species of *Rothia* genus in one patient: *R. aeria*, *R. dentocariosa*, and *Rothia mucilaginosa* in the same niche, characterized by different MALDI-TOF mass spectra. Collection of microbiota with transport swab gave a significantly narrower microbiome repertoire because strains of *S. epidermidis*, *Neisseria macacae*, *N. perflava*, as well as *R. mucilaginosa* were not detected in these conditions. The dry swab collection method and anaerobic growth conditions allowed us to detect a significantly higher abundance of oral microbiota, but without *Rothia* spp. (**Table 1**).

3.2 Clinical Sequel

Because of mucositis, dysbiosis state, and clinical manifestation of adverse ICS reaction (**Figure 2**), the immunoparameter analysis was performed (**Table 2**). Although a quadrupled dose was not continued over the 10-day period (**Figure 1**), insignificant adrenocortical suppression was observed (i.e., with a mild increase of ACTH) and a slight decrease of serum IgG and IgA. Iron deficiency was not observed (**Table 2**). Further immunological analysis excluded primary immunodeficiency, especially deficiency of IgA, which was at a normal level in blood, but a quantitative deficit of saliva was later observed. Physical examination showed no significant eye dryness (i.e., Schirmer's test 10 mm in 5 min) nor any further abnormalities, but submandibular lymph glands were slightly swollen and oral mucosa showed WHO stage 3 of oral mucositis and 3–8 mm erosion/ulcers and a bitter taste on the back of the tongue (**Figure 2**). Hypertrophy of the tongue (macroglossia) with consequences and malfunction of the upper respiratory tract caused breathing and speech problems. Tongue base-induced

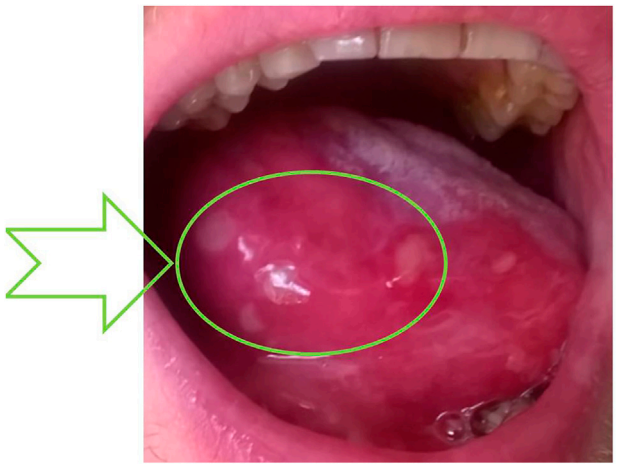

FIGURE 2 | WHO stage 3 of oral mucositis caused by dysbiosis after quadrupling fluticasone therapy. Macroglossia—enlargement of the tongue with acute epithelial disruption with fibrin, called epithelitis—and many ulcers were observed as a sign of lymphocytic infiltration of the epithelia. Formation of a fibrin coating—the place of subsequent sampling (dysbiosis state) —is indicated by an arrow. The probable ADRs are reported as L453/2020 in Polish Pharmacovigilance Service, collectively with data presented in the upper part of **Table 2**.

obstructive sleep apnea was observed. After the withdrawal of ICS in quadrupled dose, the oral ulcer disappeared, but prolonged WHO stage 2/3 of oral mucositis was observed. It required topical therapy with mucoprotectants, drugs usually used for chemotherapy-induced oral mucositis. The duration of quadrupled treatment was as short as possible and was not continued after the symptoms disappeared (**Figure 1**). Although higher doses were not continued over a 10-day period, insignificant adrenocortical suppression was observed (i.e., with mild increase of ACTH and sodium retention) as well as secondary immunodeficiency (i.e., weak decrease of serum IgG level and destruction of mucosal barrier with epithelitis) (**Table 2**). After discontinuation of ICS, the oral mucositis prolonged as mild signs of seronegative primary Sjögren's syndrome with major organ involvement (Tezcan et al., 2017). Three months later, the patient showed no serum abnormality, but prolonged lymphadenopathy, splenomegaly, saliva deficiency, and positive autoantibody such as rheumatoid factor, SS-A, were observed (**Figure 1**; **Table 2**). On the basis of the clinical presentation and formal criteria, which require the presence of immunologic abnormalities (i.e., SS-A antibody or lymphocytic infiltration in labial salivary gland), the SS diagnosis was made (Sandhya et al., 2017). After ICS discontinuation, the patient did not develop asthma exacerbation within 6 months (**Figure 1**). The *Rothia* spp. diversity was not observed later—streptococcal growth predominated (**Figure 1**). Contrary to the first microbial analysis (presented in **Table 1**), the second one revealed *S. salivarius*, *Streptococcus oralis*, *S. pneumoniae*, *Neisseria* spp., as well as *Candida albicans*. However, this could be due to the inflammatory response, changes in immunity, and the

TABLE 2 | Laboratory data of patients with quadrupling inhaled glucocorticoid (i.e., fluticasone 1,000 μg/day) and patient's characteristics.

Parameter	Initial (May)	Normal value	Control visit 3 months later (July)	WHO-UMC causality term[b] (Naranjo's score[a])
ACTH (at 7 a.m.)	40→55 mg/L = 11μMol	<50	38	Certain Definite (10)
Ferrum (Fe)	15 mM/l	10–40	NA	NA
IgG	750 mg/dl	800–1,600	1,100	Certain Definite (10)
IgM	169 mg/dl	34.0–210.0	135	NA
IgA	77 mg/dl	88.0–410.0	92	Certain Definite (10)
CRP	11.8 mg/l	0.00–5.00	NA	NA
K^+	3.65 mM/l	3.5–4.5	4.0	Possible Possible (4)
Na^+	148 mM/l	137–143	142	Certain Definite (10)
Cl	112 mM/l	100–110	115	NA
HBA_{1c}	5%	<6.5%	4%	NA
Anti-SS-A (Ro)	Negative	—	Positive	Possible
Anti-SS-B (La)	Negative	—	Negative	NA
RF (rheumatoid factor)	Negative	—	Positive (IgG class)	Possible
Anti-dsDNA	Negative	—	Negative	NA
Schirmer's test (wetting of the paper after 5 min)	10 mm	>15 mm	9 mm	Possible
Spleen length	13 cm	<12 cm	16 cm	Possible

[a]*The upper part of the table shows the biochemical/laboratory parameters that are part of the predictable ADRs and therefore allow the use of the WHO-UMC system and Naranjo's algorithm. The causality for ICS was assessed following prior epidemiologic data, comparison initial, and control visit.*

[b]*The lower part of the table presents the serological and leading parameters (criteria of Sjögren's syndrome) as category B ADRs, classified according to the WHO-UMC system (qualitative data) and reported separately (L452/2022).*

microbiome seen in Sjögren's syndrome as described previously (Almståhl et al., 2003).

3.3 Causality Assessment

The clinical course to a limited extent allowed for unambiguous answers in individual parts of the questionnaires. Some symptoms can be attributed to the increased dose (quadrupled) and some to steroids. The change in ACTH (and other biochemical/laboratory parameters presented in **Table 2**) is an indirect indicator of ICS toxic concentrations. Basing on prior epidemiologic studies (prior knowledge) and information obtained from a given case (especially dechallenge), these ADRs were classified as predictable and certain according to the WHO-UMC system. Although rechallenge was difficult to perform ethically (especially in quadrupled dose of ICS), discontinuation of ICS (dechallenge) corresponded with oral reconstitution (**Figure 1**) without *Rothia* overgrowth. Furthermore, dysbiosis and macroglossia were in strict time relationship with quadrupled dose (onset in January), and the response to withdrawal was observed (May) (**Figure 1**), and the ADRs was classified as probable. The answer to the survey question in Naranjo's algorithm (Was the mucositis more severe when the dose was increased?) is positive in the first period (i.e., when the patient escalated their ICS doses). However, the follow-up question (Was the mucositis less severe when the dose was decreased?) is not so clear after ICS withdrawal (SS prolonged). Therefore, oral mucositis may be classified as probable or possible according to the WHO-UMC system. Formoterol (LABA) and temporary short-acting beta-agonists (e.g., salbutamol) were still used; therefore, the ADRs were unlikely the cause of biochemical parameters **Table 2**.

4 DISCUSSION

4.1 Microbiological Methods of Microbiota Analysis and Methodological Difficulties

The studies of the human microbiome have revealed that healthy individuals differ remarkably in the oral cavity microbiota composition. Based on the analysis of 2,589 16S rRNA clones, the bacterial diversity of the microflora from nine different sites of five clinically healthy subjects revealed the genus *Rothia* among many others (Aas et al., 2005). In further study of 10 healthy human individuals, Bik et al. (2010) found that the genus *Rothia* was abundant in the oral cavity and was present in all 10 individuals. However, *Rothia* spp. are not described as a typical commensal in "Structure, function and diversity of the healthy human microbiome" (Human Microbiome Project Consortium, 2012). Microbiome encompasses the microbiota and its host environment, but the latter is rarely included in the analysis (Zdziarski, 2020; Raffatellu and Bäumler, 2021; Zdziarski et al., 2017b). In other words, there is no "healthy" microbiome (Yong, 2014), and an integrated approach is crucial (Brinker et al., 2019). Interestingly, higher abundance of *R. mucilaginosa* was observed previously in periodontal patients (Camelo-Castillo et al., 2015), but another publication shows the three most active microbial players, e.g., *Porphyromonas gingivalis*, *Treponema denticola*, and *Fusobacterium nucleatum* (Deng et al., 2018). Noteworthy, in several case reports (the description of four *Rothia* species is the last decade's finding), the isolation of only one species of *Rothia* predominates (Falcone et al., 2012; Zhou and Li, 2015). Our observation of three different *Rothia* species in the same sample from the patient is the first in the literature, regarding the described tissue and disease conditions. Appropriate specimen collection and storage before arrival at the molecular diagnostic laboratory are crucial (Huse et al., 2014). Furthermore, DNA/RNA false-negative results are minimized by avoiding the use of swabs with wooden shafts or cotton tips (the swab that has been validated for the amplification assay must be used). It prompts the use of a dry swab (without transport medium) or brush sampling method rather than lavage or biopsy in microbiome sampling as described elsewhere for the assessment of gut microbiota (Huse et al., 2014). The concerns raised above indicate that the pre-analytical stage of sample management is crucial to get credible results. However, the first and second elements of the diagnostic chain seem to be crucial for the final result (**Table 1**), which is partly the answer why the composition of the oral microbiome in various publications is so diverse (e.g., Human Microbiome Project Consortium, 2012; Zhou and Li, 2015). An identification method using MALDI-TOF-MS turned out to be very efficient in identification down to the species level. This is crucial because identification at the genus level is insufficient for proper classification (Nouioui et al., 2007). Therefore, without strict uniform diagnostic chain, it is impossible to define a healthy microbiome by itemizing microbial species or cataloguing their genes (Zdziarski 2020; Raffatellu and Bäumler, 2021).

Based on our observations, we propose a widely available and reliable diagnostic chain for monitoring pharmacovigilance that should consist of the following:

- Sample collection (near laboratory) with dry swab (transport medium is not useful—**Table 1**)
- Aerobic as well as anaerobic (Gas-Pack) culture of microorganisms (various media)
- Identification with MALDI-TOF-MS
- ADR reporting and terminology (dysbiosis, macroglossia, immunodeficiency, and secondary inflammatory disease)
- Modification of therapeutic regimen (e.g., microbiota transplantation, pre–pro-biotics, and ICS withdrawal), nomenclature, and guidelines, e.g., the Global Initiative for Asthma (2019).

4.2 Clinical Repercussion in Therapeutic Regimen

Our data may be important for patients with long-term oxygen therapy as well as for storing and transporting of clinical material to the laboratory (i.e., with access to oxygen). In hypoxic patients, shifts and changes in the microbiome and alpha diversity may occur when oxygen therapy (or the use of oxygen for ICS nebulization) is administered, similar as presented in **Table 1**.

Charlson et al. (2011) suggest that the lung microbiome reflects micro-aspiration of the oral flora. The risk of microbial transition is much higher after aerosol delivery, especially ICS (O'Malley, 2015). Risk factors for *R. mucilaginosa* bacteremia include prolonged and profound neutropenia, malignancy, and an indwelling vascular foreign body. Unfortunately, most of the literature indicates the risk factor, but the retrospective study identified no qualitative or statistically significant differences between the two groups for any of the variables collected, including recent corticosteroid use (6% versus 11%, p = 1.0) and the presence of neutropenia (88% versus 89%, p= 1.0) (Ramanan et al., 2014).

4.3 DATA Collection and Discrepancy: Non-Adequate Nomenclature for Infectious Complications After ICS

There are major challenges in specifying relevant outcomes and study designs for evaluating adverse drug reactions (Zdziarski et al., 2017a). High diversity in reporting, as well as variation in their definition, methods of ascertainment, and grading, is an important problem in clinical studies (Peryer et al., 2020). One of the crucial limiting factors in clinic-based causality assessment in clinical immunology practice is the long latency of many immunomediated ADRs (Zdziarski, 2019) as presented previously for anaphylaxis: IgE-mediated (type 1—"immediate") allergic reactions were observed 5 or 14 days after drug administration (Zdziarski et al., 2017a). Naranjo's algorithm was not useful (Pande, 2018). In our observation, it is difficult to unambiguously associate biochemical changes with the chronic use of ICS or a quadrupled dose, and there may have been an accumulation of them (**Figure 1**; **Table 2**). On the other hand, macroglossia and mucositis are symptoms directly and continuously observed by the patient, and the time relationship is clearer (**Figure 1**). Although oral mucositis is prolonged, the type of inflammation (bacterial to autoimmune) and the clinical picture (i.e., presented in **Figure 2** to dry mucositis in SS syndrome) changed. The Common Terminology Criteria for Adverse Events (CTCAE) and Naranjo's algorithm do not provide for such specific and qualitative scenario. Information taken from published reports may be incomplete or may lack specificity because of usually observed differences in coding and categorization of adverse effects between studies (Pande, 2018). Most of the studies do not differentiate between adverse event (harmful outcome that occurs, not necessarily caused by a drug) and adverse effect (causal relation between the drug and the event is at least a reasonable possibility). Following CTCAE, the infectious complications are, therefore, described usually as localized or life-threatening colitis, pneumonia, etc., which is an inflammatory rather than a strictly infectious process. For example, the last meta-analysis of 17 randomized controlled trials (20,478 patients) showed a significantly increased risk of upper respiratory tract infections in COPD patients with ICS therapy (Chen et al., 2020). These studies were dedicated to assessing the efficacy and safety of ICS treatment rather than the infectious profile. Upper respiratory tract infection (URTI) and pneumonia were not accurately defined as an infection without microbiological analysis, species identification, or at least type (opportunistic vs. pathogen-related). For example, there were eight deaths among patients from pneumonia as such in the combination therapy group, seven in the placebo group, nine in the salmeterol group, and 13 in the fluticasone group. Surprisingly, the URTI rate is higher in the combination therapy group than in the placebo group and in patients with fluticasone monotherapy (i.e., 0.11, 0.1, and 0.09 rate per year, respectively) (Calverley et al., 2007). The infection is, therefore, difficult to link with ICS (incidence rate lower than placebo), especially endogenous and opportunistic infections. Moreover, the meta-analysis corresponds with our report:

- Fluticasone was observed with an increased risk of URTI in comparison with other ICS (e.g., mometasone);
- High-dose fluticasone treatment was associated with a significantly higher risk of URTI but not low dose.

