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Direct or indirect endocrine and metabolic consequences of malignancies

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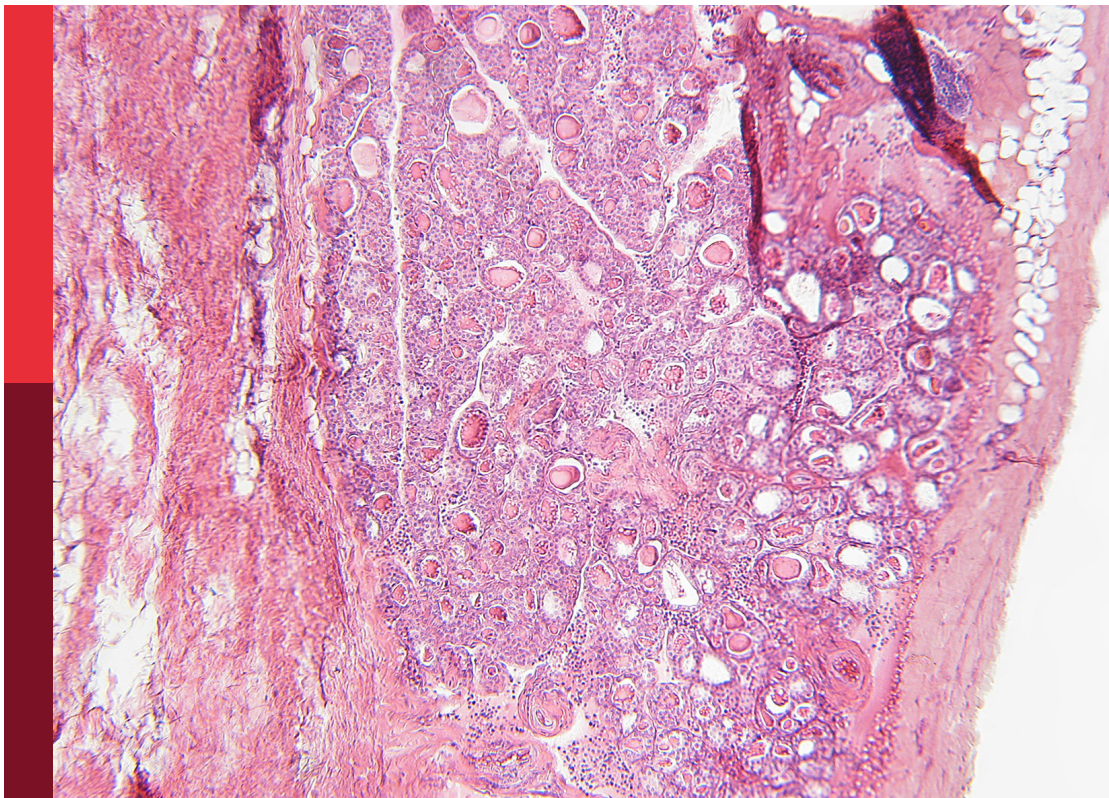

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Direct or indirect endocrine and metabolic consequences of malignancies

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Editorial: Direct or indirect endocrine and metabolic consequences of malignancies

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Editorial on the Research Topic
Direct or indirect endocrine and metabolic consequences of malignancies

Malignancies of endocrine organs, such as the pituitary, thyroid, parathyroid, and adrenal glands, significantly affect the metabolism of patients, leading to poor prognosis and high mortality; In addition, malignancies of other organs (such as the pancreas and lung) also cause endocrine or metabolic syndromes, such as Cushing's syndrome, hypoglycemia, hypercalcemia, male mammogenesis, which are called para-neoplastic syndrome. The above mentioned situations are named direct endocrine consequences of malignancies (Figure 1). Moreover, the commonly used chemo-, targeted- and immune therapeutic agents (especially immunocheckpoint inhibitors) also have non-negligible impacts on endocrine homeostasis, sometimes life-threatening, through various potential mechanisms, which is named indirect endocrine consequences of malignancies.

Among the 9 publications on this Research Topic, one article is a case report, one is a mini-review, one is a systematic review, and six are original research articles. Among them, two describe the direct impact of malignant tumors on endocrine and metabolism, and four describe the impact of immunotherapy for malignant tumors on endocrine and metabolism. Regarding the direct impact of malignant tumors on endocrine function, Li et al. summarized the pathogenesis and differential diagnosis of paraneoplastic Cushing's syndrome(PCS) caused by small cell lung cancer (SCLC) and proposed a differential method for identifying PCS through immunohistochemical (IHC) staining. SCLC can directly secrete adrenocorticotropic hormone (ACTH) and corticotropin-releasing hormone (CRH). Abnormal secretion of ACTH and CRH can lead to PCS, which is reported to account for 1.6%-6% of all SCLC cases but has a worse prognosis among all SCLC patients. Regarding the direct impact of malignant tumors on metabolism, Xie et al. proposed that serum lipoprotein A-I levels are associated with the prognosis of patients with colorectal cancer(CRC).The article discusses that serum apolipoprotein A-I levels decrease in CRC patients, and patients with lower serum apolipoprotein A-I levels have lower progression-free survival and overall survival than those with higher levels. In addition, treatment of malignant tumors also affects patient metabolism, Zhu et al. mentioned that the incidence

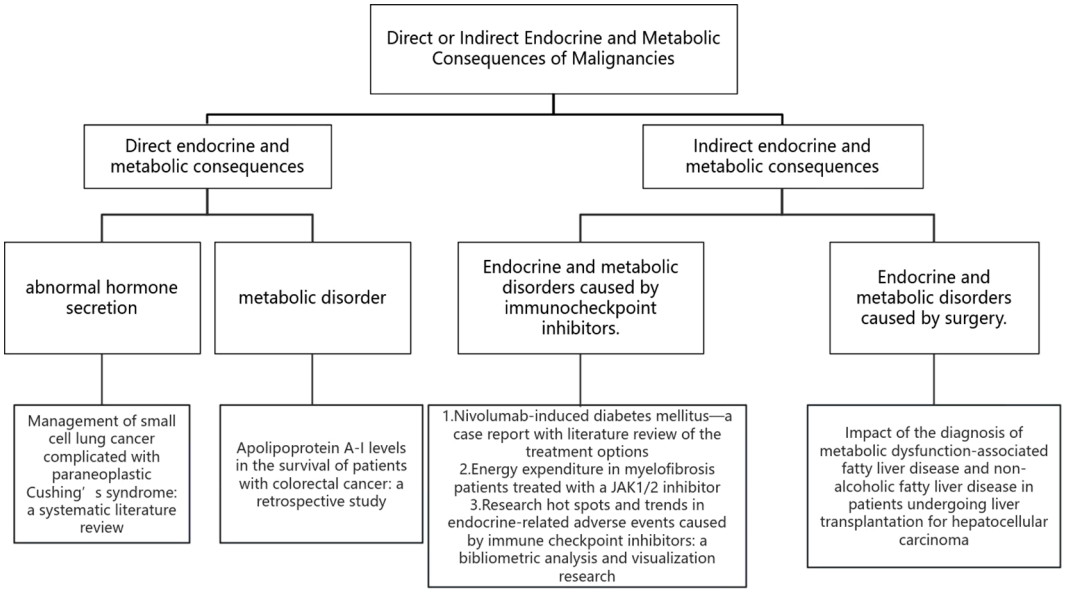

FIGURE 1
Direct or indirect endocrine and metabolic consequences of malignancies.

of metabolic dysfunction-related fatty liver disease(MAFLD) and non-alcoholic fatty liver disease (NAFLD) increases in patients with hepatocellular carcinoma(HCC) after liver transplantation surgery. The diagnosis of MAFLD is more strongly associated with metabolic abnormalities than the diagnosis of NAFLD. Regarding the impact of immunotherapy for malignant tumors on metabolism, Tremblay et al. elucidated that weight gain in patients with myelofibrosis treated with the JAK1/2 inhibitor ruxolitinib may be related to changes in systemic energy expenditure. The JAK1/2 inhibitor ruxolitinib has been found to affect patient metabolism during the treatment of myelofibrosis, resulting in weight gain. This weight gain is not only related to changes in appetite due to impaired hypothalamic JAK/STAT signaling but also to changes in overall energy expenditure. As for the impact of immunotherapy for malignant tumors on endocrine function, Daetwyler et al. reported a case of diabetes induced by nivolumab monotherapy and suggested the use of insulin therapy as early as possible in the treatment of diabetes induced by immune checkpoint inhibitors, because treatment with infliximab failed to improve β-cell function, but insulin treatment was effective. Additionally, immune checkpoint inhibitors can produce various endocrine toxicities during treatment, Zhao et al. summarized the research hotspots and trends of endocrine-related adverse events caused by immune checkpoint inhibitors in recent years, identify the current research hotspots include the management of endocrine-related adverse events, hypophysitis, thyroid dysfunction, type I diabetes mellitus, and the impact of endocrine adverse events on survival of patients in this field. In addition, Dong et al. described the relationship between thyroid dysfunction and the risk of cutaneous malignant melanoma(CMM), and indicated that hypothyroidism might be a protective factor for CMM. Lai et al. described a causal relationship between hypothyroidism and rheumatoid arthritis(RA), while no causal relationship was found between hyperthyroidism and RA. Wang et al. described the relationship between hypothyroidism and endometrial cancer(EC) and indicated through MR analysis that there is a lack of causal relationship between hypothyroidism and EC.