Our observation indicates the need for the implementation of microbiological testing and species identification in the coding and reporting of adverse effects in clinical trials. Identification using MALDI-TOF-MS as a relatively inexpensive and increasingly accessible method should be the standard. The species diversity of the cultured bacteria (**Table 1**) indicates that the dysbiotic state precedes the subsequent general symptoms and should, therefore, be described as a separate category or at least a separate grade in infectious complications in CTCAE. Regretfully, clinical trials of inhalation drugs, as well as all therapeutic regimens, do not implement microbiome analysis.

4.3.1 Patient Risk Factors and ICS: Friend or Foe?

Although *Rothia* were described as health-associated genera, these bacteria can cause disease in severe immunodeficiency. *Rothia* spp. are increasingly being recognized as emerging opportunistic pathogens (Abidi et al., 2016). Our observation shows the clinical repercussion of inhalators, especially steroid overuse. Unfortunately, routine checks for microbial colonization and surveillance cultures from patients are not recommended (O'Malley, 2015), but asthma exacerbation is frightening for a patient, and self-management of ICS prompts overdosing. The concept and strict plan for patients, which included a temporary quadrupling of the ICS dose, were described previously (McKeever et al., 2018). The finding showed five events of severe pneumonia (0.5%) with one death in the quadrupling group of 957 patients. Our findings (**Table 2**) indicate that inhaled corticoids are absorbed with systemic side effects. Furthermore, the post-ICS epithelitis and macroglossia (**Figure 2**) caused breathing perturbation. The oral cavity can act as the site of origin of dissemination of pathogenic organisms to distant body sites in immunocompromised hosts, especially those suffering from malignancies, diabetes, and rheumatoid arthritis, or SS immunosuppressive treatment. Only one study shows lung microbiome alteration in patients treated with ICS (Pragman et al., 2012). Acquired causes of macroglossia may include endocrine disorders and inflammatory or infectious diseases. However, this rare symptom has not been described

in more detail, especially in the light of ADRs and the microbiome as well as endocrine and immune system disorders (Genetic and Rare Diseases Information Center, 2022).

4.3.2 Inhaled Glucocorticoid-Induced Immune Perturbation

Innate immune response is disturbed in our patient (**Table 2**) (Bishop and Gleeson, 2009). Our observation corresponds with the data that only high-dose ICSs are associated with a significantly increased risk of infection of the upper respiratory tract but preferentially endogenous with dysbiosis (**Figure 3**). Furthermore, invasive or recurrent pneumococcal diseases were observed only in immunodeficient child with antibody deficiency (Butters et al., 2019). The ICS-induced transient humoral immunodeficiency observed here requires reflection. Until now, ICS has not been described as a risk factor for *Rothia* opportunistic complication. This is the first description and, what is noteworthy, of three different species simultaneously in the same niche. The genome-based taxonomic classification of the phylum Actinobacteria as well as *Rothia* spp. is still an open issue (Nouioui et al., 2007). We hypothesize that alterations in the oral mucosa microbiome and/or its interactions with the host immune system (e.g., low IgG, lymphadenopathy, and splenomegaly (**Table 2**; **Figure 3**) may lead to disordered immune tolerance and the development of an inflammatory state that accelerates the progression of asthma as well as induces autoimmune-lymphoproliferative disorder, i.e., Sjögren's syndrome (**Figure 3**). Unfortunately, strong alloantigenic stimulation by microbiome and narrow lymphocyte repertoire prompt lymphoproliferative disease (Zdziarski et al., 2017c). T cell receptor (TCR) threshold activity leading to such drastically opposing signaling outcomes (life or death) is modulated in part by glucocorticoids (**Figure 3**) (Wiegers et al., 2011). Dysbiosis and the role of microbiota in Sjögren's syndrome clinical presentation are the area for further research. Endogenous glucocorticoids, to some extent blocked by ICS with the increase of ACTH (**Table 2**), are required for a robust adaptive immune response because of their promotion of the selection of T cells, and the absence of thymocyte glucocorticoid signaling results in an immunocompromised state with alterations in the TCR repertoire of polyclonal T cells (Mittelstadt et al., 2012). Only through an integrated approach that considers influences of multiple interacting factors we will be able to gain a better understanding of host–microbe associations (Brinker et al., 2019; Zdziarski, 2020).

4.3.3 Sjögren's Syndrome Pathogenesis and Overlapping With Asthma/COPD

Sjögren's syndrome is a chronic autoimmune disease that classically affects the lacrimal and salivary glands and can affect almost any organ system in the body including the lungs (Sandhya et al., 2017). Lung involvement in primary SS

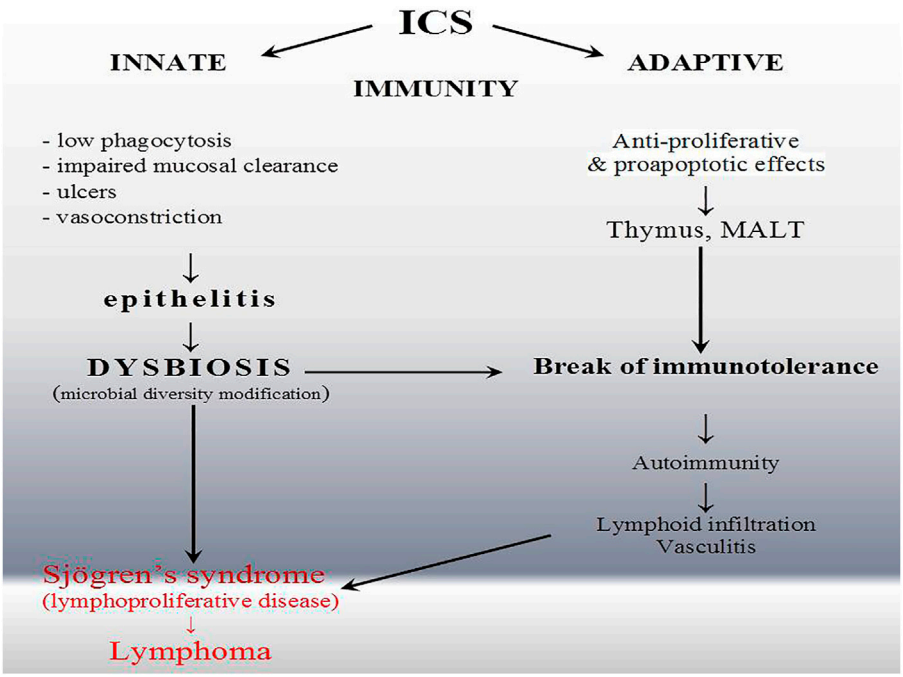

FIGURE 3 | Inflammatory complication of inhaled corticoid (ICS) overuse. The wide spectrum of immunomodulatory effects of ICS and influence on epithelial barrier prompt lymphocyte selection in thymus (T cell) or mucosa-associated lymphatic tissue (MALT) (B and T cells) intensified by non-specific perturbation and dysbiosis. ICS, unlike drugs typically associated with drug-induced lupus, have a direct influence on the immune system. Nevertheless, they also induced systemic rather than organ-specific autoimmunity.

is mainly related to the small airway disease (Papiris et al., 1999). Intriguingly, even in SS, the gut microbiome is more likely to be studied than the respiratory one, as reviewed elsewhere (Tsigalou et al., 2018). It revealed significant compositional differences compared to controls, while *Firmicutes/Bacteroidetes* ratio and *Actinobacteria* decreased (Moon et al., 2020). A similar observation was presented in our case. As SS developed, a wide variety of microbes (**Table 1**) became dominated by streptococci. Whether it is an effect or a cause, most studies are not conclusive, and indirect pieces of evidence are the only elements in this puzzle thus far (Tsigalou et al., 2018). MALT activation in preclinical phase of SS overlaps with inflammatory symptoms, and clinical presentation of inflammatory respiratory diseases usually treated with ICS (Zdziarski and Gamian, 2019), bronchial hyper responsiveness, cough, and bronchiolitis or bronchiectasis, are reported in SS with a prevalence between 7% and 61% (Flament et al., 2016). There are currently no SS treatments available that address the underlying disease etiology, and systemic or topical steroids are not effective. Initial autoimmune inflammation and epithelitis—a deregulated immune response—are the first phase of SS development (Tapinos et al., 1999) (**Figure 2**). The overuse of ICS and quadrupling of therapeutic regimen in asthma may be paradoxically the source of microbial dysbiosis, systemic inflammatory complication, and systemic autoimmunity (relatively rare seronegative SS with major organ involvement). The lack of hyper-gammaglobulinemia and low IgG observed here show that patients receiving ICS may develop secondary immunodeficiency as well as atypical SS presentation (**Table 2**). In our observation, dysbiosis and *Rothia* spp. overgrowth were not observed after ICS withdrawal (patient recovers well after ICS stopping), but contrary to early immunoabnormalities (top of **Table 2**), late immunoabnormalities and SS are difficult for causality assessment (qualified as possible as presented in the bottom part of **Table 2**). ICS show extraordinary pleiotropic effects, and SS is an unknown-etiology disease (**Figure 3**).

4.4 Limitations

Our research had several limitations. Firstly, the baseline samples were not collected prior to treatment initiation. The study was not prospective, and the method and diagnostic chain were presented in one, which is the most transparent case. It would be very difficult to obtain patients with a newly diagnosed disease, then to observe them with waiting for a similar situation and a quadrupling ICS dose. Besides, it would never be certain whether the starting sample is a native microbiome or disease altered (e.g., asthma). Secondly, the limitation of the presented diagnostic chain is transport and culture (**Table 1**). These do not allow the detection and identification of organisms that have not been cultured in the laboratory, e.g., Archaea, which are involved in periodontal disease (Vianna et al., 2006). Thirdly, there is no simple and direct evidence that the observed therapy and microbiome are the direct cause of the development of Sjögren's syndrome, a relatively rare autoimmune disease. However, apart from classical rheumatic fever and Group A streptococcal infection, the early stages of autoimmune diseases (the primary immune response) and the induction phase of the disease are not known. This accidental finding in the later observation of the patient, however, may be a sufficient example of the undiscovered role of the respiratory microbiome. Most of the studies, including in Sjögren's syndrome, are of the gut microbiome (Tsigalou et al., 2018).

5 CONCLUSION

Our findings shed a new light on an adverse effect of ICS and the initial phase and possible pathogenesis of Sjögren's syndrome. ICS and their overuse or quadrupling prompt oral and respiratory tract microbiome discrepancy (dysbiosis) and secondary immunodeficiency; therefore, opportunistic infections with microorganisms such as from the genus *Rothia* are erroneously omitted. Such dysbiosis should be considered a preclinical phase of the disease, and an area for further research should be provided. Identification with MALDI-TOF-MS as a cheap and increasingly accessible method may be a prototype approach.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: All data generated or analyzed during this study are included in this article. The clinical isolate was deposited in a publicly accessible culture collection—Polish Collection of Microorganisms.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PZ and MP conceived and designed the experiments. PZ was responsible for the clinical diagnosis and patient management. MP performed the microbiological and MALDI-TOF analysis. PZ, MP, and AG analyzed the data. AG contributed reagents/materials/analysis tools. PZ wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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Practical Considerations of PRN Medicines Management: An Integrative Systematic Review

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Background and objectives: Highly widespread use of *pro re nata* (PRN) medicines in various healthcare settings is a potential area for improper medication prescription and administration leading to patient harm. This study aimed to summarize and integrate the findings of all relevant individual studies regarding the practical considerations of PRN medicines management including strategies and interventions by healthcare professionals for safe prescription, dispensing, administration, monitoring, and deprescription of PRN medicines in healthcare settings.

Methods: An integrative systematic review on international databases were performed. Electronic databases including Web of Knowledge, Scopus, PubMed (including MEDLINE), and Cinahl were searched to retrieve articles published until end of May 2021. Original qualitative, quantitative, and mixed methods studies written in English were included with a focus on PRN medicines management in healthcare settings. Research synthesis using the narrative method was performed to summarise the results of included studies.

Results: Thirty-one studies on PRN medicines in healthcare settings by different healthcare providers were included after the screening of the databases based on eligibility criteria. They were published from 1987 to 2021. The majority of studies were from Australia, the United States, Canada, and the United Kingdom and were conducted in psychiatric settings. Given variations in their purposes, methods, and outcomes, the research synthesis was conducted narratively based on diversities and similarities in findings. Eight categories were developed by the authors as follows: "PRN indications and precautionary measures," "requirements of PRN prescription," "interventions for PRN administration," "monitoring and follow up interventions," "deprescription strategies," "healthcare professionals' role," "participation of patients and families," and "multidisciplinary collaboration." Each category consists of several items and describes what factors should be considered by healthcare professionals for PRN medicines management.

Conclusion: The review findings provide insights on the practical considerations of PRN medicines management in clinical practice. The suggested list of considerations in our

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review can be used by healthcare professionals for optimal PRN medicines management and safeguarding patient care.

Keywords: clinical practice, medication, medicines management, patient safety, pro re nata

1 INTRODUCTION

Medication therapy is the most common therapeutic intervention (World Health Organization, 2019). Medicines management is the process of the evaluation of the patient's health status and the need for medications' prescription and dispensing, and the administration and monitoring of medication's effectiveness (Car et al., 2017; Mishore et al., 2020). Medication errors are significantly prevalent and happen in up to 67% of patients during hospitalization (Nguyen et al., 2017). They are major international contributors to healthcare complications and the increased costs of healthcare (Acheampong et al., 2014). Therefore, the safe use of medications has a top priority for healthcare systems with an calculated annual burden of $ 42 billion worldwide (World Health Organization, 2018).

Given the frequency and potential association of preventable medication errors with adverse patient outcomes, the development of strategies through medicines management for the reduction of their clinical magnitude are common (Kwan et al., 2013; Basey et al., 2014). Prevention and reduction of medication errors are the primary goals of healthcare organizations through participation in quality improvement initiatives. They are also intertwined with ethical healthcare (Smith, 2013; Pitkänen et al., 2016).

1.1 *Pro re nata* Medicines Management in Healthcare Settings

"*Pro re nata*" (PRN), "when required," or "as needed" is defined as the prescription and administration of medications based on the immediate patients' needs instead of prescheduled administration times (Martin et al., 2017). PRN medications often are used for relieving symptoms rather than treating patients' underlying diseases (Harper et al., 2017). Common medications used as PRN are psycholeptic and psychotropic medications including antipsychotics, anxiolytics, sedatives and hypnotics; painkillers; gastrointestinal medications; and other drugs used for mitigating physical and psychological symptoms (Allers et al., 2017; Dörks et al., 2019; Vaismoradi et al., 2020).

PRN medications are prescribed and administered at least once to 68–83.9% of patients suffering from mental health issues (Vaismoradi et al., 2018). In mental healthcare settings, PRN prescriptions have major contributions to the frequency of dangerous high and combined doses of antipsychotic medications that patients receive (Baker et al., 2008). It has been reported that 62–97% of patients treated in mental health wards receive PRN medications especially antipsychotics and psychotropics (Baker et al., 2008; Fujita et al., 2013; Martin et al., 2017; Hipp et al., 2020; Saito et al., 2020). The use of psychotropic medications as PRN is associated with abuse, polypharmacy, increased risks of morbidity, dependency, and risk of falls, which complicate its safety (Hilton and Whiteford, 2008; Nyborg et al., 2017). Therefore, the potential of patient harm should be carefully evaluated (Stroup and Gray, 2018).

This is the nurse responsibility to administer PRN medications based on the patient health condition after receiving the physician's prescription order (Dörks et al., 2019). Although PRN medications can improve care efficiency, they are accompanied with potential medication safety issues (Oh et al., 2014). Medication errors can happen during the prescription, dispensing, and administration of PRN medications (Vaismoradi et al., 2019). In the intensive care unit, medication errors have been reported in 89% of PRN medication orders (Alaqqad et al., 2016). Improper prescription and administration of PRN medications can cause medication interactions, adverse drug reactions (ADRs), overuse and abuse (Davies et al., 2007; Vaismoradi et al., 2018). PRN medications increase the complexity of medication regimens (Picton et al., 2021). Frequent administration of PRN medications can hide the signs and symptoms of underlying diseases (Vaismoradi et al., 2018).

PRN is considered an unsafe mechanism for medication delivery because the chain of accountability between the decision to prescribe PRN medications and the decision to administer them is unclear (Price and Baker, 2013). The safety of PRN medicines management is influenced by healthcare professionals' knowledge and skills, and the healthcare culture (Morkunas et al., 2016). The decision for the use of PRN medications are taken in collaboration with the physician, nurse, patient, and families, but it is accompanied by the risk of errors due to their distinct interpretations of the medication process (Hogan et al., 2019). Ambiguities in PRN medicines management including indication for prescription, method of administration, and complete documentation can adversely impact patient care outcomes, increase the risk of polypharmacy, adverse drug events, and abuse (Friedman et al., 2012; Oh et al., 2014; Hammer et al., 2019). The decision for the administration of PRN medications is a nearly independent component of the nursing role after prescribing, and nurses require a clear articulation in clinical practice associated with PRN medication administration (Molloy et al., 2012).

1.2 Significance of Understanding the Practical Considerations of PRN Medicines Management

Discrepancies in medicines management between healthcare professionals in various healthcare settings indicate the potential concerns for the use of PRN medications (Stubbings et al., 2019). Nurses often interpret PRN orders for painkillers to be the least amount of PRN medication use. Also, the practice of

PRN medications has setting-specific characteristics rather than being evidenced-based (Lellan, 1997; Sonntag et al., 2006). Moreover, there are disparities in the perspectives of healthcare professions especially nurses with regard to the appropriate indications for PRN medications use in patients with different health conditions (Baker et al., 2007b). Furthermore, the monitoring of PRN medications by nurses after administration and related documentation are not properly performed (Friedman et al., 2012; Ross et al., 2021). Making decisions on the use of PRN medications usually is not guideline-based and rather is based on habits and previous experiences in clinical practice (Douglas-Hall and Whicher, 2015; Walsh et al., 2021).

Due to the highly widespread use of PRN medications in various healthcare settings, and the growing concern regarding the use of PRN medications as the first-line choice for relieving patient's suffering, there is a need for the introduction of evidenced-based protocols and procedures with regard to prescription, dispensing, administration, and monitoring of PRN medications. Also, reviews on PRN medications in terms of indication, frequency, and interdisciplinary collaborations for PRN medicines management is insufficient (Martin et al., 2017; Vaismoradi et al., 2021a). Therefore, this study aimed to summarize and integrate the findings of all relevant individual studies regarding the practical considerations of PRN medicines management including strategies and interventions by healthcare professionals for prescription, dispensing, administration, monitoring, and deprescription of PRN medicines in healthcare settings. The review question was as follows: What are the practical considerations in terms of strategies and interventions by healthcare professionals including nurses, pharmacists and physicians for PRN medicines management in short-term, long-term and acute healthcare settings?

2 MATERIALS AND METHODS

2.1 Design

An integrative systematic review was conducted. This is a review method that allows for the inclusion of studies with qualitative and quantitative methodologies and considers a narrative approach for the synthesis of data from a wide range of research designs (Whittemore and Knafl, 2005; Souza et al., 2010). The review protocol was developed by the authors prior to the study, and all steps of the review were conducted accordingly (**Supplementary File S1**). In addition, PROSPERO was searched to identify ongoing or recently completed similar systematic reviews.

The review question was framed using the PICO statement as follows:

P (Population): healthcare providers including nurses, pharmacists, and physicians who are involved in PRN medicines management; I (Interest): practical considerations in terms of interventions and strategies by healthcare professionals for prescription, dispensing, administration, monitoring, and deprescription of PRN medicines; Co. (Context): all contexts in healthcare consisting of child, adult, physical and mental health.

The review process was informed by the Preferred Reporting Items Systematic Reviews and Meta-analysis (PRISMA) statement (Liberati et al., 2009) (**Supplementary File S2**).

2.2 Search Process

Search keywords and phrases were determined by the research team consisting of the nurse (AM, PP, MV), physician (CW), and pharmacist (SJ) through the review of relevant literature and based on a pilot search in general and specialized databases. The Boolean search method was used with the inclusion of the following keywords:

(PRN OR "pro re nata" OR "as needed" OR "as required") AND (guideline OR "practice guideline" OR "clinical practice guideline" OR "clinical guideline" OR "critical pathway" OR "clinical pathway" OR "critical path" OR "clinical path" OR "patient care planning" OR instruction OR technique OR program*) AND (medication OR drug OR medicines OR "pharmaceutical preparations" OR pharmaceuticals OR "medicines management").

The online databases of Web of Knowledge, PubMed (including MEDLINE), Cinahl, and Scopus were searched to retrieve empirical studies published by peer-reviewed scientific journals up to end of May 2021. In addition, cross-references from bibliographies and manual search in the references lists of selected studies were performed to expand the search coverage.

Inclusion criteria for the selection of studies to our review were: qualitative, quantitative, and mixed methods studies with a focus on PRN medicines management; use of any practical consideration in terms of interventions and strategies by healthcare professionals for prescription, administration, monitoring, and management of the side effects and ADRs of PRN medications; published in peer-reviewed scientific journals.

Studies without exact relevance to PRN medicines management were excluded. Also, exclusion encompassed non-empirical studies such as reviews, letters, commentaries, conference proceedings, theses, dissertations, books, and governmental documents that did not provide appropriate data to our review.

The phases of review were carried out separately by two review authors (AM, MV). They shared results and conducted online conversations to make decisions on the subsequent search steps. The studies' titles, abstracts and full-texts were screened step by step by them. The review authors held discussions in case of discrepancies in their perspectives to reach agreement, and also sought the perspective of the third review author.

2.3 Quality Appraisal and Risk of Bias Assessment

Quality of selected studies was evaluated in terms of the appropriateness of research structure and reporting using the Enhancing the Quality and Transparency of Health Research (EQUATOR). According to the studies' designs, the following tools were used: 1) the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) for observational and cross-sectional studies; 2)

the Standards for Reporting Qualitative Research (SRQR) for qualitative research; 3) Consolidated Standards of Reporting Trials (CONSORT) for experimental and quasi-experimental studies; 4) the Good Reporting of A Mixed Methods Study (GRAMMS) for Mixed-methods studies (EQUATOR Network, 2019).

For making the final decision on whether or not to include studies in the research synthesis, the authors considered scores obtained by the quality appraisal tools and their collective opinions with regard to the significance and the methodological quality of each study.

The Cochrane Collaboration's tool for assessing the risk of bias for randomized clinical trials was used and the review authors classified their judgments as low, high, and unclear risk of bias (Higgins and Altman, 2011). The Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) tool was also used along with the categorization of judgments as follows: low, moderate, serious, critical, and no information regarding risk of bias (Sterne et al., 2016). The risk of bias assessment for cross-sectional studies was adapted from the Newcastle-Ottawa Quality Assessment Scale with the judgment's classification of low, probably low, probably high, and high risk of bias (Herzog et al., 2013).

2.4 Data Extraction and Knowledge Synthesis

For data extraction, a table was developed comprising the following sections: 1) the first author's surname, publication year, and the country where the study was conducted; 2) study design, sample size, and setting; 3) data relating to the practical considerations of PRN medicines management; 4) name and dose of PRN medications and patients' age group; and 5) healthcare providers involved in PRN medicines management.

To ensure that the data extraction table could gather the required information on the characteristics of selected studies, a pilot test was conducted on a couple of included studies. The review findings were presented narratively, because the presence of heterogeneities in the methods, aims, and results of the studies hindered us to conduct meta-analysis. Therefore, the findings of the included studies were reviewed and based on diversities and similarities in their findings, appropriate categories were developed. The authors discussed to reach agreement on the allocation of the studies' findings into the relevant categories.

3 RESULTS

3.1 Search Results and Selection of Studies

The search results on the databases were reported in **Table 1**. In total, 4,972 articles were retrieved. After removing irrelevant and duplicate titles and carrying out abstract and full-text readings, 31 studies were picked out to be considered for data analysis and research synthesis.

Figure 1 presents the study flow diagram based on the PRISMA.

3.2 Quality Assessment and Risk of Bias

The quality appraisal of the selected articles was performed on the full-text of the selected studies (**Table 2**). Since all studies were judged to have an acceptable level of quality in terms of methodology, theoretical and conceptual framework, no study was ruled out from our review.

The results of risk of bias evaluation for two randomized controlled studies (McCarthy et al., 2013; McCarthy et al., 2019) were presented in **Supplementary Figure S1**. In terms of bias in random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, and selective outcome reporting, the studies presented insufficient data leading to the judgement of unclear risk of bias. In addition, they were judged to have a low risk of bias in terms of bias in incomplete outcome data.

The risk of bias assessment in quasi-experimental (Edwards et al., 2001) and qualitative studies (Procaccini et al., 2020) were described in **Supplementary Figure S2**. In terms of bias due to confounding and selection of participants into the study, one study was judged to have a low risk of bias and another one had a serious risk of bias. In terms of bias in the classification of interventions and bias due to missing data, one study was judged to have a low risk of bias and another had a critical risk of bias. In addition, both studies had low risk of bias in the view of bias due to deviations from intended interventions and failure to provide information in terms of bias in the measurement of the outcome and bias in the selection of the reported result.

Furthermore, the results of risk of bias for 20 observational studies were presented in **Supplementary Figure S3**. The selected studies mostly had a low risk of bias in terms of the assessment of exposure (100%), development of the outcome of interest (95%), selection of cases (85%), and controls (85%). In terms of the control of prognostic variable, 40% of the studies had low risk of bias, 20% probably low risk of bias, 15% high risk of bias, and 25% probably high risk of bias.

3.3 Characteristics of Selected Studies

A summary of selected studies (n = 31) has been presented in **Table 2**. All studies published in English from 1987 until 2021. Twelve studies were from Australia (Edwards et al., 2001; Geffen et al., 2002; Curtis et al., 2007; Stein-Parbury et al., 2008; Kaur et al., 2009; Usher et al., 2009; Usher et al., 2010; Mullen and Drinkwater, 2011; Russell et al., 2014; Barr et al., 2018; Stasinopoulos et al., 2018; Sharma et al., 2021), five from the United States (Gordon et al., 2008; Carder, 2012; McCarthy et al., 2013; McCarthy et al., 2019; Procaccini et al., 2020), four from Canada (Craven et al., 1987; Swart et al., 2011; Martin et al., 2018a; Walsh et al., 2020), three from the United Kingdom (Di Giulio and Crow, 1997; Baker et al., 2007a; Griffiths et al., 2019), two from Saudi Arabia (Al-Sughayir, 2014; Al-Sughayir, 2017), one from Germany (Dörks et al., 2016), one from Ireland (Jimu and Doyle, 2019), one from Norway (Nilsen et al., 2020), one from Scotland (Akram et al., 2014), and one from Thailand (Chaichan, 2008).

Regarding their methods, 11 studies used the chart review method (Craven et al., 1987; Curtis et al., 2007; Chaichan, 2008; Stein-Parbury et al., 2008; Kaur et al., 2009; Mullen and

TABLE 1 | The result of search process.