From these nine articles, we can gain insight into the impact of malignant tumors on the body's metabolism and endocrine function, which can predict patient prognosis. For instance, the article mentions that serum apolipoprotein A-I in CRC patients can be used as an effective biomarker for patient prognosis. In SCLC, PCS can be used as one of the prognostic indicators for patients. Similarly, MAFLD in HCC liver transplant patients can be used as an independent predictor of a high risk of HCC recurrence. For early identification of endocrine disorders in malignant tumors, IHC staining can be used as an effective diagnostic tool for distinguishing between PCS and Cushing's disease. It is recommended that in the future, the application of IHC staining for unique hormones (ACTH or CRH) should be strengthened in clinical practice for early differential diagnosis of PCS. In addition, the treatment of diabetes caused by immune checkpoint inhibitors may differ from other immune-related adverse events. While other immune adverse events are typically treated with immunosuppressants, this approach is often ineffective in diabetes, and early use of insulin is the key to treatment. Furthermore, it should be noted that most endocrine adverse reactions caused by immunotherapy are due to the involvement of a single gland. However, with the gradual increase in the clinical use of immune checkpoint inhibitors, reports of involvement of two or more glands are becoming more common. According to studies, the combination of thyroid and pancreatic injury is the most common multi-gland injury. Patients receiving immunocheckpoint inhibitor therapy must continue to monitor these endocrine-related events. It is also crucial to actively seek effective biomarkers in clinical practice to predict the risk of endocrine adverse reactions, which will help early detection and management of patients' immune adverse reactions.

Author contributions

CW: Writing – original draft, Writing – review & editing. OR: Writing – review & editing. JW: Writing – review & editing.

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Energy expenditure in myelofibrosis patients treated with a JAK1/2 inhibitor

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Weight gain is a known adverse effect of ruxolitinib, a JAK1/2 inhibitor that is the mainstay of treatment for many patients with myelofibrosis. The mechanisms behind weight increase with ruxolitinib is incompletely understood, although decreased adipose tissue lipolysis and increased appetite due to blocking the effects of leptin in the hypothalamus have been proposed. In order to explore the metabolic changes in ruxolitinib-treated patients with myelofibrosis, we performed a pilot study to assess the feasibility of using a portable indirect calorimeter to quantify energy expenditure before and during ruxolitinib treatment and report the results of two patients. Waist circumference increased during ruxolitinib treatment in both patients. Energy expenditure initially increased followed by a decrease and then increase again, but to levels below baseline. These results suggest that weight gain secondary to ruxolitinib may be related to changes in whole body energy expenditure.

KEYWORDS

ruxolitinib, energy expenditure, weight gain, intolerance, leptin

Introduction

Myelofibrosis (MF) is a hematologic malignancy clinically characterized by abnormal cytokine production, splenomegaly, and constitutional symptoms that include weight loss (1). Ruxolitinib (Jakafi, Incyte) is a JAK1/2 inhibitor and was the first Food and Drug Administration approved therapy for MF, based on the results of the COMFORT-I and -II studies that demonstrated efficacy in terms of spleen reduction and symptoms improvement (2, 3). Interestingly, ruxolitinib is associated with statistically and clinically significant weight gain. In the pivotal, phase 3 COMFORT-I and COMFORT-II trials, patients treated with ruxolitinib gained an average of 3.9 kg after 24 weeks (COMFORT-I) and 4.4 kg after 48 weeks (COMFORT-II) of ruxolitinib therapy (2–4). Weight gain occurs regardless of pretreatment body mass index (BMI), suggesting weight gain is not solely

attributable to resolution of MF symptoms (e.g., early satiety, abdominal discomfort) in cachectic patients (5).

We have previously shown in a retrospective analysis of 179 patients with MPNs treated with ruxolitinib that 69% of patients experienced weight gain during treatment and the average gain for those patients was 12% of pretreatment body weight, with over 50% of patients gaining > 5% of their pretreatment body weight (5). There is uncertainty as to the pathogenesis of ruxolitinib-mediated weight gain. In a murine model, ruxolitinib reduced the normal JAK2/STAT3 phosphorylation in neuronal cells in mouse hypothalamus that occurs in response to feeding or to administration of exogenous leptin, which typically signals satiety, suggesting that increased appetite may be responsible (5). However, we have also demonstrated that ruxolitinib inhibits JAK/STAT signaling in the adipose tissue, which is an important regulator of adipose tissue lipolysis (6, 7).

To gain further insight into the metabolic changes that occur during ruxolitinib therapy, we performed a pilot and feasibility study of patients with MF using a portable indirect calorimeter to determine energy expenditure before and during treatment with ruxolitinib. Here, we describe two MF patients that highlight metabolic changes with this JAK1/2 inhibitor.

Methods

MF patients without prior JAK inhibitor exposure who were intending to start ruxolitinib as standard of care therapy were prospectively enrolled. Patients with chronic obstructive pulmonary disease, untreated endocrinopathy aside from diabetes, heart failure, or additional malignancies were excluded. Enrolled patients had resting energy expenditure measured by indirect calorimetry at screening and an additional baseline visit. Patients then had repeat measurements performed 2, 4, 8, 16, and 24 weeks after starting ruxolitinib. Weight, height, and waist circumference were recorded at each study visit, in addition to standard of care laboratory evaluation. A portable indirect calorimeter was kindly provided by PNOE (Palo Alto, California) (8). This device includes a wearable facemask, where the subject breathes through the Micro-Electro-Mechanical Systems based hot film anemometer flow sensor that operates on a breath-by-breath mode that continuously measures volume and determines expired gas concentrations simultaneously. Heart rate was measured using a heart rate monitor (Polar Electro, Lake Success, NY). Respiratory and cardiac measurements were transmitted *via* telemetry. Patients were required to fast for at least 8 hours prior to the testing, and not to exercise or use nicotine containing products for at least 4 hours prior to testing. They were seated at rest for at least 10 minutes before measurements were taken.

The Myeloproliferative Neoplasms Symptom Assessment Form Total Symptom Score (MPN-SAF-TSS) was calculated at each visit (9). The MPN-SAF-TSS includes ten symptoms: fatigue; early satiety; abdominal discomfort; inactivity; impaired concentration; night sweats; pruritis; bone pain; fever; unintentional weight loss over preceding 6 months. Each symptom is rated on a scale of 0-10, with 0 being absence of symptoms, and 10 being the worst imaginable symptoms. The MPN-SAF-TSS has a range from 0-100, with 100 being the most severe symptom severity.

All subjects signed a written informed consent prior to their participation in the study. The study was approved by the Program for the Protection of Human Subjects Institutional Review Board at the Icahn School of Medicine at Mount Sinai. All procedures were in accordance with the Declaration of Helsinki.