Search Keyworks	Databases	Total in each database	Selection based on title	Selection based on abstract	Selected based on full text reading	Selection based on quality appraisal and risk of bias assessment
(PRN OR "pro re nata" OR "as needed" OR "as required") AND (guideline OR "practice guideline" OR "clinical practice guideline" OR "clinical guideline" OR "critical pathway" OR "clinical pathway" OR "critical path" OR "clinical path" OR "patient care planning" OR instruction OR technique OR program*) AND (medication OR drug OR medicines OR "pharmaceutical preparations" OR pharmaceuticals OR "medicines management")	PubMed (including MEDLINE)	414	33	6	4	4
	Scopus	2,127	49	17	14	14
	Cinahl	1,301	16	0	0	0
	Web of Science	941	17	4	2	2
	Backtracking references of selected articles	189	32	23	11	11
	Total	4,972	147	50	31	31

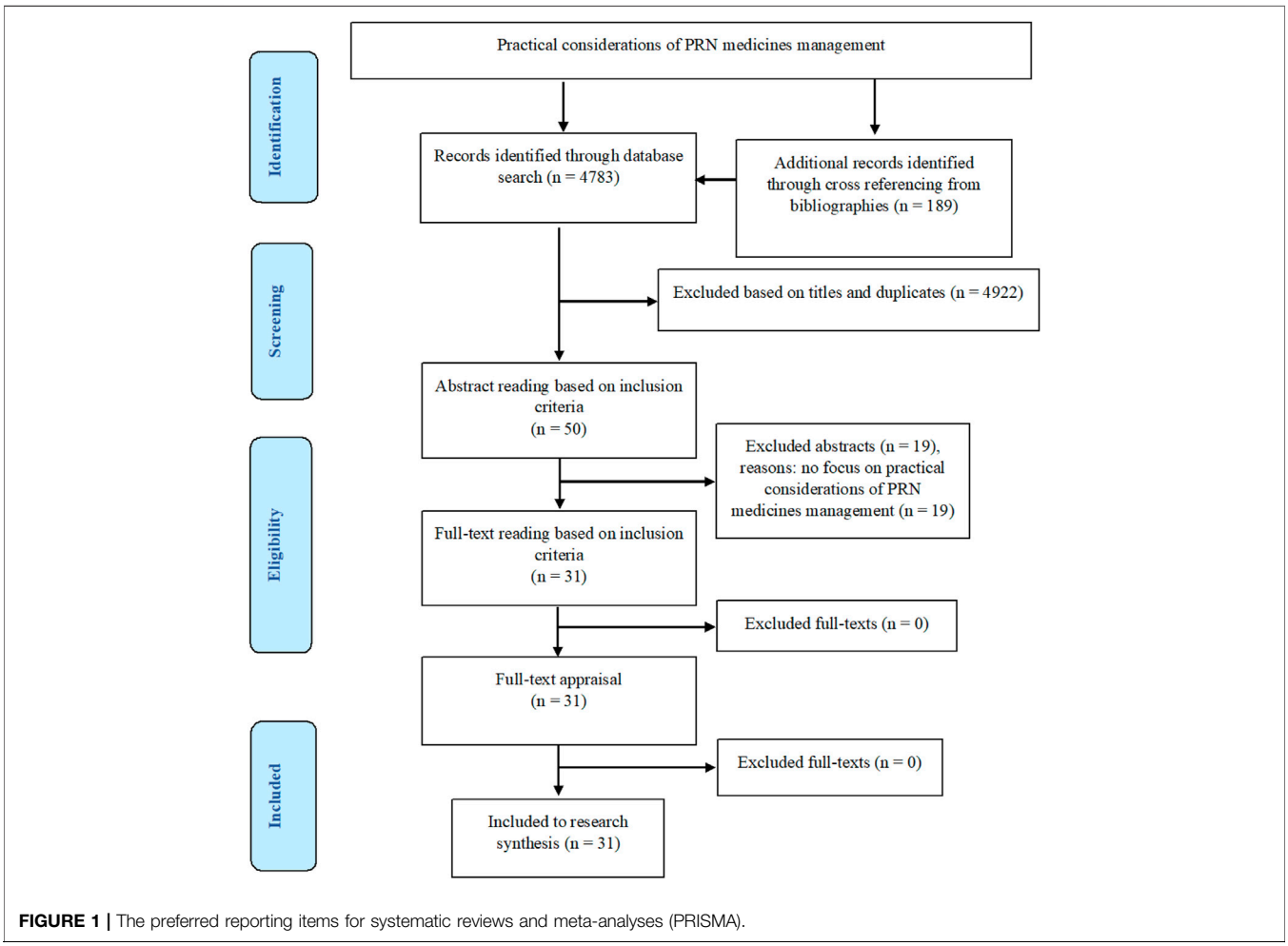

FIGURE 1 | The preferred reporting items for systematic reviews and meta-analyses (PRISMA).

Drinkwater, 2011; Swart et al., 2011; Akram et al., 2014; Al-Sughayir, 2014; Russell et al., 2014; Al-Sughayir, 2017), six were cross-sectional (Di Giulio and Crow, 1997; Geffen et al., 2002; Gordon et al., 2008; Dörks et al., 2016; Barr et al., 2018; Griffiths et al., 2019), six were qualitative (Usher et al., 2009; Usher et al., 2010; Carder, 2012; Jimu and Doyle, 2019; Nilsen et al., 2020; Walsh et al., 2020), three were interventional (Edwards et al., 2001; McCarthy et al., 2013; McCarthy et al., 2019), two were secondary analysis (Stasinopoulos et al., 2018; Sharma et al., 2021), one was Delphi technique (Baker et al., 2007a), one was mixed-methods (Martin et al., 2018a), and one was qualitative improvement (Procaccini et al., 2020).

TABLE 2 | General characteristics of the included studies to our data analysis and knowledge synthesis.

Author (year), country	Aim	Methods	Sample and settings	Outcome measurement	Main finding	Conclusion	Quality appraisal
Craven et al. (1987), Canada	To investigate the frequency and indications of the PRN prescription and administration of psychotropic medications in a psychiatric teaching hospital	Chart review	100 patients in general psychiatry wards of a psychiatric teaching hospital	Frequency and indications of PRN prescription and administration	88 patients had PRN prescription (total: 1,041); 75 patients received PRN administration (total: 1,522); diagnosis of personality disorder and age ≥50 years significantly associated with PRN prescription and administration	Hospitals should monitor PRN psychotropic medications use among inpatients and discover reasons for such use; instructions for PRN prescriptions should be obvious and detailed	STROBE Statement/ 14 from 34
Di Giulio and Crow (1997), United Kingdom	To describe cognitive processes used by nurses and doctors to decide on the administration of PRN analgesics to postoperative cancer patients	Descriptive-comparative	5 nurses and 5 doctors in an oncological digestive surgery department	Cognitive processes used when deciding to administer PRN analgesics to postoperative cancer patients	Wider use of theory and/ or experience as the source of information by doctors compare to nurses	Doctors' main concern was to make the right diagnosis, but the nurses' main concerns were patients' reactions and collaboration	STROBE Statement/ 15 from 34
Edwards et al. (2001), Australia	To investigate the effect of the Peer Intervention Program on nurses' beliefs, attitudes, subjective norms, self-efficacy, perceived control, and intentions in the management of pain using PRN narcotic analgesia	Quasi-experimental	61 nurses in 21 surgical wards spread across four hospitals	Beliefs, attitudes, subjective norms, perceived control and intention in relation to the management of pain using PRN narcotic analgesia	The peer intervention program changed nurses' beliefs, self-efficacy, and perceived control in relation to the administration of PRN narcotic analgesia to patients with pain	To improve pain management, a pain management educational program through the utilization of peers can be adopted	CONSORT 2010 checklist/ 21 from 37 (5 items were N/A)
Geffen et al. (2002), Australia	To examine the knowledge and beliefs of doctors and nurses in inpatient psychiatric units about PRN medications for psychotic disorders	Cross-sectional	80 nurses and 47 doctors in two inpatient psychiatry units	Knowledge and beliefs about PRN medications for psychotic disorders	Nurses selected more indications for PRN antipsychotics than doctors; doctors selected more indications for PRN benzodiazepines	Educational interventions should be devised for both nurses and doctors to achieve the best practice in PRN medication use	STROBE Statement/ 18 from 34
Baker et al. (2007a), United Kingdom	To explore expert opinion concerning issues and the best practice for the prescription and administration of psychotropic PRN medications within acute inpatient mental health settings	Delphi technique	18 experts (four psychiatrists, 13 nurses and a pharmacist) *via* online discussions	The best practice for the prescription and administration of psychotropic PRN medications within acute inpatient mental health settings	13 clinical practice recommendations were established	Generated items provide useful and practical guidance for prescribers and administrators of PRN psychotropic medications	STROBE Statement/ 18 from 34
Curtis et al. (2007), Australia	To explore the occurrence of PRN medication administration and the type of alternative therapeutic interventions that are documented as accompanying its administration	Retrospective chart review	64 patients in a mental health facility in an acute admission unit	Occurrence of PRN medication administration, the type of alternative therapeutic interventions that are documented as accompanying PRN administration	47 patients (73.4%) received PRN medications at least once; for nearly three-quarters (73%) of PRN medication administrations, no other therapeutic intervention was documented as occurring prior to administration	Teaching patients and nurses to learn individual techniques to recognize and cope with symptoms than rely on medication as a quick fix	STROBE Statement/ 17 from 34

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TABLE 2 | (*Continued*) General characteristics of the included studies to our data analysis and knowledge synthesis.

Author (year), country	Aim	Methods	Sample and settings	Outcome measurement	Main finding	Conclusion	Quality appraisal
Chaichan (2008), Thailand	To evaluate the use of the Positive and Negative Syndrome Scale-Excited Component (PANSS-EC) to evaluate the control of agitation and aggression among inpatients with schizophrenia as a criterion for the administration of PRN medications	Retrospective review of medical records	35 patients prior to the use of PANSS-EC scores/41 patients after its use in two acute inpatient adult psychiatric units	Assessing the effect of adoption of the PANSS-EC as a criterion for the administration of PRN medications for agitation	No statistically significant difference in the mean number of doses of PRN medication administered for agitation before and after adopting the PANSS-EC; lower number of episodes of aggression in the group assessed with the PANSS-EC	The use of criteria based on PANSSEC scores for decision-making for administering psychotropic medications to agitated patients with schizophrenia	STROBE Statement/ 20 from 34
Gordon et al. (2008), United States	To document nurses' opinions of the appropriate implementation of PRN opioid analgesic orders for acute pain	Cross-sectional	602 nurses in an academic medical center and a multihospital system with five operating units	Opinions of appropriate analgesic administration practices	Participants mainly chose appropriate responses; attending pain management courses associated with appropriate responses, sedation level, pain intensity rating, respiratory rate, and the patient's prior response to dosing choose to be considered in opioid administration	Significance of conducting a multidisciplinary examination of range order practices and the need to educate prescribers in how to write appropriate range orders and nurses in how to implement them to provide effective and safe analgesic	STROBE Statement/ 20 from 34
Stein-Parbury et al. (2008), Australia	To provide a detailed description of circumstances surrounding the use of PRN medications	Retrospective chart review	420 patients in four inpatient units	Prescriptions and administrations of PRN medications	97% were prescribed PRN medications and benzodiazepine was the most frequently prescribed one; 84% received at least one PRN medication; agitation was the most common reason for PRN administration	PRN medication use has endured as standard practice; the combination of second-generation antipsychotics as regular medications and benzodiazepines for PRN medication is consistent with recommended treatment guidelines	STROBE Statement/ 18 from 34
Kaur et al. (2009), Australia	To examine psychiatric nurses' responses to patients' requests for PRN medications and to examine whether these requests were interpreted as "drug-seeking"	Retrospective chart review	38 patients in a secure inpatient hospital	Patients' history of drug use, the frequency with which they requested PRN medications, how often staff administered PRN medications following requests, and how often patients were labelled "drug seeking"	44.7% of patients were described as 'drug-seeking'; patients with the history of amphetamine and opiate use were more frequently labelled "drug-seeking"	Need to education to highlight the influence of negative causal attributions on helping behaviours; provision of guidelines to improve the practice of PRN medication administration	STROBE Statement/ 19 from 34
Usher et al. (2009), Australia	To explore the medical and nursing decision-making process associated with the prescription and administration of PRN psychotropic medications	Qualitative	16 nurses and 3 doctors in three mental health units	Decision-making process associated with the prescription and administration of PRN psychotropic medications	Decision-making processes, factors influencing the administration and prescription of "as needed" medications, individual protocols, improving practice	Need to in-service education for mental health nurses on psychotropic medications and PRN medications; extensive review of PRN medication prescription and administration compared to best practice guidelines	SRQR/17 from 20

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TABLE 2 | (*Continued*) General characteristics of the included studies to our data analysis and knowledge synthesis.

Author (year), country	Aim	Methods	Sample and settings	Outcome measurement	Main finding	Conclusion	Quality appraisal
Usher et al. (2010), Australia	To explore doctors' and nurses' decision making surrounding appropriate PRN psychotropic administration practices within inpatient mental health settings	Qualitative	16 nurses and 3 doctors in three mental health units	Decision-making process associated with the prescription and administration of PRN psychotropic medications	Checking patients' physical health prior to the administration of PRN medications, caution about administering psychotropic drugs to elderly people, de-escalation prior to a range of further PRN medications	Decisions regarding PRN medication administration are often based upon previous experiences and levels of knowledge. Variable practices associated with when, how much and which drug to administer	SRQR/12 from 20
Mullen and Drinkwater (2011), Australia	To report the rate of PRN medication use in a psychiatric intensive care unit	Retrospective chart review	A psychiatric intensive care unit	Trends in the overall rate of PRN medication administration, time of administration, and type of medication given during the study period	A gradual decline in the total number of given PRN medications, but the typical number of patients per month receiving any PRN did not change	Offering noteworthy insights into the situations that can allow nurses to routinely investigate alternatives to PRN medications and save PRN to a minimum	STROBE Statement/ 15 from 34
Swart et al. (2011), Canada	To identify patterns for the use of PRN medications given PRN or statim and their efficacy in controlling aggressive behaviors in the mental health services environment	Retrospective chart review	338 youth in a regional children's MH center	PRN or statim medications were given to control aggressive behaviours	Those youth who received PRNs had a significantly longer period of residential treatment. Those in the Axis II program and had a developmental disability were more likely to receive PRN medications	The Axis II diagnosis of mental retardation in youth influences reasons for the administration of PRN medications, the level of supervision during PRN medication administration, and the total number of times of receiving PRN	STROBE Statement/ 22 from 34
Carder (2012), United States	To identify how unlicensed staff members decide to administer PRN medications prescribed to the residents of assisted living settings designated for persons with dementia	Qualitative	16 med aides in 3 assisted living	Decision-making regarding the administration of PRN medications	Residents' request, interpretation of resident-specific behaviours, experience and training, setting-specific practices to guide med aides' decisions regarding PRN medication administration	Training should identify the implicit knowledge of practicing medication aides; need to understand how other healthcare providers are involved in medication treatment	SRQR/17 from 20
McCarthy et al. (2013), United States	To investigate the patient-centered PRN label instructions, referred to as "Take-Wait-Stop," versus standard label	Experimental	87 patients in an emergency department	Incorrect dosing	Use of the Take-Wait-Stop label caused a reduction in going beyond the maximum daily dose	Use of the Take-Wait-Stop method significantly reduces maximum daily dose	CONSORT 2010 checklist/ 16 from 37 (6 items were N/A)
Akram et al. (2014), Scotland	To determine the frequency and nature of PRN practice	Retrospective chart review	75 patients in 10 psychiatric intensive care units	Frequency and nature of PRN practice	The most frequently administered PRN medication were lorazepam, haloperidol, and zuclopenthixol; the mean number of PRN administrations per patient per day was 0.4	Inadequate monitoring and documentation of PRN medications; possible insufficient understanding of prescribers regarding differences in bioavailability between oral and injectable forms of medications	STROBE Statement/ 16 from 34

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TABLE 2 | (*Continued*) General characteristics of the included studies to our data analysis and knowledge synthesis.

Author (year), country	Aim	Methods	Sample and settings	Outcome measurement	Main finding	Conclusion	Quality appraisal
Al-Sughayir (2014), Saudi Arabia	To investigate whether the mental health accreditation program drives improvements in the clinical practice of giving PRN antipsychotic medications for psychiatric inpatients	A record-based pre-post assessment	177 patients during the pre-accreditation period/182 patients during the post-accreditation period in a psychiatric inpatient adult unit	Number of PRN antipsychotic medications administered and indications for use	12.10 ± 7.0 and 7.47 ± 3.2 of PRN antipsychotics were administered per patient pre- and post-accreditation, respectively	Implementation of clinical practice guidelines during the mental health accreditation program significantly reduces the frequency of PRN antipsychotic medications and can enhance patient safety	STROBE Statement/ 14 from 34
Russell et al. (2014), Australia	To document PRN prescribing practices and to identify patterns with respect to clinical characteristics and medications prescribed	Prospective consecutive case note review	203 individuals in two hospices and palliative care services	PRN prescribing practices and associated factors	Mean number of PRN medications prescribed was 3.0. Higher rates of PRN medications in the last week of life and during the terminal phase of disease was observed	The trends of increasing numbers of PRN prescriptions and worsening the clinical status show the flexibility in prescribing PRN medications and to respond rapidly to changing clinical symptoms or circumstances	STROBE Statement/ 20 from 34
Dörks et al. (2016), Germany	To examine characteristics of PRN drug use and potential predictors in nursing homes	A cross-sectional study	852 residents in 21 nursing homes	Characteristics and potential predictors of PRN medication use	74.9% of residents received at least one PRN medication; more length of stay and polypharmacy, with five or more long-term medications were associated with a higher number of PRN prescriptions	Physicians should regularly review the need for any PRN medication in the medication plan. The high prevalence of PRN medications and its relationship with the length of stay underscore the importance an accurate documentation	STROBE Statement/ 26 from 34
Al-Sughayir, (2017), Saudi Arabia	To investigate whether hospital accreditation drives improvements for administered PRN benzodiazepines in psychiatric inpatients	Record-based pre-post assessment	177 patients during the pre-accreditation period/182 patients during the post-accreditation period in a psychiatric inpatient adult unit	Number of administrations of PRN benzodiazepines	Average number of PRN benzodiazepines' administrations per patient post-accreditation was 4.83 ± 2.1 compared to 6.19 ± 3.4 pre-accreditation	Accreditation may have a positive influence on the process of administering PRN benzodiazepines' medications in psychiatric inpatients	STROBE Statement/ 17 from 34
Barr et al. (2018), Australia	To identify mental health nurses' attitudes towards the use of PRN medications with mental health consumers in a forensic and non-forensic acute mental health setting in Australia	Survey	70 nurses in three acute mental health units	Nurses' attitudes towards the use of PRN medications with mental health consumers	Practice differences between forensic and other acute mental health settings were related to the use of PRN medications to manage symptoms from nicotine, alcohol and other drug withdrawals, use of comfort rooms, and conducting comprehensive assessments of consumers' psychiatric symptoms	Need for services for regular monitoring and reviewing medication prescribing and administration practices at the service level to reduce reliance on PRN medication administration	STROBE Statement/ 19 from 34

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TABLE 2 | (*Continued*) General characteristics of the included studies to our data analysis and knowledge synthesis.

Author (year), country	Aim	Methods	Sample and settings	Outcome measurement	Main finding	Conclusion	Quality appraisal
Martin et al. (2018a), Canada	To describe and compare the documentation of PRN medications for anxiety at two psychiatric hospitals, one that used paper charts and another that used electronic health records; to examine congruence between nursing documentation and their verbal reports	Mixed-methods	400 administrations of PRN medications for anxiety in two psychiatric hospitals	Documentation of PRN medications for anxiety; congruency between nursing documentation and their verbal reports	Nurses using electronic health records documented more information in comparison to those using paper charts. There were some diversities between written and verbal reports	Calls for improving the quality of nursing documentation; supporting the shift to the use of electronic health records	GRAMMS/ 4 from 6
Stasinopoulos et al. (2018), Australia	To determine the frequency of, and factors associated with PRN medication administration in residential aged-care services	Secondary analysis of cross-sectional data	383 residents in 6 residential aged-care services	Frequency and factors associated with PRN medication administrations	94% residents charted ≥1 PRN medication and 99 (28%) were administered PRN medications at least once; residents with greater dependence with the activities of daily living and a greater number of regular medications were more likely to be administered PRN medication	The portion of PRNs to medication burden in residential aged care services may be lower than previously thought	STROBE Statement/ 20 from 34
Griffiths et al. (2019), United Kingdom	To describe the prescription and administration rates of PRN medications for people with dementia in United Kingdom care homes	Cross-sectional study	728 participants with dementia or memory problems in 50 care homes	Prescription and administration of PRN medications for the treatment of behaviours associated with neuropsychiatric symptoms and pain	The total number of PRN medication prescriptions was 317. The most commonly prescribed PRN medications (35.3%) were analgesics	Low levels of medication prescriptions and even lower levels of administrations are observed for the management of neuropsychiatric symptoms	STROBE Statement/ 23 from 34
Jimu and Doyle (2019), Ireland	To explore the process of PRN medication administration by mental health nurses	Qualitative	19 nurses in an acute inpatient service	Process of PRN medication administration	Undertaking an assessment of the patient before administering PRN medications; need for service improvements in terms of the use of alternative strategies than PRN use	There is a potential for improvement in relation to how PRN medications is prescribed and administered	SRQR/17 from 20
McCarthy et al. (2019), United States	To assess the implementation of a patient-centered PRN label entitled Take-Wait-Stop (TWS) with three deconstructed steps replacing traditional wording	Experimental	211 patients in an emergency department	Prescriptions labels	12% one step wording; 26% two-step wording; 44% three-deconstructed steps	Higher implementation reliability for new instructions such as Take-Wait-Stop (TWS) requires additional supports	CONSORT 2010 checklist/ 18 from 37 (6 items were NA)

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TABLE 2 | (*Continued*) General characteristics of the included studies to our data analysis and knowledge synthesis.

Author (year), country	Aim	Methods	Sample and settings	Outcome measurement	Main finding	Conclusion	Quality appraisal
Nilsen et al. (2020), Norway	To describe healthcare personnel perceptions of factors affecting PRN medicines management in sheltered housing for older adults	Qualitative	22 healthcare personnel in sheltered housing from four municipalities representing urban, suburban and rural districts	Factors affecting PRN medicines management	Four main factors including the medication, the resident, the healthcare personnel, and the organisation affecting PRN medicines management	Safe PRN medicines management requires inter-professional collaboration and professional practice with appropriate medical competence and knowledge, practical experience and skills, and communication and documentation competency	SRQR/20 from 20
Procaccini et al. (2020), United States	To increase compliance of PRN sedative and analgesic orders with the use of failure mode and effects analysis and human factors risk assessment methodologies in a pediatric intensive care unit	Quality improvement	A pediatric intensive care unit	Proportions of compliant PRN analgesic and sedative orders based on the Joint Commission Medication Management standards	After staff education, weekly average PRN orders compliance increased from 62.0 to 77.7%; after order set implementation, weekly average compliance further increased to 93.2%	Interdisciplinary collaboration and a combined failure mode and effects analysis and human factors risk assessment are effective strategies for identifying the failure modes of PRN medication orders	CONSORT 2010 checklist/ 12 from 37 (6 items were NA)
Walsh et al. (2020), Canada	To understand how acute care nurses make decisions about administering PRN psychotropic medications to hospitalised people with dementia	Qualitative	8 nurses in three medical units	Decision making about administering PRN psychotropic medications to hospitalised people with dementia	Legitimising control (medicating undesirable behaviours to promote the nurses' perceptions of safety), making the patient fit (maintaining routine and order), and future telling (pre-emptively medicating to prevent undesirable behaviours from escalating) were developed	Need for better understanding of how to improve nursing practice in relation to PRN medication administration to hospitalised people with dementia	SRQR/17 from 20
Sharma et al. (2021), Australia	To determine the prevalence and factors associated with PRN medication administration in residential aged-care facilities and examine changes over 12-months	Secondary analysis	242 residents in 8 residential aged care facilities	Prevalence and factors associated with PRN medication administration	87.2% residents were prescribed ≥1 PRN medication; PRN administration was less likely among residents with more severe dementia symptoms and greater dependence with activities of daily living	Contribution of PRN medications to entire medication use in residential aged-care facilities is small and PRN is relatively static over 12-months	STROBE Statement/ 22 from 34

3.4 Practical Considerations of *Pro re nata* Medicines Management

Characteristics of PRN medicines management including name and dose of PRN medications, patient's age group, healthcare providers who were involved in PRN medicines management, and related practical considerations for each of the included studies were presented in **Table 3**.

During the narrative research synthesis, eight categories in relation to the practical considerations of PRN medicines management were identified: "PRN indications and precautionary measures," "requirements of PRN prescription," "interventions for PRN administration," "monitoring and follow up interventions," "deprescription strategies," "healthcare professionals' role," "participation of patients and families," and "multidisciplinary collaboration". The initial list of items corresponding to each category has been presented in **Supplementary File S4**.

3.4.1 *Pro re nata* Indications and Precautionary Measures

Prescription of PRN medications should be based on the thorough assessment of patients and collection of data about their medical history (Usher et al., 2009). For the prescription of PRN medications, appropriate indications and purpose of medication use should be specified and fully described (Craven et al., 1987; Baker et al., 2007a). Craven et al. (1987) reported that physicians had not specified any indication for 47% of PRN prescriptions for psychiatric inpatients.

When healthcare providers prescribe a new atypic medication as PRN, they should have more concerns and give more attention to its efficacy and side effects (Usher et al., 2009). Healthcare providers require to find clinical indicators for the continuation or discontinuation of PRN medications in the patient's treatment plan (Procaccini et al., 2020) and consider underlying health condition and diagnosis for PRN medication prescription (Barr et al., 2018).

The use of regularly prescribed medications in a suitable dose at the time of hospitalization instead of PRN orders has been shown to help with the reduction of the use of PRN medications (Barr et al., 2018). Effectiveness of this method in psychiatric wards has been shown (Al-Sughayir, 2014; Al-Sughayir, 2017).

3.4.2 Requirements of *Pro re nata* Prescription

Medication reconciliation and documentation of current medications should be performed soon after admission to the hospital (Al-Sughayir, 2014; Al-Sughayir, 2017). A specifically designed sheet containing headings for medication name, dose, route of administration, and an empty space for the physician's instructions should be devised for the prescriptions of PRN medications (Craven et al., 1987). Also, details of the reason for PRN medication use (Chaichan, 2008), the time interval between the doses of medications (Craven et al., 1987; Chaichan, 2008), maximum dosage limit per 24 h (Craven et al., 1987), and sequencing PRN medications for the same healthcare problem, if applicable, (Procaccini et al., 2020), should be specified.

Besides the patient's health condition, his/her preference should guide healthcare providers for the prescription of appropriate PRN medications. When the patient is at risk of self-harm, the use of fast-acting PRN medications is suggested (Walsh et al., 2020). In addition, the use of PRN medications to reduce agitation in patients who are unable to follow their previous habits including smoke cigarettes, drink alcohol, and access to illicit drugs have been recommended (Barr et al., 2018).

Controlling the undesirable behaviors of patients is a legitimate reason for the prescription and use of PRN medications when non-pharmacological strategies do not work properly (Walsh et al., 2020). Oral PRN medications have been recommended rather than injections when the patient accepts that the required response can be achieved via this method (Al-Sughayir, 2014; Al-Sughayir, 2017).

3.4.3 Interventions for *Pro re nata* Administration

Having clear goals and ration underpinning the use of PRN medications is required (Baker et al., 2007a; Curtis et al., 2007). The study by Curtis et al. (2007) reported that the rationale for the administration of 42% of PRN psychotropic medications within acute mental health settings was not stated. The PRN medication use should be supported with logic and reasons (Akram et al., 2014). It helps healthcare providers ensure the match between the indication of prescription and administration of PRN medications (Baker et al., 2007a). They require to communicate this ration to the patient and families involved in patient care along with the provision of information about any perceived risks. Answers to their questions should be given and their consent before medication administration should be sought (Baker et al., 2007a).

Timing of PRN medication use should be considered (Mullen and Drinkwater, 2011) and PRN administration should be avoided when the specified minimum time between the doses of the medication would be violated (Chaichan, 2008). Adequate attention should be paid to the interval and dose of re-administration of a similar PRN medication (Gordon et al., 2008).

The route of PRN medication administration and its dose are the important aspects of medicines management. Plasma levels of medications from oral ingestion are notably lower than those of an intramuscular or intravascular injection. Therefore, side effects are more likely to happen when the comparative doses of medications are administrated *via* injection instead of oral use (Akram et al., 2014).

Being prepared and having PRN medication orders when a patient is involuntarily admitted and is at risk of harm to themselves or others help healthcare providers to administer medications to control a potentially violent incident (Jimu and Doyle, 2019).

Making a decision on PRN medication administration should be based on collected data and the assessment of patients and their healthcare background (Di Giulio and Crow, 1997; Usher et al., 2009). Accordingly, healthcare providers should monitor patients' physical and psychological symptoms (Usher et al., 2010; Swart et al., 2011; Jimu and Doyle, 2019; Walsh et al., 2020), check their laboratory test results, evaluate their vital signs (Di Giulio and Crow, 1997), assess their allergies (Baker et al., 2007a), consider their behaviors, concerns and requests (Di Giulio and Crow, 1997; Usher et al., 2009; Barr et al., 2018; Walsh et al., 2020). In this respect, the use of both subjective assessment such as interviewing and objective assessment such as observation help with making decisions on PRN medication use (Geffen et al., 2002). Healthcare providers can make a decision on the administration of PRN medications through the interpretation of the patient's actions and non-verbal clues (Carder, 2012). Specifically, prior to the administration of opioid medications as PRN, the sedation level, pain intensity, respiratory rate, and prior response to medications should be assessed (Gordon et al., 2008). Severity of the patient's health condition and related symptoms indicate the need for medications (Usher et al., 2009). Therefore, a collective decision-making on PRN medications can be made based on the healthcare provider's perspectives and the patient's behaviors and symptoms (Walsh et al., 2020). Perceived harm and the probability of risk should be detected by healthcare providers as

TABLE 3 | PRN medicines management and related practical considerations based on the findings of each included study.