Results

Baseline characteristics

We report observations from two patients enrolled in this study. Additional patient enrollment was ceased because of the COVID-19 pandemic. Baseline metabolic characteristics are shown in Table 1. While Patient 1 was normal weight with a BMI of 20.5 kg/m^2, Patient 2 had Class III obesity (BMI 40.6 kg/m^2) and a waist circumference of 122 cm. Both patients were newly diagnosed and treatment naive. Patient 1 had baseline leukocytosis with a white blood cell (WBC) count of 94.1 x 10^9/L, hemoglobin of 8.9 g/dL and platelet count of 172 x 10^9/L while Patient 2 had a WBC of 9.8 g/dL, hemoglobin of 7.3 g/dL and a platelet count of 389 x 10^9/L. The Dynamic International Prognosis Scoring System (DIPSS) risk category was high and intermediate-2 for Patient 1 and Patient 2, respectively. Both patients were symptomatic with an MPN-SAF-TSS) of 31 and 49, respectively. Patient 1 had a baseline palpable spleen length of 15cm below the left costal margin while Patient 2 did not have palpable splenomegaly. Both patients were initiated ruxolitinib at 5mg twice daily, a dose selected considering baseline disease-related anemia.

Changes in metabolic parameters during treatment

The body weight of Patient 1 increased from 75.6kg to 83.9kg at 24 weeks, a gain of 8.3kg. Patient 2 experienced mild weight loss, with a change from110.8kg to 109.3kg at 24 weeks, a loss of 1.5kg. However, waist circumference increased in both patients: Patient 1 increased from 99.5cm at baseline to 104cm at 24 weeks, and Patient 2 increased from 122cm to 128cm at 24 weeks.

Indirect calorimetry readings displayed interesting dynamics in both patients. The resting energy expenditure for Patient 1 initially increased from a baseline of 3158 kcal/day to 3355 kcal/day at week 2 and then dropped to 2303 kcal/day at week 4 before rebounding to 2686 kcal/day at week 8 and then plateaued. Patient 2 had a baseline resting energy expenditure of 1870 kcal/day which increased to 2530 kcal/day on week 4 before decreasing to 1420 kcal/day on week 8 before ending at 1983 kcal/day at week 24.

Figures 1A, B show the energy expenditure over time plotted with changes in waist circumference. In both cases, there was an initial increase in resting energy expenditure followed by a decrease followed by an increase before returning to levels below baseline.

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TABLE 1 Baseline patient characteristics.

	Patient 1	Patient 2
Age/sex	67 male	40 female
Race/Ethnicity	Black	Hispanic
Comorbidities	Chronic photosensitive dermatitis	Coronary artery disease, hypothyroidism (controlled)
Diagnosis	Primary myelofibrosis	Post-essential thrombocythemia myelofibrosis
DIPSS risk category	High risk	Intermediate-2
Driver mutational status	Triple negative	Triple negative
Palpable spleen length below left costal margin (cm)	15	0
Weight (kg)	75.4	110.6
BMI (kg/m^2)	20.5	40.6
Waist circumference (cm)	99.5	122
Albumin (g/dL)	3.3	3.5
LDL (mg/dL)	50	70
HDL (mg/dL)	11	22
Triglycerides (mg/dL)	85	171
LDH (units/dL)	810	473

Changes in hemoglobin levels over time somewhat mirrored energy expenditure with initial decrease followed by an increase (Supplemental Figure 1).

Consistent with prior reports, albumin increased in both patients, with Patient 1 increasing from 3.3 g/dL to 3.9 g/dL at week 24 and increasing from 3.6 g/dL to 3.7 g/dL at week 24. Triglyceride levels also increased from a baseline of 85 mg/dL to 143 mg/dL and 171 mg/dL to 259 mg/dL for Patient 1 and 2, respectively. Patient reported outcome measure of early satiety during the treatment course steadily decreased from 8 out of 10 at baseline to 5 at week 24 in Patient 1, and from 8 to 7 in Patient 2.

Discussion

Our results suggest that weight gain due to ruxolitinib may not only be mediated by changes in appetite due to impaired JAK/STAT signaling in the hypothalamus, but may also related to changes in whole body energy expenditure.

In this pilot study, we used a portable indirect calorimeter, which allowed us to bring the device to the patients, facilitating point of care testing in the oncology rather than requiring separate visits to a metabolic testing location. Having the ability to perform indirect calorimetry in the oncology center could allow us to perform larger future studies to develop a greater understanding of the links between systemic metabolism and cancer.

Leptin receptors are found not only in the hypothalamus, but are expressed in other parts of the brain, and also in other tissues including the liver, spleen, kidneys, and adipose tissue. Leptin signaling in the brain and in adipose tissue has been found to increase energy expenditure in pre-clinical models (10). Interestingly, in pre-clinical models with intact leptin signaling in the hypothalamus, but adipose tissue leptin receptor deficiency, mice gained weight despite normal food intake, and developed insulin resistance and hypertriglyceridemia (11). Growth hormone binding to the growth hormone receptor also activates the JAK/STAT pathway. Interestingly, both chronic growth hormone excess and deficiency have been associated with increased energy expenditure (12). People with growth hormone receptor deficiency have higher percent body fat; however, in contrast to the leptin deficient models, do not develop insulin resistance or hypertriglyceridemia. Therefore, the effects of ruxolitinib on energy expenditure and triglycerides may be mediated through the leptin receptor. Interestingly, none of the previous studies have found biologically significant hyperglycemia with ruxolitinib despite the weight gain. This lack of hyperglycemia could be related to inhibition of the antagonistic effects of growth hormone on insulin signaling.

Ruxolitinib treatment has also been shown to increase muscle mass in MF patients (13). It is possible that this increase in muscle mass may be responsible for return of energy expenditure to near baseline as skeletal muscle is a significant driver of resting energy expenditure (14). Unfortunately, abdominal imaging was not obtained in our study and thus we are unable to explore this possibility.

Our study has several limitations that limit generalizability, including the fact that both patients were negative for MPN driver mutations, a rare subset of MF patients. In addition, the limited sample size and observational nature of our study did not allow us to evaluate the impact of ruxolitinib dosing on metabolic parameters. Overall, this pilot study shows the potential value of

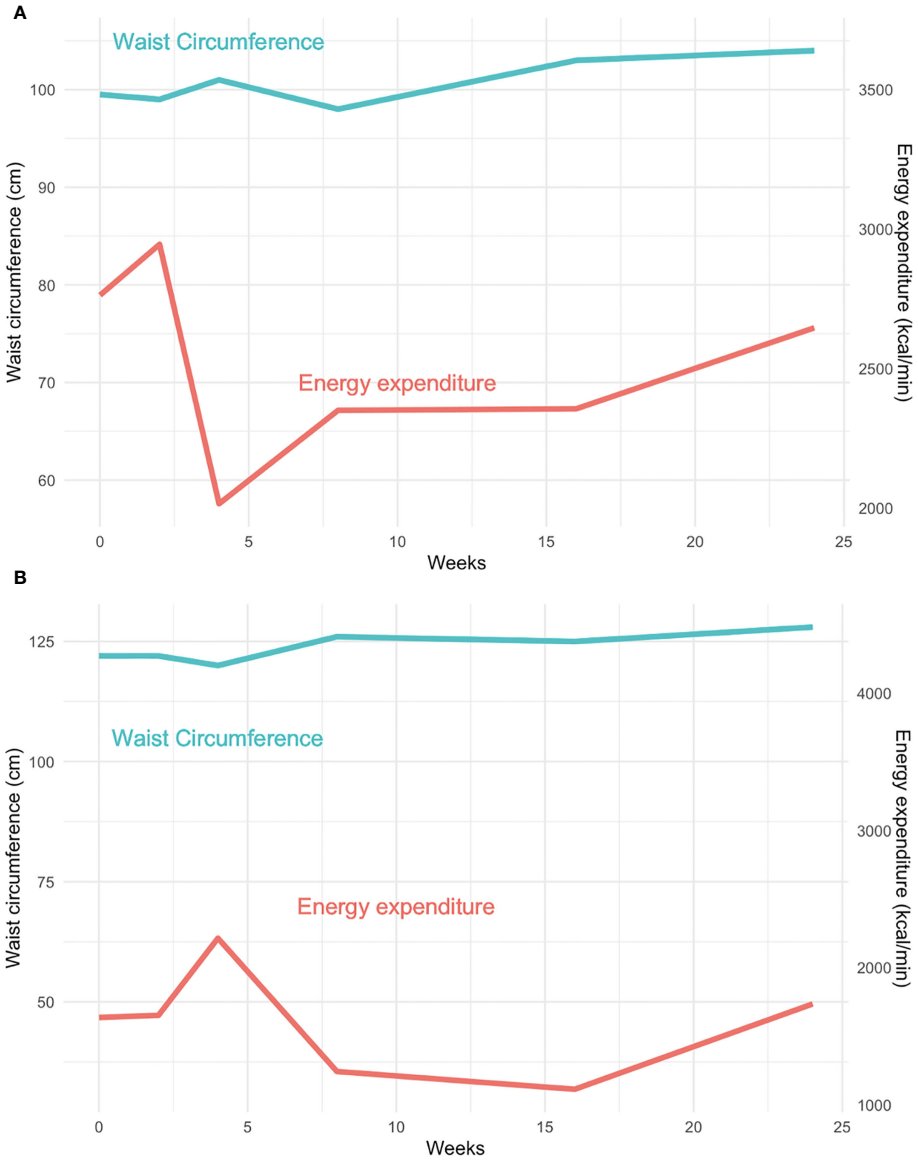

FIGURE 1
Changes in energy expenditure and waist circumferences over time. For patient 1 **(A)** and 2 **(B)**, energy expenditure initially increased after initiation of ruxolitinib then decreased which was followed by an increase in waist circumference.