Author, year, country	Name and dose of PRN medications	Patient's age group	Healthcare providers involved in PRN medicines management	Practical considerations
Craven et al. (1987), Canada	Total PRN prescriptions: 1,041; most prescriptions were neuroleptics (32%), antiparkinsonians (31%) and sedative-hypnotics (30%); total PRN administration: 1,522; most administrations were neuroleptics (32%), antiparkinsonians (17%), and sedative-hypnotics (45%)	Men: 34 years (range = 17 to 69 years)	Nurses and physicians	The use of specifically designed sheet for PRN medicines management for medication name, dose, route of administration, and a space for the physician's instructions; use of stop-order policy after 7 days, reassessment of prescription needs by the physician; documenting the reason for PRN medication administration; specifying the indication for PRN prescriptions; stating the time interval between the doses of PRN medications and maximum dosage limit per 24 h during medication prescriptions; deprescribing PRN medications when they are no longer needed
Di Giulio and Crow (1997), United Kingdom	Analgesics	N/A	Nurses and physicians	Making decisions on PRN based on collected data; relying on theoretical and practical knowledge for PRN medication prescription and administration; consideration of patient's symptoms, behaviours, and preferences for PRN use; consideration of laboratory test results; collecting data on vital signs; having a closer look at psychological symptoms and a broader perspective rather than problem-specific for medication use; being worried about the administration of wrong medications that can hamper diagnosis; interference of medications in patient's collaboration with the treatment plan
Edwards et al. (2001), Australia	Narcotics	N/A	Nurses	Having a positive attitude toward the administration of PRN medications; having a good intention for PRN medication administration; positive attitude by the patient, family members and healthcare providers toward PRN medications use; ability to administer PRN medications
Geffen et al. (2002), Australia	Antipsychotics, benzodiazepines, anticholinergics	N/A	Physicians and nurses	Use of both subjective (internal state) and objective (behaviour) assessment methods to make decisions on medication use; consideration of alternative interventions instead of PRN medications
Baker et al. (2007a), United Kingdom	Psychotropic medications	N/A	Physicians, nurses and pharmacists	Clear purpose for PRN medications; being aware of the potential side effects of PRN medications; ensuring the match between the indication for PRN prescription and administration; consideration of side effects and additional medication interactions/allergic reactions; finding allergies prior to administration; having the clear goal underpinning the use of PRN medications; clear description of indications for PRN; joint decision making about the prescription wherever possible –including translating/agreeing the rational/indication for the prescription into the language of/with the service user; time-limited prescription of PRN medications, with regular reviews; gaining knowledge of any advance directive related to PRN medications; clear documentation of circumstances leading to the administration of PRN medications and its beneficial or detrimental impact on behaviour; regular and systematic evaluation of the use and effects of PRN medications for individual patients; communicating the rational to the service user as well as information about any perceived risks, answering questions, and seeking consent
Curtis et al. (2007), Australia	309 psychotropic medications were administered on 268 occasions including 1. Benzodiazepines (n = 188, 60.8%) 2. Atypical antipsychotic (n = 31, 10%) 3. Typical antipsychotic (n = 87, 28.1%) 4. Other (n = 2, 0.6%)	<19: 8 20–29: 12 30–39: 17 40–49: 18 50+: 9	Nurses	PRN administrations based on rational and reason; documentation of PRN medication effects; description of the method used for the evaluation of PRN medication effects; documentation of any additional pre- or post-intervention when PRN medications are used

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TABLE 3 | (*Continued*) PRN medicines management and related practical considerations based on the findings of each included study.

Author, year, country	Name and dose of PRN medications	Patient's age group	Healthcare providers involved in PRN medicines management	Practical considerations
Chaichan (2008), Thailand	Psychotropic medications for agitation in patients with schizophrenia; prescriptions: all study participants were prescribed at least one PRN medication; the most frequently prescribed medication was haloperidol in the control group and in the Positive and Negative Syndrome Scale-Excited Component (PANSS-EC) group Administration: in the control group, 23 patients (65.7%) received 54 doses of PRN psychotropic medications, while 23 patients (56.1%) in the PANSS-EC group received 56 doses	Mean (SD): control group: 32.49 (8.67) years PANSS-EC group: 35.54 (9.33) y	Nurses and physicians	Inclusion of the medication name, dose, route of administration, reason for use, and shortest time allowed before and the dose can be repeated in the physician order; use of assessment tools during admission to determine the need for PRN medications, avoiding PRN administration when the minimum time specified between doses of the medication is violated
Gordon et al. (2008), United States	Opioids	N/A	Nurses	Consideration of the sedation level, pain intensity, respiratory rate, prior response for the selection of opioids; paying attention to the interval and dose of the re-administration of a similar PRN medication
Stein-Parbury et al. (2008), Australia	97% of the patients (408/420) were prescribed PRN medications; total prescription: 139. The most frequently prescribed medications 1. Benzodiazepines (52.2%) 2. First-generation antipsychotic (FGAs): 16.6% administrations for 420 admissions, 3,868 episodes of PRN medications; types of administrated 1. Benzodiazepines: 70.7% 2. FGAs: 18.1% 3. Benztropine: 4.3%	Mean: 38.63 years	Nurses and physicians	Administration of more than one PRN medications without the description of its clear indication; documentation of the indication of the administration of PRN medications; documentation of the outcome of PRN medication administration
Kaur et al. (2009), Australia	N/A	N/A	Nurses	Assessing the drug dependency and abuse
Usher et al. (2009), Australia	N/A	N/A	Nurses and physicians	Consideration of the patient's behaviour, concerns and requests; having concerns about the prescription of the new atypical medications as PRN; PRN medication use only after trying alternatives; not interpreting the patient's request as the drug-seeking behaviour; prescription and administration based on thorough assessment of patients and getting knowledge of his/her background; concerns about ineffectiveness of medications, and related side effects; being looked a like unwell to receive PRN medications; patient's willingness and previous effectiveness to choose alternative methods; severity of the patient's health condition and symptoms as the factor affecting medication use; staffing pattern and shortages and inexperienced staff to affect the medication use; personal perspective and philosophy by nurses for PRN medication use; presence of the individual medication protocol to decide on PRN medication administration; need to clear and up-to-date prescription information; being ensured of patient safety in the caring environment; clear writing of medication orders by the doctor
Usher et al. (2010), Australia	Psychotropics	N/A	Nurses and physicians	Regular patient's checking in terms of physical health before and after medication use; de-escalation using restraints and seclusion before PRN medication use

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TABLE 3 | (*Continued*) PRN medicines management and related practical considerations based on the findings of each included study.

Author, year, country	Name and dose of PRN medications	Patient's age group	Healthcare providers involved in PRN medicines management	Practical considerations
Mullen and Drinkwater (2011), Australia	50–60% of patients in the psychiatric intensive care unit received at least one PRN medication during their stay; the most frequently administered PRN medication during all four periods was diazepam	N/A	Nurses	Timing of PRN medication use
Swart et al. (2011), Canada	50.3% of patients received one or more PRNs; three most medications were chlorpromazine, lorazepam, and olanzapine	Mean SD: 12.3 years (2.68)	Nurses and physicians	Assessing techniques for reducing PRN medication use including counselling, prompt to calm, redirection, planned ignoring, offering alternative choices, and reminder of consequences; assessing the reason for PRN medication administration such as gesture of treat
Carder (2012), United States	N/A	N/A	Med aides	Expression of symptoms and request for medications by the patient; provision of instructions with enough detail for the appropriate use of PRN medications such as the dosage guideline; provision of training to healthcare providers in relation to PRN medications; giving information in relation to patients' medication during shift handoff interpreting the patient's non-verbal behavioural clues; regulations for PRN medications use in terms of reasons for use, schedule and route, circumstances for use, maximum dose, when to call the resident's physician, and when to discontinue; appropriate storage of medications to facilitate access to medications
McCarthy et al. (2013), United States	Pain medication containing acetaminophen	Mean (SD): 39.8 (12.9)	Pharmacists	Use of the Take-Wait-Stop label design consisting of explicit, deconstructed instructions and simplified text (numeric characters instead of words, e.g., "1 tab" instead of "one tab", and "carriage returns" to place each part of the instructions on separate lines; use of word "stop" instead of "do not exceed" to convey the maximum daily dosage to patients in plain language; deconstructing instructions so that each action or intended behavior was separate and would potentially allow patients to be more cognizant of each step to be taken
Akram et al. (2014), Scotland	65% of patients were administered psychotropic PRNs (total 396 doses); number of most frequently administrated psychotropic PRNs 1. Oral forms of lorazepam (n = 198) 2. Oral form of haloperidol (n = 66) 3. Oral form of zuclopenthixol (n = 22) 4. Injection form of lorazepam and quetiapine (both n = 14)	Male patients: 37 years; female patients: 40 years	Nurses	Patient's request for medications or nurses' decision making on PRN; assessing the peak time of medication administration in the day; administration route of medications; assessing the reason of PRN medication use as rapid tranquilisation; simultaneous use of PRN medications and restrains; documentation of post medication administration monitoring
Al-Sughayir (2014), Saudi Arabia	Antipsychotics	<25: 109 (30.3%) 25–50: 208 (57%) >50: 42 (11.7%)	Nurses and physicians	Reconciliation of medications soon after patient admission and their documentations; use of regular medications for individual patients as PRN; avoiding polypharmacy; consideration of alternative methods such as counselling when handling the patient's difficult behaviour before resorting to PRN medications; completing PRN regimen order among the treating psychiatrist as soon as possible; use of oral PRN medications when the patient accepts them and when the required response is achieved rather than injections; documenting administered PRN medications and the patient's response to them; monitoring vital signs for side effects such as extrapyramidal side effects after administering PRN medications; informing the treating psychiatrist and asking for a medical evaluation in case of any concern

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TABLE 3 | (*Continued*) PRN medicines management and related practical considerations based on the findings of each included study.

Author, year, country	Name and dose of PRN medications	Patient's age group	Healthcare providers involved in PRN medicines management	Practical considerations
Russell et al. (2014), Australia	606 total PRN prescriptions including 1. Opioid: 178 (29.4%) 2. Antiemetic: 112 (18.5%) 3. Benzodiazepine: 99 (16.3%) 4. Laxative: 82 (13.5%) 5. Acetaminophen: 56 (9.2%) 6. Other: 79 (13%)	Mean (SD): 72.9 years (12.6 years)	Physicians and nurses	Assessment of polypharmacy and over-prescription of medications; considering inappropriate PRN medications prescription; prescribing and administering PRN medications according to the patients' condition
Dörks et al. (2016), Germany	Total 2117 PRN prescriptions; most commonly used PRN drugs, *n* (%) 1. Acetaminophen: 299 (14.1%) 2. Metamizole: 272 (12.8%) 3. Ibuprofen: 124 (5.9%) 4. Macrogol: 110 (5.2%) 5. Loperamide: 103 (4.9%) 6. Lactulose: 101 (4.8%) 7. Melperone: 84 (4.0%) 8. Metoclopramide: 74 (3.5%) 9. Lorazepam: 69 (3.3%) 10. Bisacodyl: 60 (2.8%)	Mean (SD): 83.5 years (10.5 years)	Physicians and nurses	Monitoring the number of medications in patients with a long duration of hospitalisation
Al-Sughayir, (2017), Saudi Arabia	Benzodiazepine	<25: 109 (30.3%) 25–50: 208 (57%) >50: 42 (11.7%)	Nurses and physicians	Reconciliation and documentation of current medications after admission; use of regular medications for individual patients as PRN; avoiding polypharmacy; consideration of alternative methods such as counselling when handling the patient's difficult behaviour before resorting to PRN medications; completing the PRN regimen among the treating psychiatrist as soon as possible; use of oral PRN medications when the patient accepts them and when the required response is achieved rather than injections; documentation of administered PRN medications and the patient's response to it; monitoring vital signs for side effects after PRN medication administration; informing the treating psychiatrist and asking for a medical evaluation in case of any concern
Barr et al. (2018), Australia	Psychotropics	N/A	Nurses	Consideration of underlying diagnosis in PRN prescription and administration; attention to the patient's request for PRN medications; use of PRN medications for reducing agitation in patients who are unable to follow their previous behaviours such as smoke cigarettes, drink alcohol or access illicit drugs; accurate assessment *via* appropriate tools to determine the need for PRN medications and reduce PRN medication use; consideration of alternative methods such as music and relaxation to reduce PRN use; regular medication prescription instead of PRN orders; multidisciplinary team collaboration for the management of behaviour and reduction of medication use; taking more responsibility in the prescription and administration of PRN medications and being aware of issues resulting from high dose and poly-pharmacy; combination of PRN medications with other methods to improve its effectiveness; use of PRN medications to reduce the use of seclusion and restrictive measures damaging therapeutic relationships; use of alternative methods such as behaviour therapy and relaxation instead of PRN to improve self-care
Martin et al. (2018a), Canada	400 administrations of PRNs for anxiety; 80% of the prescriptions were lorazepam	—	Nurses	Identification and documentation of symptoms related to the need for PRN medication use; documentation of PRN medications when it is administered; documentation of the reason for PRN medication administration; documentation of the effect and side effect of PRN medications; trying non-pharmacological interventions prior to administering PRN medications

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TABLE 3 | (*Continued*) PRN medicines management and related practical considerations based on the findings of each included study.

Author, year, country	Name and dose of PRN medications	Patient's age group	Healthcare providers involved in PRN medicines management	Practical considerations
Stasinopoulos et al. (2018), Australia	94% of residents were charted at least one PRN medication (median: 4); the most prevalent charted PRN medications, number of residents who had charted PRN (%) were 1. Paracetamol: 178 (46.5) 2. Docusate sodium ± senna: 143 (37.3) 3. Bisacodyl: 82 (21.4) 4. Oxycodone: 72 (18.8) 5. Metoclopramide: 72 (18.8) 6. Glyceryl trinitrate: 69 (18.0) 7. Macrogol: 62 (16.2) 8. Betamethasone: 56 (14.6) 9. Temazepam: 54 (14.1) 10. Oxazepam: 50 (13.1) 11. Salbutamol (inhaled): 49 (12.8)	Median (interquartile range (IQR): 88 (84–92)	Nurses and physicians	Assessment of over-medication and polypharmacy; assessment of over-prescription of PRN medications in patients with more dependency levels
Griffiths et al. (2019), United Kingdom	317 PRN prescriptions; 180 PRN medications were administrated 1. Antipsychotic, 10 prescribed, 2 administrated 2. Benzodiazepine, 39 prescribed, 19 administrated 3. Non-benzodiazepine hypnotic, 6 prescribed, 5 administrated 4. Antidepressant, 3 prescribed, 3 administrated 5. Analgesic, 259 prescribed, 151 administrated	Mean (SD): 85.6 years (7.64 years)	Physicians and nurses	Evaluation of the effects of PRN medications on the symptoms of the underlying health conditions; consideration of polypharmacy with PRN medications administration; association between the severity of the symptoms experienced and the amount of prescribed PRN medications
Jimu and Doyle (2019), Ireland	N/A	N/A	Nurses	Assessing the patient in terms of physical and psychological symptoms; undertaking a risk assessment with regard to the patients and others; preparing the patient with regard to when PRN medications should be administered; discussing changes in PRN medication use between the physician and nurse; consideration of over-medication and poly-pharmacy; consideration of alternative treatment methods; need for senior nurses to get involved in the PRN medication process and discuss administration
McCarthy et al. (2019), United States	Hydrocodone-acetaminophen	Mean (SD): 44.3 years (14.3 years)	Pharmacists	Developing the Take-Wait-Stop label, following the patient-centered prescription label design; deconstructing prescription wording regarding the core components of PRN instructions to explicitly convey the dose, interval between doses, and maximum daily dose; PRN instruction emphasis on deconstructing actions and behavioural steps that support understanding and recall; employing numeric characters instead of words, e.g., "1 tab" instead of "one tab," and "carriage returns" place each section of the instructions on different lines; use of simplified text and plain language, "Stop" to replace the typical wording "do not exceed," to convey maximum daily dosing among patients with limited literacy
Nilsen et al. (2020), Norway	N/A	N/A	Nurses, healthcare workers, apprentices in health and social work, social educators	Judgement of the patients' symptoms for PRN medication use; creating a consensus on PRN medication use through interprofessional medication review; patients' participation in decision making on PRN medications; patients' knowledge of list of medications; communication and cognitive abilities of patients to assess the necessity of PRN medication use; reaching agreements by the healthcare providers and families on PRN; healthcare staff's knowledge of medicines management; seeking for complementary competency

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TABLE 3 | (*Continued*) PRN medicines management and related practical considerations based on the findings of each included study.

Author, year, country	Name and dose of PRN medications	Patient's age group	Healthcare providers involved in PRN medicines management	Practical considerations
				through asking for the second opinion; significance of practical knowledge; skills for the assessment of the effects of PRN medications; appropriate staffing pattern in the ward; sharing verbal and written information; appropriate storage of medications to facilitate access; culture of medication use as the use of non-pharmacological methods prior to medication use
Procaccini et al. (2020), United States	Sedative and analgesic medications	N/A	Physicians, nurses, pharmacists	Consideration of clinical indications for the use or discontinuation of PRN medications; sequencing PRN medications for the same healthcare problem; communication with prescriber in case of unsuccessful outcome of PRN use; education of healthcare staff to comply with PRN medication standards; use of decision support tools
Walsh et al. (2020), Canada	Psychotropics	N/A	Nurses	Perceived harm and the probability of risk of patients by healthcare providers as the indicator of PRN medication use; patient's preference and compliance with PRN medication use; use of fast acting medications to prevent patient's self-harm; close monitoring of the patient's behaviours and symptoms to find indication for medication use; controlling undesirable behaviours to legitimate PRN medication use; use of non-pharmacologic strategies such as restrain before medication use; use of PRN medications based on the hospital's protocol to prevent the use of restraints; time-consuming identity of nonpharmacologic interventions such as distraction and redirection; more PRN use due to higher workloads and staff shortages; use of PRN medications to manage sleep disturbances and help adjust with the work unit; PRN medication administration to the best interest of the patients; collective decision making on PRN medications based on the nurse's perspectives and the patient's behaviour and symptoms; use of PRN medications based on predicting the patient's pattern of behaviours and knowing the patient; consideration of the disease's general pattern and the underlying cause of behaviours for PRN medication use; misinterpretation of the patient's behaviours due to communication issues and PRN medication use
Sharma et al. (2021), Australia	1090 PRN prescribed; the most prevalent PRN medications prescribed were paracetamol (54.1% of residents), docusate and sennosides (40.9%) and metoclopramide (26.8%)	Median (Interquartile range): 87.0 (81.0–92.0)	Physicians and nurses	Assessment of daily dose recommendation of medications; assessment of the PRN medication administration in patients with severe cognitive issues

an important indicator of PRN medication administration (Walsh et al., 2020).

There is a need to have a closer look at psychological symptoms and having a broader perspective rather than problem-oriented one for the appropriate use of PRN medications (Di Giulio and Crow, 1997). It helps predict the patient's pattern of behaviors by knowing the patient and empower him/her to safely use PRN medications for the prevention of dangerous behaviors and related harm (Walsh et al., 2020). Healthcare providers' judgment of the patient's symptoms is decisive for PRN medication administration. Some symptoms such as heavy breathing or constipation clearly have an obvious cause, which facilitate decision-making regarding PRN medication use. On the other hand, a single night insomnia is not judged to be an indication for the use of hypnotics as PRN (Nilsen et al., 2020).

Communication challenges between the patient and healthcare providers contribute to the increased use of PRN medications. It hinders the assessment of patients' underlying health problems or unmet needs leading to undesirable behaviors. Therefore, the patient's behaviors due to communication issues should not be misinterpreted and hastily decisions on the use of PRN medications should not be made (Walsh et al., 2020).

Undertaking a formal risk assessment is an important step for making a decision on the use of PRN medications. It consists of the assessment of the risk to the patient themselves, to other

patients, and to healthcare providers (Jimu and Doyle, 2019). Relevant assessment tools at the hospital admission determines the need for PRN medication use. For example, the Positive and Negative Syndrome Scale-Excited Component (PANSS-EC) can be used to evaluate the control of agitation and aggression in people with schizophrenia during the first 3 days of admission. Its score influences the decision on the administration of PRN medications (Chaichan, 2008). The study by Chaichan (2008) showed that the mean number of episodes of aggression in patients with schizophrenia during the period of hospitalization was remarkably lower among those assessed with the PANSS-EC. The Positive and Negative Symptom Scale (PANSS) used for the accurate assessment of psychiatric patients with appropriate tools can help determine the need for PRN medications and reduce PRN medication use during hospital stays (Barr et al., 2018). Use of decision support tools for evaluating pain and sedation can optimize PRN medication administration (Procaccini et al., 2020).

In addition to screening tools, the general pattern of the disease, underlying cause of behaviors (Walsh et al., 2020), and underlying diagnosis (Barr et al., 2018) can be helpful for decision-making about PRN medication administration. Nurses have the best position to use their knowledge about patients with long-term health conditions and observe distinctive behavioral patterns and help with the determination of the patient's needs for PRN medications (Walsh et al., 2020).

Healthcare providers should note that the administration of wrong PRN medications can hamper the diagnosis. Particularly analgesics and antispasmodics can conceal the patient's symptoms (Di Giulio and Crow, 1997). PRN medication administration should be to the best interest of patients. Healthcare providers should follow a middle ground with regard to how to manage disruptive behaviors using PRN medications without causing medication toxicity (Walsh et al., 2020).

Alternative treatment strategies including non-pharmacologic methods such as redirection and distraction, and physical restraint as the last resort can be considered before the use of PRN medications (Walsh et al., 2020). Restraint, time out, and seclusion can be used to help de-escalation before further PRN medication administration (Usher et al., 2010). Although the use of restrains is outlawed, a balance should be present between the administration of PRN medications and avoiding the use of seclusion, because seclusion is restrictive and has the potential of damaging the therapeutic relationship between healthcare professionals and patients (Barr et al., 2018).

3.4.4 Monitoring and Follow up Interventions

The use and effects of PRN medications should be regularly and systematically evaluated (Baker et al., 2007a). Healthcare providers should be aware of the potential side effects of PRN medications (Baker et al., 2007a) and have a concern about both their ineffectiveness and side effects (Usher et al., 2009). The patient should be regularly checked in terms of physical health before and after PRN medication use (Usher et al., 2010) and probable medication interactions/allergic reactions (Baker et al., 2007a). Side effects of PRN medications can be identified through the monitoring of vital signs and the patient's symptoms such as extrapyramidal complications (Al-Sughayir, 2014; Al-Sughayir, 2017). In addition, there is a need to assess the effects of PRN medications on the patient's underlying health condition (Griffiths et al., 2019). The nurse should communicate with the physician as prescriber in case of unsuccessful outcome of PRN medication use (Procaccini et al., 2020). The treating physician should be informed and asked for a medical evaluation in case of any concern regarding the use of PRN medications (Al-Sughayir, 2014; Al-Sughayir, 2017). Assessing the peak time of PRN medication administration during the day can help take appropriate measures to optimize PRN medication use (Akram et al., 2014).

Monitoring and documentation of related data when PRN medications are administered (Al-Sughayir, 2014; Al-Sughayir, 2017; Martin et al., 2018a) and post medication administration are of utmost importance (Akram et al., 2014). Documentation of PRN medications should be clear in terms of the reason (Craven et al., 1987; Martin et al., 2018a) and indication of use (Stein-Parbury et al., 2008), circumstances and symptoms leading to administration (Baker et al., 2007a; Martin et al., 2018a), related effects (Baker et al., 2007a; Curtis et al., 2007; Stein-Parbury et al., 2008; Al-Sughayir, 2014; Al-Sughayir, 2017; Martin et al., 2018a), negative consequences and side effects (Martin et al., 2018a), and the method used for the evaluation of expected outcomes (Curtis et al., 2007). Martin et al. (2018a) in their study reported that in 15% of cases, the administration of psychotropic PRN medications were not documented and in 79% of cases, a reason for it was mentioned. In another study, only in 63.2% of episodes a reason for PRN medication administration was documented (Stein-Parbury et al., 2008). In the study by Curtis et al. (2007), the effect of PRN medications was documented only in 38.8% of occasions.

When PRN medications are used, any additional pre- or post -intervention should be recorded (Curtis et al., 2007). According to the Curtis et al.'s (2007), additional pre- or post -intervention was documented only in 28% of occasions of PRN medication administration. If more than one PRN medications is administered, indications should be clearly explained (Stein-Parbury et al., 2008).

Healthcare providers should take more responsibility for the prescription and administration of PRN medications and should be aware of issues resulting from high doses and polypharmacy especially in patients with mental health problems (Barr et al., 2018; Griffiths et al., 2019; Jimu and Doyle, 2019), and avoid polypharmacy if possible (Al-Sughayir, 2014; Al-Sughayir, 2017).

In those patients who are at the risk of high doses of medications including in long-term care facilities and with severe cognitive issues, the administration of PRN medications and daily recommended dose of PRN medications should be monitored (Sharma et al., 2021). Assessment of over-prescription, over-medication, and polypharmacy of PRN medications should encompass patients with more dependency levels (Stasinopoulos et al., 2018). Monitoring the number of medications in patients with a longer duration of hospital stay is required (Dörks et al., 2016).

3.4.5 Deprescription Strategies

The PRN medication regimen should be completed and its use should be discontinued by the treating physician as soon as possible (Al-Sughayir, 2014; Al-Sughayir, 2017). The end date should be clearly stated at the beginning of prescription (Usher et al., 2009). As a rule, PRN medications should be deprescribed when they are no longer needed (Craven et al., 1987). A time-limited prescription of PRN medications requires the regular review of medication use (Baker et al., 2007a). The use of a stop-order policy after 7 days can help avoid unnecessary PRN medication use and the early deprescription of PRNs. Accordingly, the prescriber has to reassess the PRN medication order and decide on the need or for continuation for more than 7 days. In case of the needs for continuation, the prescriber repeats the order with the consideration of the prescription requirements (Craven et al., 1987). Drug dependency and abuse should be considered when making such a decision (Kaur et al., 2009).

Alternative interventions such as non-pharmacologic strategies on appropriate occasions prior or instead of PRN medication administration or in combination with them not only help achieve an optimal response, but also prepare the ground for discontinuation (Geffen et al., 2002; Usher et al., 2009; Swart et al., 2011; Al-Sughayir, 2014; Al-Sughayir, 2017; Martin et al., 2018a; Barr et al., 2018; Jimu and Doyle, 2019). The feasibility of their use depends on that the patient is identified by healthcare professionals to have a low risk level for use along with having a positive attitude toward such interventions (Usher et al., 2009). Behavior therapy, music, counselling, relaxation, redirection, and planned ignoring have been shown helpful in the reduction of PRN medication use and improvement of self-care (Swart et al., 2011; Al-Sughayir, 2014; Al-Sughayir, 2017; Barr et al., 2018). Combination and the simultaneous use of PRN medications with alternative interventions improve the effectiveness of medication use (Akram et al., 2014; Barr et al., 2018). However, the alternative and non-pharmacologic interventions are time-consuming and their practice requires appropriate expertise (Walsh et al., 2020).

3.4.6 Healthcare Professionals' Role

Healthcare providers should show their good intentions and positive attitudes toward PRN medication use to be able to perform related caring measures (Edwards et al., 2001). They need to rely on their theoretical and practical knowledge (Di Giulio and Crow, 1997). Having sufficient pharmacotherapeutic knowledge is important for PRN medication use, post-PRN monitoring, and its documentation (Nilsen et al., 2020). Practical experience and knowledge are important and refers to having knowledge about how PRN medications can impact on the patient's health condition (Nilsen et al., 2020).

Healthcare providers should gain knowledge of any advance directive with regard to PRN medications (Baker et al., 2007a). Appropriate education to healthcare providers can empower them to comply with PRN medication standards such as the dosage guideline (Carder, 2012; Procaccini et al., 2020). Healthcare providers should know about the regulations of PRN medication use in terms of the reason for use, schedule and route, circumstances of use, maximum dose, when to contact the physician, and when to discontinue medications (Carder, 2012). Experienced healthcare providers can teach newly staff regarding the facility-specific systems for PRN medication order, stock, documentation, and administration (Carder, 2012). Personal skills of healthcare providers can contribute to the assessment of the effects of PRN medications (Walsh et al., 2020).

Having access to a senior healthcare provider who is involved in PRN medicines management and discussion on its administration improve medication safety (Jimu and Doyle, 2019). Healthcare providers can seek a second opinion prior to the final decision making regarding PRN medication administration as complementary to their own competence (Nilsen et al., 2020). Sufficient information sharing in both written and oral formats influences PRN medicines management. Quality of the documentation is a significant element in the decision-making process with regard to the PRN medication use. Oral information sharing during shift handoff can inform the next healthcare provider about challenges during the work shift, the patient's health condition, and how to face issues with PRN medication use (Carder, 2012; Nilsen et al., 2020). Clear, accurate, and up-to-date prescription information avoids uncertainty between the prescriber and the administrator, improves optimal PRN medicines management, and prevents misinterpretations (Usher et al., 2009).

Environmental factors also can influence decisions regarding PRN medication administration. Appropriate staffing on each work shift improves high-quality PRN medicines management (Nilsen et al., 2020). In contrast, staff shortages and heavy workloads increase the inappropriate use of PRN medications (Walsh et al., 2020). When staff shortages are present, healthcare providers are busy and do not have enough time for the patient's assessment. Therefore, they may give PRN medications more regularly to patients without attempting to take more time and use alternative strategies (Usher et al., 2009; Walsh et al., 2020). Inexperienced healthcare providers who may not be quite familiar with the healthcare setting are the reason for the higher rate of PRN medication use (Usher et al., 2009).

The appropriate storage of PRN medications can facilitate access to medications and is a contributing component of safe PRN medicines management (Carder, 2012; Nilsen et al., 2020). Other aspects are placing medications in a labeled container inside the locked cabinet, adding a direction about conditions in which the medication can be administered, and informing the healthcare provider about the availability of medications (Carder, 2012). Where PRN medications are in storage and a healthcare provider has the key, other healthcare providers have to discuss with her/him and explain the situation before asking for medications, thereby regulate PRN medicines management (Nilsen et al., 2020).

TABLE 4 | The suggested list of the practical considerations of PRN medicines management.