portable indirect calorimeters for measuring energy expenditure in individuals with malignancies, and suggests that the changes in energy expenditure may contribute in part to the weight gain associated with ruxolitinib.

Author contributions

DT, JM and EG designed the study, enrolled the patients, performed the indirect calorimetry and wrote the manuscript. MD performed regulatory functions and assisted with enrollment and design of the study. All authors contributed to the article and approved the submitted version.

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Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1141029/full#supplementary-material

References

1. Mascarenhas J, Gleitz HFE, Chifotides HT, Harrison CN, Verstovsek S, Vannucchi AM, et al. Biological drivers of clinical phenotype in myelofibrosis. *Leukemia* (2022). doi: 10.1038/s41375-022-01767-y

2. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, Dipersio JF, et al. Placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med* (2012) 366:799–807. doi: 10.1056/NEJMoa1110557

3. Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med* (2012) 366:787–98. doi: 10.1056/NEJMoa1110556

4. Mesa RA, Verstovsek S, Gupta V, Mascarenhas JO, Atallah E, Burn T, et al. Effects of ruxolitinib treatment on metabolic and nutritional parameters in patients with myelofibrosis from COMFORT-I. *Clin Lymphoma Myeloma Leuk* (2015) 15:214–221.e211. doi: 10.1016/j.clml.2014.12.008

5. Molle N, Krichevsky S, Kermani P, Silver RT, Ritchie E, Scandura JM. Ruxolitinib can cause weight gain by blocking leptin signaling in the brain *via* JAK2/STAT3. *Blood* (2020) 135:1062–6. doi: 10.1182/blood.2019003050

6. Sapre M, Tremblay D, Wilck E, James A, Leiter A, Coltoff A, et al. Metabolic effects of JAK1/2 inhibition in patients with myeloproliferative neoplasms. *Sci Rep* (2019) 9:16609. doi: 10.1038/s41598-019-53056-x

7. Xu D, Yin C, Wang S, Xiao Y. JAK-STAT in lipid metabolism of adipocytes. *JAKSTAT* (2013) 2:e27203. doi: 10.4161/jkst.27203

8. Tsekouras YE, Tambalis KD, Sarras SE, Antoniou AK, Kokkinos P, Sidossis LS. Validity and reliability of the new portable metabolic analyzer PNOE. *Front Sports Act Living* (2019) 1:24. doi: 10.3389/fspor.2019.00024

9. Emanuel RM, Dueck AC, Geyer HL, Kiladjian JJ, Slot S, Zweegman S, et al. Myeloproliferative neoplasm (MPN) symptom assessment form total symptom score: prospective international assessment of an abbreviated symptom burden scoring system among patients with MPNs. *J Clin Oncol* (2012) 30:4098–103. doi: 10.1200/JCO.2012.42.3863

10. Harris RB. Direct and indirect effects of leptin on adipocyte metabolism. *Biochim Biophys Acta* (2014) 1842:414–23. doi: 10.1016/j.bbadis.2013.05.009

11. Huan JN, Li J, Han Y, Chen K, Wu N, Zhao AZ. Adipocyte-selective reduction of the leptin receptors induced by antisense RNA leads to increased adiposity, dyslipidemia, and insulin resistance. *J Biol Chem* (2003) 278:45638–50. doi: 10.1074/jbc.M304165200

12. Salomon F, Cuneo RC, Hesp R, Morris JF, Poston L, Sonksen PH. Basal metabolic rate in adults with growth hormone deficiency and in patients with acromegaly: relationship with lean body mass, plasma insulin level and leucocyte sodium pump activity. *Clin Sci (Lond)* (1992) 83:325–30. doi: 10.1042/cs0830325

13. Lucijanic M, Galusic D, Soric E, Sedinic M, Cubela M, Huzjan Korunic R, et al. Ruxolitinib treatment improves muscle mass in patients with myelofibrosis. *Ann Hematol* (2021) 100:1105–6. doi: 10.1007/s00277-020-04243-8

14. Zurlo F, Larson K, Bogardus C, Ravussin E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J Clin Invest* (1990) 86:1423–7. doi: 10.1172/JCI114857

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Management of small cell lung cancer complicated with paraneoplastic Cushing's syndrome: a systematic literature review

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Paraneoplastic Cushing's syndrome (PCS) is a rare, but clinically important feature of small cell lung cancer (SCLC) that is associated with even worse prognosis. To identify key considerations in comprehensive management of SCLC patients complicated with PCS, we conducted a systematic review of relevant reports on PubMed and Web of Science, focusing on SCLC with PCS cases. The systematic review analyzed 61 reports published between 1985 and 2022 with a total of 157 SCLC patients included. Out of the 157 patients, 132 (84.1%) patients across 58 (95.1%) reports were diagnosed with ectopic Cushing's syndrome. The immunohistochemical (IHC) staining for adrenocorticotropic hormone (ACTH) was performed on 30 (19.1%) patients across 22 (36.1%) reports and demonstrated encouraging performance. For treatment, chemotherapy and ketoconazole were utilized in 50 (81.97%) and 24 (39.34%) reports, respectively. Regarding cause of death, infection and cancer were equally frequent, each being recorded in 17 (27.87%) reports. To conclude, the majority of PCS cases in SCLC patients were caused by ectopic hormone secretion. In order to make a differential diagnosis, it is recommended to utilize IHC staining for a specific hormone such as ACTH or corticotropin-releasing hormone. In the comprehensive treatment of SCLC with PCS patients, effective management of hypercortisolism and potent safeguarding against infection play two crucial roles. Ultimately, further confirmations are required regarding the specificity and accuracy of IHC staining technique as well as the efficacy and safety of immunotherapy in the treatment of SCLC with PCS patients.

KEYWORDS

small cell lung cancer, paraneoplastic Cushing's syndrome, neuroendocrine tumor, management, immunohistochemistry, infection

1 Introduction

Small cell lung cancer (SCLC), a highly aggressive subtype of lung cancer, is characterized by rapid proliferation, high growth fraction, and early development of metastases. It accounts for approximately 14% of all lung cancer cases and possesses a particularly poor prognosis (1, 2). Likewise, ectopic Cushing's syndrome (ECS), hypercortisolism due to ectopic hormone secretion, is estimated to account for 5%–10% of all Cushing's syndrome (CS) cases (3–5). Furthermore, the SCLC patients complicated with paraneoplastic Cushing's syndrome (PCS), mostly caused by ectopic hormone secretion from tumor tissues, comprise a smaller proportion [reported as 1.6%–6% of all SCLC cases (6–9)] but possess an even poorer prognosis among all SCLC patients (6, 8). Retrospective studies have shown that median survivals of SCLC patients with PCS were less than 7 months (6–11).

Regarding the management of SCLC with PCS patients, although some effective hypercortisolism controlling methods exist (3, 12, 13), there was little advancement in treating SCLC for over three decades before the advent of immune checkpoint inhibitors (ICIs) modestly improved its overall survival (14–16). However, several reports have emerged on immunotherapy-induced CS, drawing much attention to the adverse effect (17–20). Considering PCS being a poor prognosis marker for SCLC patients, early and further differential diagnosis of CS is relevant for evaluating prognosis of SCLC patients.