Category	Item
PRN indications and precautionary measures	Prescription based on the diagnosis and the assessment of the patient and his/her medical history Specification of appropriate indications and the purpose of medication use Consideration of the efficacy and side effects of new atypic medications Attention to clinical indications for the continuation or discontinuation of medications Replacement of PRN medications by regular medications with suitable doses
Requirements of PRN prescription	Medication reconciliation immediately after admission Documentation of the medication name, dose, route of administration, and the physician's instructions Inclusion of prescription details such as the reason for use, shortest time allowed before dose repetition, time intervals, maximum dose per 24 h, and sequencing PRN medications for the same healthcare problem Consideration of the patient's preferences in the prescription of medications Setting undesirable patients' behaviors and ineffectiveness of non-pharmacological methods as legitimate reasons for medication prescription Prioritizing oral medications to injections when the required response can be achieved
Interventions for PRN administration	Setting clear goals and having ration underpinning medication administration Administration of rapid tranquilizations along with logic and reasons Setting concordance between the indication of prescription and administration of medications Involvement of patients and informal caregivers through informing them about the rationale of PRN medication use, related perceived risks, and seeking consent before medication administration Avoiding the violation of the minimum time specified between doses Selection of the best route for medication administration Medication administration when there is the risk of patient harm Making decisions on medication administration after thorough assessment of patients and related health history Interviewing and observation of the patient before medication use Interpretation of the patient's actions and non-verbal clues Consideration of the severity of the patient's health condition and related symptoms Collective decision-making based on collected data and personal judgments by all healthcare providers Incorporation of probable risks into the indications of medication administration Going beyond problem-specific symptoms for medication administration Avoiding the misinterpretation of the patient's behaviors and taking hastily decisions Risk assessment for decision making on medication administration Prevention of administration of medications that cause toxicity and hamper diagnosis Administration of medications in the best interest of patients Use of alternative and non-pharmacologic methods before medication administration Use of restraint, time out, and seclusion to help with de-escalation before medication administration
Monitoring and follow up interventions	Regular and systematic evaluation of the effects of medications on the symptoms of the underlying health condition Being aware of the potential side effects of PRN medications and having concerns about their ineffectiveness and side effects Regular checking of the patient's physical health and probable medication interactions/allergic reactions before and after medication use Communication of the unsuccessful outcome of PRN medication use and any concern to the prescriber Assessing the peak time of daily medication use to take appropriate measures for medication optimization Detailed documentation of the medication procedure in terms of indication for use, circumstances and symptoms leading to administration, related effect, negative consequences and side effects, and methods used for expected outcomes' evaluation Knowledge improvement about issues resulting from high doses and polypharmacy Close monitoring of medications use in patients who are at the risk of polypharmacy, dependency, overdose and showing allergic reactions Monitoring of the number of medications in patients with a longer duration of hospitalization
Deprescription strategies	Completing the medication regimen and its early discontinuation Determining the end date for medication use at the beginning of prescription Time-limited prescription of PRN medications using a regular review The use of a stop-order policy after 7 days to avoid unnecessary medication use Consideration of drug dependency and abuse to make deprescription decision Use of alternative and non-pharmacologic methods on appropriate occasions instead of medications or in combination
Healthcare professionals' role	Appropriate individualized philosophical perspectives and positive attitudes toward medication use Improving theoretical and practical knowledge of medicines management Education of healthcare staff to comply with standard medication use Education of new staff by experienced and senior ones with regard to medication order, stock, documentation, and administration Seeking a second and expert opinion prior to medication administration

(Continued on following page)

TABLE 4 | (*Continued*) The suggested list of the practical considerations of PRN medicines management.

Category	Item
	Sharing information between healthcare providers in both written and oral formats regarding PRN medicines management Clear, accurate, and up-to-date information sharing to avoid ambiguity between the prescriber and administrator Appropriate staffing pattern on each work shift for medication administration Appropriate storage of medication, e.g., in a labelled container inside the locked cabinet and direction regarding the conditions in which the medication can be administered Establishing the culture of non-pharmacological interventions before medication use Use of medications to facilitate patients' adjustment to the requirements of the work environment during hospitalization
Participation of patients and families	Creating positive attitudes in the patient and informal caregivers about medication use Attention to the patient's preferences and compliance with medication use Involvement of the patient in the decision process for medication use Joint decision-making about the prescription of medications and translating/agreeing the rational/indication into the patient's language Improvement of the patients' knowledge regarding the medication process Encouraging the patient to replace medications with alternative and non-pharmacological methods Resolving conflicting understanding of medication use between healthcare providers, patients, and informal caregivers Connecting the severity of symptoms and medication doses Use of instructions on the medication bottles under the name of the Take-Wait-Stop label for outpatient and ambulatory patients
Multidisciplinary collaboration	Collaboration by healthcare professionals from the moment that PRN medications are prescribed Identifying and highlighting nurses' roles for medicines management Interprofessional medication review on the patient's medication list to reach consensus on medication use Involvement of the multidisciplinary team in the management of patients' behavioral problems

The culture of applying non-pharmacological interventions before administering PRN medications can prevent inappropriate medication use (Nilsen et al., 2020). Clinical protocols where they restrict the physical restraint policy and the use of chemical restraint when non-pharmacological strategies such as distraction and redirection fail to alleviate unfavorable behaviors are supported (Walsh et al., 2020).

Additionally, healthcare professionals' disciplines or philosophical perspectives regarding the use of PRN medications impact medication use. Some clinical protocols enforce healthcare providers to administer PRN medications or to apply an alternative strategy. However, some healthcare providers may prefer to discuss with their patients and seek alternative strategies and resolve the problem without the use of PRN medications (Usher et al., 2009). On the other hand, some healthcare providers may use PRN medications to facilitate patients' adjustment to the requirement of the work environment during hospitalization (Walsh et al., 2020).

3.4.7 Participation of Patients and Families

Positive attitudes of the patient and his/her family members toward PRN medications influence PRN medicines management (Edwards et al., 2001). The patient's preference and compliance with PRN medication use influence healthcare provider's decisions on the administration of PRN medications (Di Giulio and Crow, 1997; Walsh et al., 2020). The patients' involvement is a substantial aspect of the decision process for PRN medication use (Nilsen et al., 2020). If the patient can reliably express his/her symptoms and request for medications, it is easiest for healthcare providers to decide about PRN medication use (Carder, 2012; Akram et al., 2014). However, patients' self-request for PRN medications may not be completely to their best interest, specifically for medications that increase the risk of dependence and abuse (Akram et al., 2014). Wherever possible, joint decision-making about the prescription of PRN medications is recommended on translating/agreeing the rational/indication for the prescription into the language of/with the patient (Baker et al., 2007a).

The patients' knowledge regarding their medications also is important. For instance, when healthcare providers improve their patients' knowledge of the side effects of a particular PRN medication, it is more likely that the patient accepts non-administration of medications (Nilsen et al., 2020). The patient's willingness can influence the replacement of medications with alternative and non-pharmacological interventions (Usher et al., 2009).

Communication and cognitive abilities of the patient to assess the necessity of PRN medication use also have been emphasized. For example, the patient's wellness informs healthcare providers of the patient's ability to convey the situation that raises the need for the administration of PRN medications (Nilsen et al., 2020). The patient's ability to cooperate may be influenced by the administration of some PRN medications such as analgesics (Di Giulio and Crow, 1997). Conflicting understanding between healthcare providers and family members regarding the patient's need for PRN medications should be resolved through reaching agreement by all parties involved in patient care (Nilsen et al., 2020).

When the patient looks unwell and for instance expresses the signs of aggression, agitation, or elated mood, PRN medication use is more likely (Usher et al., 2009). A

direct association has been shown between the severity of symptoms and the dose of PRN medications (Griffiths et al., 2019).

For outpatients and those who have to manage PRN medications themselves, applying some instructions and strategies on PRN medication bottles under the name of the Take-Wait-Stop label design is beneficial. Deconstructing prescription wording about PRN instructions can explicitly convey the dose, interval between doses, and maximum daily dose to patients and their families. It consists of explicit, deconstructed instructions, and simplified texts such as numeric characters instead of words, e.g., "1 tab" instead of "one tab," and carriage returns to place each part of the instructions on separate lines. The use of simplified text and plain language, "Stop" to replace the typical wording "do not exceed," can inform patients with limited literacy levels about the maximum daily dose (McCarthy et al., 2013; McCarthy et al., 2019).

3.4.8 Multidisciplinary Collaboration

Collaboration by healthcare professionals is needed from the moment that PRN medications are prescribed. Nurses spent the most time with patients and have the central role for identifying the patient's need for PRN medications. Physician's and nurse's collaboration regarding PRN medication use has been emphasized (Jimu and Doyle, 2019). Interprofessional medication review with the collaboration of pharmacist on the patient's medication list facilitates updating medications and changing and removing unused ones. It also creates consensus on PRN medication use (Nilsen et al., 2020). Also, involvement of the multidisciplinary team in the management of patients' behaviors using alternative methods reduces the need for PRN medication use (Barr et al., 2018).

4 DISCUSSION

In this systematic review with an integrative approach, the practical considerations of PRN medicines management were suggested. They can help with the improvement of quality and safety of the PRN medication process. Our review findings showed the need for appropriate assessment and planning for safe PRN medication use and inclusion of strategies for the improvement of multidisciplinary collaboration, monitoring of medications' effects and side effects, deprescription, use of alternative therapies, and involvement of patients and families in medication therapy.

Healthcare professionals' collaboration for making decisions on the prescription and administration of PRN medications is important. For instance, double-checking by at least two healthcare providers can prevent medication errors (Koyama et al., 2020; Vaismoradi et al., 2020). However, the role of electronic and digital solutions for improving the safety of PRN medicines management has remained unattended. Electronic prescribing and administrating of medications have the potential for reducing the risk of medication errors and adverse drug events (Ammenwerth et al., 2008; Slight et al., 2019). A systematic review and meta-analysis reported a considerable (50%) reduction in preventable adverse drug events when electronic prescribing systems in acute care settings were used in healthcare settings (Nuckols et al., 2014). It can ensure the safety of PRN prescribing through the provision of important capabilities such as decision support, specification of indications for the PRN medication use and the maximum daily dose, provision of appropriate alert, and communication between prescribers and administrators (Donyai et al., 2008; Baysari et al., 2012; Martin et al., 2017).

Since the accurate documentation of patient information is one of the primary competences of healthcare providers and facilities the monitoring of PRN medications, structured report templates regardless of the method of documentation can improve PRN medication documentation (Hammer et al., 2019). The electronic health record with the inclusion of information about effectiveness, side effects, and matching between the indication of PRN prescription and administration contributes to the high quality documentation process (Martin et al., 2017).

In addition, the significance of assessment tools regarding the effectiveness of PRN medication use was not acknowledged in the included studies to this review. In an instrument development study, Silk et al. (2013) suggested that the provision of an accurate evaluation of the effectiveness of PRN medications as a result of decreased subjective and ambiguous language improved the patient outcomes (Silk et al., 2013). The prevention of polypharmacy along with PRN medication use requires appropriate screening tools. Although such a specific tool is not available yet, the STOPP (screening tool of older persons' potentially inappropriate prescriptions) and START (screening tool to alert doctors to the right treatment) tools can be used to review medications for vulnerable people and identify potentially inappropriate medications (O'Mahony et al., 2015; Vaismoradi et al., 2020).

Our review findings highlighted the deprescription of PRN medications and its replacement with non-pharmacologic methods to prevent polypharmacy and medication abuse. The plan for deprescribing process of PRN medications should be devised based on each patient's need and under close monitoring (Renn et al., 2018; Vaismoradi et al., 2021a). Also, cost and benefit assessment with regard to the continuation and discontinuation of medications should be performed (Renn et al., 2018). Moreover, the concerns of patients and their informal caregivers about the replacement of medications by alternative therapies that can influence their collaboration with the deprescription plan should be taken seriously (Scott et al., 2015; Vaismoradi et al., 2021a). Given that the use of PRN medications reduce the inclusion of other therapeutic interventions in the therapeutic plan (Hipp et al., 2018), PRN medications should not be used when potential non-pharmacological treatment options are available (Martin et al., 2017).

According to our review findings, healthcare professionals' competencies for PRN medicines management influenced the safety of the medication process. Their pharmacological competence as having sufficient knowledge and skills to manage real-life medication circumstances and making appropriate

decisions (Sulosaari et al., 2011; Salehi et al., 2021) are affected by the complexity of the patient's medication processes (Sulosaari et al., 2011; Lichtner et al., 2016). Healthcare providers need education and training about the application of alternative and non-pharmacological interventions for relieving patients' symptoms (Molloy et al., 2012). They should be educated to avoid overreliance on PRN medications (Zeisel et al., 2016; Harper et al., 2017; Martin et al., 2018b).

Patient participation and shared decision-making was a pilar for safe PRN medicines management in our review. Patients play an active role in care planning and should have the opportunity to participate in decision making (Mikesell et al., 2016). Patients eagerly participate in decision-making if they receive sufficient knowledge about their medications, have appropriate understanding of PRN medications (Hipp et al., 2018), and are able to define PRN medications and the rational for their use (Morkunas et al., 2016). It also enhances their compliance to the medication regimen (Fernandez et al., 2006; Mardani et al., 2020). Therefore, the opportunity for asking about PRN medications and giving consent when PRN medications are offered should be given to patients (Hipp et al., 2018; Vaismoradi et al., 2021b).

4.1 Strengths and Limitations

This systematic review using international databases can improve our understanding of practical considerations that should be applied by healthcare professionals for safe PRN medicines management. We identified relevant literature with qualitative and quantitative research designs by applying multidimensional keywords for a systematic search on international databases. Therefore, our findings provide an extensive overview of the present international knowledge regarding this important clinical topic. However, our review was limited to studies published in the English language due to restriction in translation. Future studies need to consider grey literature and other sources of literature including local guidelines used in clinical settings and in other languages to improve the generatability of our review findings. Also, the majority of retrieved studies in the present systematic review was from Australia, the United States, Canada, and European countries. A limited number of studies from Asia and Africa on PRN medicines management was retrieved. Therefore, PRN medicines management should be addressed in other research contexts to improve our understanding of cultural aspects affecting medication safety.

5 CONCLUSION

The current review sought to summarize and integrate practical considerations by healthcare professionals for PRN medicines management in different healthcare settings. The findings of this review demonstrate that PRN medicines management is a complex process and many factors influence its safety. We identified a range of possible practical measures that should be taken for improving the safety of PRN medication therapy.

The synthesised knowledge in our review can be used to develop optimal PRN medicines management guidelines in different clinical settings and to investigate its effect on safe care indicators. A suggested list of practical considerations for PRN medicines management has been developed based on our review findings and has been presented in **Table 4**. After making it suitable for application in clinical practice, they can be used to guide healthcare professionals in PRN medicines management situations. Along with other medication safety measures, the suggested implications can support healthcare practitioners' decision making for improving the quality and safety of PRN medication use.

It should be acknowledged that alternative interventions such as non-pharmacologic strategies in appropriate caring occasions have priority over PRN medication use due to fewer side effects. Therefore, healthcare providers should improve their competencies to avoid overreliance on PRN medication use for relieving patients' symptoms.

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

MV: Conceptualization. AM and MV: Data curation, Formal analysis, Investigation, Methodology; Project administration, Resources, and Software. AM, MV, PP, CW, and SJ: Writing—original draft, Writing-review and editing. All authors have read and agreed to the published version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.759998/full#supplementary-material

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