Although there have been case reports, case series, and retrospective studies on SCLC complicated with PCS, no systematic review has been carried out on this topic before. Accordingly, we conducted one, incorporating relevant reports of SCLC with PCS available on PubMed and Web of Science. Through this review, we aimed to illustrate the treatment status of this disease previously and to identify key considerations for comprehensive treatment of these patients.

2 Methods

The systematic review has been conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement (21).

2.1 Literature search and selection

We performed a systematic review of relevant reports of SCLC complicated with PCS on PubMed and Web of Science. The search queries are "*(cushing[Title/Abstract]) AND ((sclc[Title/Abstract]) OR (small cell lung cancer[Title/Abstract]) OR (small cell lung carcinoma[Title/Abstract])) NOT ((nsclc[Title/Abstract]) OR (non small cell lung cancer[Title/Abstract]) OR (non small cell lung carcinoma[Title/Abstract]))*" on PubMed and "*(TS=cushing) AND ((TS=sclc) OR (TS=small cell lung cancer) OR (TS=small cell lung carcinoma)) NOT ((TS=nsclc) OR (TS=non small cell lung cancer) OR (TS=non small cell lung carcinoma))*" on Web of Science, respectively. The literature retrieval was performed on 19 November 2022, without restriction on publication date or language.

Selection criteria were as follows: (1) clinical case or case series, prospective or retrospective study, systematic review, or meta-analysis; (2) special reports on this topic or relevant articles involving management of SCLC with PCS patients; (3) critical data available, at least the information on clinical presentation, therapeutic strategy, or causes of death; and (4) no preference for publication date or language. The study was supplemented by screening references of selected articles.

Selection procedures are presented in Figure 1. The evidence quality of included reports has been evaluated using the critical appraisal tools provided by the Joanna Briggs Institute (JBI).

2.2 Data extraction and analysis

The following information were collected from eligible articles: age, gender, clinical presentation, the immunohistochemical (IHC) staining for adrenocorticotropic hormone (ACTH), therapeutic strategy, survival time/follow-up time, and cause of death. For retrospective and prospective studies included, the quantitative data on age, survival time, and follow-up time were represented as median (minimum–maximum), while the descriptive information on clinical presentation, therapeutic strategy, and cause of death were recorded as percentages wherever possible. As for clinical presentation, therapeutic strategy, and cause of death, we focused on specific details of critical significance for diagnosis and management of the disease. These specific details were categorized and their frequencies were presented.

3 Results

Throughout the entire selection process, 61 articles were retrieved, comprising 44 case reports (22–65), 7 case series (66–72), 9 retrospective studies (6–11, 73–75), and 1 prospective study (76) published between 1985 and 2022 in English, Japanese, French, German, Spanish, and Korean. A total of 157 SCLC with PCS patients were involved in these 61 articles. Table 1 presents details of included reports and patients while Table 2 displays frequencies of specific details on clinical presentation, therapeutic strategies, and cause of death. The evidence quality of all 61 included reports was evaluated using JBI critical appraisal checklists for case reports, case series, and cohort studies, and results are presented in Supplementary Materials.

All 157 patients were diagnosed with SCLC through histological methods. The one reported by Bodvarsson et al. was a patient with donor-derived SCLC (31). One patient (1/10) reported by Winquist et al. was mixed SCLC with non-small cell lung cancer (NSCLC) (10). The one reported by Qiang et al. was mixed SCLC with large cell neuroendocrine carcinoma (65). The one reported by Vadlamudi et al. had combined SCLC, lung adenocarcinoma, and giant cell carcinoma of the lung (40).

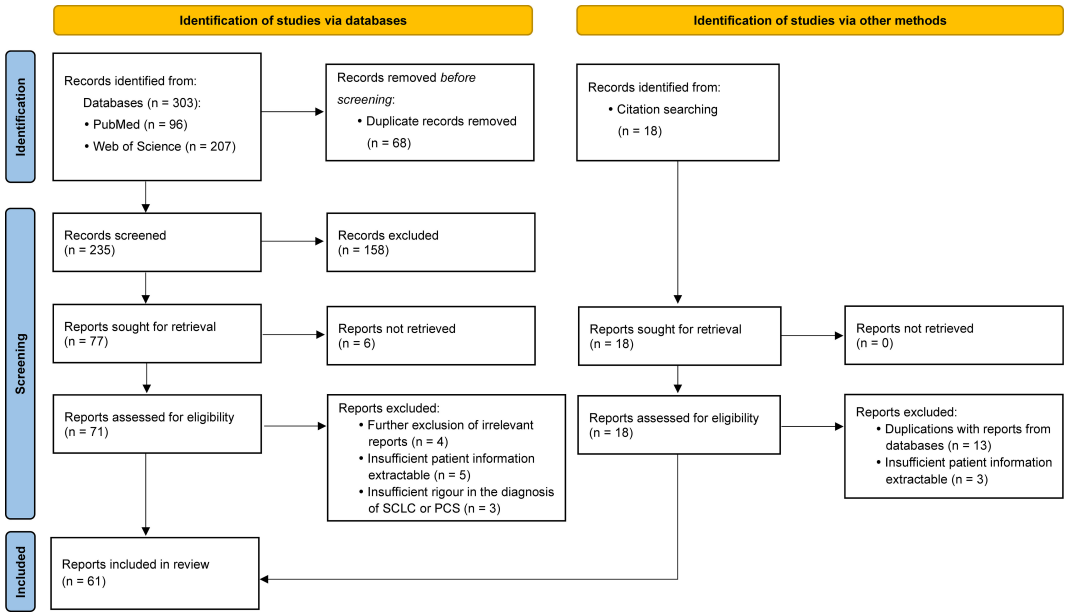

FIGURE 1
Flow diagram for publication selection procedures performed according to the PRISMA 2020 Statement.

For differential diagnosis of CS, 132 SCLC patients (84.1% of all 157 patients) in 58 reports (95.1% of all 61 reports) were diagnosed with ECS. In 9 (15.5%) reports, 40 (30.3%) patients were diagnosed without strict evidence from combining imaging examinations with laboratory tests or reported with no mention of specific procedures for the diagnosis of ectopic hormone secretion (8, 10, 22, 40, 45, 49, 57, 58, 72). The patient reported by Cabral et al. (63) and the 23 patients in the report by Nagy-Mignotte et al. (6) were merely diagnosed as PCS without further investigating the hormone origin. The patient reported by Tabata et al. (24) was diagnosed as PCS and his IHC result was negative for ACTH, while some laboratory tests revealed the opposite. In addition, the patient reported by Kosuda et al. was complicated not only by ECS but also by the syndrome of inappropriate antidiuretic hormone secretion (61). Moreover, IHC staining for ACTH was performed in 30 (19.1%) patients, 29 had ECS and 1 had PCS, across 22 (36.1%) reports. Out of these reports, four ECS patients (25, 35, 39, 57) and one PCS patient (24) showed negative results. It is noteworthy that the case reported by Auchus et al. stained negative for ACTH, but positive for corticotropin-releasing hormone (CRH), which confirmed that the patient's ECS was caused by ectopic CRH secretion rather than ACTH (25).

Regarding clinical presentation, hypokalemia was mentioned in 59 (96.72%) reports as the most frequently recorded clinical feature in PCS patients, followed by hypertension in 42 (68.85%) reports. For treatment, chemotherapy and ketoconazole were the first-line option used for SCLC patients with PCS, in 50 (81.97%) and 24 (39.34%) reports, respectively. As for cause of death, infection was recorded in 17 (27.87%) reports, equally to cancer. The remaining causes included respiratory complications in 16 (26.23%) reports, cardiovascular complications in 8 (13.11%), hormone issues in 7 (11.48%), and other causes (including general condition deterioration) in 15 (24.59%).

Regarding the survival of SCLC with PCS patients, five retrospective studies (6–9, 75) and one prospective study (76) indicated unfavorable results, with median survivals of less than 7 months. However, eight case reports with superior outcomes also existed, all showing a survival of 1 year or more (28, 31, 50, 57, 61, 68–70), with four patients having lived for over 2 years (28, 61, 68, 70). The longest survival was 117 months, reported by Sakuraba et al. (61).

General descriptions of specific cases exhibiting long-term survival, mixed pathological types of lung cancer, or negative results in IHC staining for ACTH, as well as a brief introduction to the latest retrospective study, are presented in Supplementary Materials.

4 Discussion

In this systematic review, we have incorporated relevant reports of SCLC with PCS available on PubMed and Web of Science and presented a comprehensive analysis. Through the review and analysis, we have not only reflected on opportunities to refine the differential diagnostic strategy for PCS, but also discovered key considerations to underpin the comprehensive treatment of SCLC with PCS patients.

4.1 Differential diagnosis of ECS from Cushing's disease

Since PCS is a marker of poor prognosis for SCLC patients, it is crucial to perform early differential diagnosis of CS to evaluate the prognosis of SCLC patients. Once the CS has been identified and the ACTH non-dependent type has been ruled out, the most

TABLE 1 The 61 reports of SCLC complicated with PCS from PubMed and Web of Science.

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Presentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
1 (22)	Shepherd	1985	Arch Intern Med	case report	1	ECS▲	56	M	1. hypokalemia 6. weakness		1. chemotherapy 4. ketoconazole	5 months	1. tumor progression	
2 (23)	Hoffman	1991	Cancer	case report	1	ECS	60	F	1. hypokalemia 5. edema 6. myopathy, weakness 9. polyuria		1. chemotherapy 4. ketoconazole	3 months	3. rapidly increasing ACTH and cortisol levels 6. condition deteriorated	
3(9)	Dimopoulos	1992	Cancer	retrospective	11	ECS	62(49-65)	3/8	1. hypokalemia (100%) 3. glycemia (91%) 4. metabolic alkalosis (100%) 6. weakness (55%) 7. dyspnea (27%)		1. chemotherapy 5. metyrapone	12 days (2-45)	2. infection (73%) 4. cardiac complications 5. respiratory complications 6. miscellaneous, not know	
4(8)	Shepherd	1992	J Clin Oncol	retrospective	23	ECS▲	60(43-77)	17/6	1. hypokalemia (96%) 3. hyperglycemia (59%) 4. metabolic alkalosis (96%) 5. edema (83%) 6. muscle weakness (61%)		1. chemotherapy 4. ketoconazole 5. aminoglutethimide	6.23 months (0-20)	1. progressive malignancy 2. infection 5. pneumonia 6. other causes	
5(7)	Delisle	1993	Arch Intern Med	retrospective	14	ECS	62(36-67)	7/7	1. hypokalemia (100%) 2. hypertension (14%) 3. hyperglycemia (71%) 4. metabolic alkalosis 5. peripheral edema (36%) 6. proximal myopathy (29%)		1. chemotherapy 2. radiotherapy	5.5 months (0.75-22)	1. progressive growth of tumor (79%) 2. infections (21%)	
6 (67)	Rieu	1993	Horm Res	case series	2	ECS	55	M	2. hypertension 3. hyperglycemia		1. chemotherapy 2. radiotherapy 6. octrotide	2 months	1. metastatic disease	
						ECS	34	F				8 months		

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Pre-sentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
									5. edema 7. dyspnea		1. chemotherapy 2. radiotherapy 3. thoracotomy 4. ketoconazole 5. metyrapone 6. lanreotide, octreotide		1. metastatic disease	
7 (24)	Tabata	1993	Nihon Kyobu Shikkan Gakkai Zasshi	case report	1	CS	62	M	1. hypokalemia 2. hypertension 3. hyperglycemia 4. alkalosis 9. polyuria	(-)	1. chemotherapy	5 months	1. cancer 5. pneumonia	
8 (25)	Auchus	1994	J Endocrinol Invest	case report	1	ECS★	75	F	1. hypokalemia 2. hypertension 4. metabolic alkalosis 7. dyspnea	(-)	1. chemotherapy 5. metyrapone	/	/	
9 (26)	Huang	1994	Changgeng yi xue za zhi	case report	1	ECS	25	M	1. hypokalemic 3. hyperglycemia 4. alkalosis 7. dyspnea	(+)	4. ketoconazole	13 days	1.progression of lung lesion 2. Nocardia infection	
10 (10)	Winquist	1995	J Clin Oncol	retrospective	9 +1◆	ECS▲	58.5 (44-71)	8/2	1. hypokalemia 2. hypertension 3. diabetes mellitus 4. metabolic alkalosis 5. edema 6. muscle weakness		1. chemotherapy 4. ketoconazole	/	1. progressive cancer 3. hormonal disorder	
11 (27)	Takano	1996	Nihon Kyobu Shikkan Gakkai Zasshi	case report	1	ECS	70	F	1. hypokalemia 2. hypertension 3. hyperglycemia 4. metabolic alkalosis	(+)	1. chemotherapy	11 months	1. progressive disease	
12 (28)	Sato	1997	Nihon Ronen Igakkai Zasshi	case report	1	ECS	72	M	1. hypokalemia 2. hypertension 4. metabolic alkalosis 5. edema	(+)	1. chemotherapy 2. radiotherapy 5. mitotane	26 months	5. respiratory failure	

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Pre-sentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
13 (29)	Cabezas	1998	Neth J Med	case report	1	ECS	56	M	1. hypokalemia 4. metabolic alkalosis 9. polyuria	(+)	1. chemotherapy 6. octreotide	9 months	1. meningitis carcinomatosa	
14 (31)	Bodvarsson	2001	Cancer	case report	1✖	ECS	25	F	5. edema 6. weakness		1. chemotherapy 3. nephrectomy of the transplanted kidney	/	/	18 months
15 (30)	Dubé	2001	Ann Fr Anesth Reanim	case report	1	ECS	58	M	1. hypokalemia 4. metabolic alkalosis 5. edema 7. dyspnea		/	1 week	5. respiratory deterioration	
16 (68)	Sakuraba	2003	Jpn J Thorac Cardiovasc Surg	case series	1	ECS	44	F	1. hypokalemia 6. fatigue	(+)	3. surgery (anterior lobe of pituitary, adrenal gland, right middle lobectomy)	117 months	/	
17 (32)	Agha	2005	Pituitary	case report	1	ECS	49	M	1. hypokalemia 2. hypertension 5. edema 6. weakness 7. dyspnea 9. polyuria	(+)	1. chemotherapy 4. ketoconazole	9 months	/	
18 (73)	Ilias	2005	J Clin Endocrinol Metab	retrospective	3	ECS	/	2/1	1. hypokalemia 2. hypertension 3. diabetes 5. edema 6. muscle weakness		1. chemotherapy 3. bilateral adrenalectomy (2/3) 7. with/without endocrine therapy	/	/	1–48 months
19 (34)	Hadem	2007	Z Gastroenterol	case report	1	ECS	68	F	1. hypokalemia 2. hypertension 3. hyperglycemia 4. metabolic alkalosis 6. muscle weakness 7. dyspnea		1. chemotherapy 4. ketoconazole	7 weeks	1. intracranial tumor spread 2. septic complications 3. endocrine and electrolyte disturbances 5. pneumonia	
20 (35)	Lee	2007	Endocrinol Metab	case report	1	ECS	73	F	1. hypokalemia 2. hypertension 3. hyperglycemia	(-)	1. chemotherapy 2. radiotherapy	10 days	5. neutropenic pneumonia	

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Pre-sentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
									4. metabolic alkalosis 5. edema 6. weakness					
21 (36)	Muessig	2007	Internist	case report	1	ECS	68	F	1. hypokalemia 2. hypertension 3. hyperglycemia 6. weakness 7. dyspnea	(+)	1. chemotherapy	/	/	3 courses therapy
22 (37)	Servonnet	2007	Ann Biol Clin	case report	1	ECS	41	F	1. hypokalemia 2. hypertension 3. hyperglycemia 4. metabolic alkalosis 5. edema 7. dyspnea		1. chemotherapy 5. metopirone	/	/	
23 (33)	Tanaka	2007	Nihon Kokyuki Gakkai Zasshi,	case report	1	ECS	54	F	9. polyuria		1. chemotherapy 2. radiotherapy	3 months	2. multiple intracystic infections of bilateral upper lobe 6. general deterioration	
24 (39)	Fernández-Rodríguez	2008	Arq Bras Endocrinol Metabol	case report	1	ECS	59	M	1. hypokalemia 2. hypertension 4. metabolic alkalosis 5. edema	(-)	1. chemotherapy 4. ketoconazole	<1 month	2. septic shock	
25 (38)	Guabello	2008	Am J Clin Oncol	case report	1	ECS	71	M	1. hypokalemia 2. hypertension 3. hyperglycemia 5. edema		1. chemotherapy	1 month	2. blood infection, septic shock 3. hypercortisolism 6. nephrosis	
26 (40)	Vadlamudi	2008	South Med J	case report	1•	ECS▲	76	M	1. hypokalemia 2. hypertension 4. metabolic alkalosis 6. weakness 7. dyspnea		/	several days	2. hyperinfection 3. hypercortisolism 4. cardiac arrest	

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Pre-sentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
27 (41)	Bindi	2009	Recenti Prog med	case report	1	ECS	64	M	1. hypokalemia 8. hypothyroidism		1. chemotherapy	/	/	
28 (42)	Martínez-Valles	2009	Cases J	case report	1	ECS	54	M	1. hypokalemia 2. hypertension 4. metabolic alkalosis 5. edema 6. fatigue 9. polyuria		4. ketoconazole	a few days	2. sepsis due to a right leg cellulitis 5. respiratory failure 6. bilateral pleural effusions	
29 (43)	Cicin	2010	Trak Univ Tip Fak Derg	case report	1	ECS	37	M	1. hypokalemia 4. alkalosis 5. edema 6. weakness		1. chemotherapy 4. ketoconazole	11 days	2. infection 5. pneumonia	
30 (74)	Doi	2010	Endocr J	retrospective	2	ECS	58	M	/		1. chemotherapy 2. radiotherapy	7 months	1. cancer	
						ECS	69	M	1. hypokalemia 2. hypertension 3. diabetes 6. weakness		1. chemotherapy 2. radiotherapy 5. mitotane, metyrapone	6 months	1. cancer	
31 (11)	Ejaz	2011	Cancer	retrospective	9	ECS	/	/	1. hypokalemia 2. hypertension 3. hyperglycemia 4. alkalosis	(+)	3. surgery (bilateral adrenalectomy, resection of primary tumors, combined bilateral adrenalectomy along with primary tumor resection) 4. ketoconazole 5. metyrapone	/	2. infections 3. hyperglycemia 4. venous thromboembolism	
32 (44)	Suyama	2011	Intern Med	case report	1	ECS	53	M	1. hypokalemia 2. hypertension 3. hyperglycemia 7. dyspnea		1. chemotherapy 5. mitotane	5 months	1. cancer progress	
33 (45)	Stempel	2013	BMJ Case Rep	case report	1	ECS▲	79	F	2. hypertension 4. metabolic alkalosis 5. edema 6.lethargy 7. dyspnea		5. metyrapone	1 week	4. lateral ischaemia 5. respiratory failure	

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Pre-sentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
34 (46)	Akinosoglou	2014	Ann Clin Biochem	case report	1	ECS	59	M	1. hypokalemia 5. edema		1. chemotherapy 4. ketoconazole	/	/	
35 (6)	Nagy-Mignotte	2014	J Thorac Oncol	retrospective	23	CS	62 (29-84)	16/7	1. hypokalemia (95.6%) 2. hypertension (56.5%) 3. hyperglycemia (95.6%) 4. metabolic alkalosis (69.6%) 5. edema (52.4%) 6. myopathy (55%)		1. chemotherapy 2. radiotherapy 3. surgery	6.6 months (95% confidence interval, 3.2–11.4)	1. cancer (81.8%) 2. infection (45.5%) 4. cardiac (9.1%)	
36 (48)	Nandagopal	2014	Am J Ther	case report	1	ECS	57	F	1. hypokalemia 2. hypertension 4. metabolic alkalosis 5. edema 6. weakness, fatigue		1. chemotherapy 2. radiotherapy	/	/	
37 (47)	Vega	2014	Rev Clin Esp (Barc)	case report	1	ECS	66	M	1. hypokalemia 2. hypertension 3. hyperglycemia 4. metabolic alkalosis 5. edema 7. dyspnea		1. chemotherapy 4. ketoconazole	1 month	2. escherichia coli septicemia secondary to acute perforated diverticulitis	
38 (49)	Cekerevac	2015	Acta Clin Croat	case report	1	ECS▲	63	M	1. hypokalemia 3. hyperglycemia 6. exhaustion 7. shortness of breath		/	2 weeks	4. heart failure 5. respiratory failture	
39 (76)	Ghazi	2015	Endokrynol Pol	prospective	4	ECS	61.5 (40-65)	2/2	1. hypokalemia (50%) 3. hyperglycemia (50%) 4. metabolic alkalosis (25%) 6. muscle		3. postero-lateral thoracotomy (2/4) 4.ketoconazole	1.5 months (1-3)	2. sepsis 4. intractable tachyarrhythmias, heart failure (only one patient mentioned)	

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Presentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
									weakness (100%) 9. polyuria (25%)					
40 (50)	Jeong	2015	Tuberc Respir Dis (Seoul)	case report	1	ECS	69	M	1. hypokalemia 2. hypertension 4. metabolic alkalosis 6. weakness	(+)	1. chemotherapy 4. ketoconazole	15 months	/	
41 (52)	Aoki	2016	Intern Med	case report	1	ECS	35	M	1. hypokalemia 2. hypertension 6. muscle weakness 9. polyuria	(+)	1. chemotherapy	4 courses therapy	6. suffocation due to a retropharyngeal abscess	
42 (51)	Kaya	2016	J Clin Diagn Res	case report	1	ECS	70	M	1. hypokalaemia 2. hypertension 4. metabolic alkalosis 6. weakness	(+)	3. surgery for intestinal perforation 4. ketoconazole	12 days	6. general deterioration	
43 (53)	Ohara	2016	Intern Med	case report	1	ECS	64	M	1. hypokalemia 2. hypertension 3. hyperglycemia 4. metabolic alkalosis 5. edema 6. weakness 7. dyspnea	(+)	5. metyrapone	1 month	5. idiopathic pulmonary fibrosis, respiratory failure	
44 (54)	Hine	2017	J Emerg Med	case report	1	ECS	62	M	1. hypokalemia 2. hypertension 3. diabetes 5. edema 6. weakness	(+)	1. chemotherapy 2. radiotherapy	/	/	
45 (56)	Wilkins	2017	Clin Schizophr Relat Psychoses	case report	1	ECS	56	M	2. hypertension 3. hyperglycemia		1. chemotherapy 2. radiotherapy 4. ketoconazole 5. metyrapone	/	/	4 courses therapy
46 (55)	Zhang	2017	Thorac Cancer	case report	1	ECS	74	M	1. hypokalemia 3. hyperglycemia 4. metabolic alkalosis		1. chemotherapy	3 months	6. liver failure	

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Pre-sentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
									5. edema 6. muscle weakness					
47 (66)	Deldycke	2018	Acta Clin Belg	case series	1	ECS	71	F	1. hypokalemia 3. hyperglycemia, diabetes 5. peripheral edema 6. muscle weakness		1. chemotherapy 6. somatostatin analogue	/	/	
48 (57)	Ferreira	2018	BMJ Case Rep	case report	1	ECS▲	42	M	1. hypokalemia 2. hypertension 4. metabolic alkalosis	(-)	1. chemotherapy 2. radiotherapy 5. metyrapone	12 months	4.5. cardiopulmonary arrest 6. clinical deterioration persisted with hypotension and prostration	
49 (58)	Foray	2018	Respir Med Case Rep.	case report	1	ECS▲	66	F	1. hypokalemia 3. diabetes mellitus 4. metabolic alkalosis 5. edema 6. weakness 8. hypothyroidism		/	12 days	6. comorbid	
50 (69)	Richa	2018	Endocrinol Diabetes Metab Case Rep	case series	2	ECS	46	F	1. hypokalemia 2. hypertension 4. metabolic alkalosis 6. lethargy, fatigue 8. hyperthyroidism		1. chemotherapy 2. radiotherapy 4. ketoconazole	12 months	/	
						ECS	51	M	1. hypokalemia 2. hypertension 3. diabetes mellitus 4. metabolic alkalosis 5. edema		1. chemotherapy 4. ketoconazole	several months	/	

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Pre-sentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
									6. lethargy, fatigue 7. dyspnea					
51 (59)	Kamijo	2019	Intern Med	case report	1	ECS	72	M	1. hypokalemia 3. hyperglycemia 6. fatigue	(+)	/	11 days	6. liver failure, hepatic and renal dysfunction	
52 (70)	Zhou	2019	World J Clin Cases	case series	2	ECS	54	F	1. hypokalemia 2. hypertension 3. hyperglycemia 6. weakness 8. hypothyroidism		1. chemotherapy	/	/	2 years
						ECS	50	M	1. hypokalemia 2. hypertension 3. diabetes mellitus 5. edema 8. hypothyroidism 9. diuresis		1. chemotherapy 2. radiotherapy	/	/	4 courses therapy
53 (63)	Gerhardt	2020	Dtsch Med Wochenschr	case report	1	ECS	58	M	1. hypokalemia 2. hypertension 4. metabolic alkalosis 5. edema	(+)	1. chemotherapy 4. ketoconazole	/	/	
54 (61)	Kosuda	2020	Intern Med	case report	1	ECS✦	70	F	1. hypokalemia 5. edema 6. fatigue		1. chemotherapy 2. radiotherapy	40 months	1. cancer progression	
55 (63)	Cabral	2020	Eur J Case Rep Intern Med	case report	1	CS	49	M	1. hypokalemia 2. hypertension 4. metabolic alkalosis 5. edema 6. asthenia 7. dyspnea 9. polyuria		5. etomidate, metyrapone	50 days	2. infection 5. pneumonia, respiratory failure	
56 (60)	Pingle	2020	ESC Heart Fail	case report	1	ECS	64	M	1. hypokalemia 2. hypertension 3. hyperglycemia, diabetes mellitus 5. edema		1. chemotherapy 5. metyrapone	/	/	5 courses therapy

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TABLE 1 Continued

ID	First Author	Year	Journal	Study Design	N	PCS	Age (median)	Gender (M/F)	Clinical Presentation	IHC staining for ACTH	Treatment	Survival (median)	Cause of Death	Follow-up
57 (75)	Lopez-Montoya	2021	Arch Endocrinol Metab	retrospective	3	ECS	68(50-72)	2/1	1. hypokalemia 2. hypertension 3. diabetes mellitus 6. proximal myopathy		1. chemotherapy (2/3) 2. radiotherapy (1/3) 3. bilateral adrenalectomy (1/3) 4. ketoconazole (2/3)	2 months (22 days-3 months)	1. disease progression 2. febrile neutropenia, septic shock, sepsis 5. respiratory failure	
58 (65)	Qiang	2021	BMC Endocr Disord	case report	1■	ECS	64	M	1. hypokalemia 3. hyperglycemia 4. metabolic alkalosis 5. edema 6. weakness	(+)	1. chemotherapy 7. mifepristone	<1 month	6. dyscrasia	
59 (64)	Senarathne	2021	BMJ Case Rep	case report	1	ECS	56	M	1. hypokalemia 3. hyperglycemia 5. edema 6. weakness 7. dyspnea	(+)	1. chemotherapy 4. ketoconazole	3 weeks	5. neutropenic pneumonia	
60 (72)	Piasecka	2022	Front Med (Lausanne)	case series	1	ECS▲	81	M	1. hypokalemia 2. hypertension 5. edema 6. muscle weakness 7. dyspnea		/	1 week	6. condition deteriorated	
61 (71)	Rosales-Castillo	2022	Hipertens Riesgo Vasc	case series	2	ECS	59	M	1. hypokalemia 2. hypertension 3. hyperglycemia 4. metabolic alkalosis 9. polyuria		1. chemotherapy	1 courses therapy	6. massive hematemesis	
						ECS	47	M	1. hypokalemia 2. hypertension 3. hyperglycemia 4. metabolic alkalosis 5. edema 6. weakness		4. ketoconazole	2 months	3. metabolic alterations	

1.▲suspected; 2.★CRH induced; 3.✦combined with SIADH; 4.✖donor-derived SCLC; 5.◆;mixed with NSCLC; 6■mixed with large cell neuroendocrine carcinoma; 7.•mixed with adenocarcinoma and giant cell carcinoma of the lung.
N, number of patients; M, male; F, female; CS, Cushing's syndrome; PCS, paraneoplastic Cushing's syndrome; ECS, ectopic Cushing's syndrome; IHC, immunohistochemical; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; SIADH, syndrome of inappropriate antidiuretic hormone secretion; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.

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TABLE 2 The frequency of specific details recorded in all 61 reports.

Category	Specific Detail	No. of Articles	Rank
Clinical Presentation			
1	hypokalemia	59(96.72%)	1
2	hypertension	42(68.85%)	2
3	hyperglycemia or diabetes mellitus	37(60.66%)	4
4	metabolic alkalosis	37(60.66%)	4
5	edema	36(59.02%)	5
6	myopathy, weakness, fatigue, or lethargy	41(67.21%)	3
7	dyspnea	19(31.15%)	6
8	hypothyroidism or hyperthyroidism	5(8.20%)	8
9	diuresis, polyuria, polyuresis or diabetes insipidus	11(18.03%)	7
Treatment			
1	chemotherapy	50(81.97%)	1
2	radiotherapy	17(27.87%)	3
3	surgery (lung, adrenal gland or elsewhere)	9(14.75%)	5
4	ketoconazole	24(39.34%)	2
5	metyrapone, mitotane, etomidate or other steroidogenesis inhibitors	15(24.59%)	4
6	octreotide, lanreotide or other somatostatin analogues	4(6.56%)	6
7	mifepristone or other treatments for hypercortisolism	2(3.28%)	7
Cause of Death			
1	cancer	17(27.87%)	1
2	infection	17(27.87%)	1
3	hormone issues	7(11.48%)	5
4	cardiovascular complications	8(13.11%)	4
5	respiratory complications	16(26.23%)	2
6	other causes (included general condition deteriorated)	15(24.59%)	3

The percentage was calculated as the proportion of the articles with records of corresponding details out of the all 61 articles.

challenging part is distinguishing ECS from Cushing's disease (CD), where the pituitary gland releases ACTH (12, 57, 66). In this context, IHC staining for ACTH in the tumor tissue could be an efficient diagnostic tool.

No individual imaging examination or laboratory test has been explicitly recommended to definitively differentiate between pituitary and ectopic CS (3, 66, 77). Despite the fact that high-dose dexamethasone suppression test and CRH stimulation test may individually yield inaccurate outcomes, their combination has shown a better diagnostic performance (3, 12, 77–80). Following the Pituitary Society's guideline, in the event of negative outcomes for both tests, consideration should be given to diagnosing ECS. Conversely, if both tests yield positive results, CD should be acknowledged. If the results are mismatched, a bilateral inferior petrosal sinus sampling is necessary for a definite diagnosis (77). Furthermore, a conclusion drawn in collaboration with imaging techniques must be more convincing. The guideline recommended magnetic resonance imaging (MRI) as the preferred modality for imaging ACTH-secreting pituitary adenomas (77) despite its high rate of false negative or false positive (12, 66, 81). Moreover, emerging data have suggested that the CRH/desmopressin stimulation test in collaboration with pituitary MRI, subsequently followed by a whole-body computed tomography scan, could be a reliable alternative (77, 82, 83). However, some investigators suggested that a conclusive diagnosis of an ACTH-secreting tumor should only be made post-surgery. After surgical removal of the tumor, the resolution of hypercortisolism symptoms and the positive IHC staining for ACTH or its precursor in excised tissues could indicate an ECS diagnosis (66, 81).

IHC staining for ACTH has demonstrated a high degree of reliability for its consistency with outcomes from the combination of laboratory tests and imaging examinations within our reviewed reports. However, none of the three guidelines from the American Endocrine Society, European Society of Endocrinology, or the Pituitary Society contained any histological diagnosis-relevant contents on ECS diagnosis in SCLC patients (13, 77, 78, 84). We

look forward to this technique being evaluated by a proficient multidisciplinary team in the future and the latest guidelines shedding some light on this diagnostic method. The IHC staining of a distinctive hormone (either ACTH or CRH) allows us to make an early diagnosis of ECS in conjunction with the pathological diagnosis of SCLC and to take prophylactic measures against hypercortisolism, which can exacerbate cancer-induced immunosuppression and cause severe infectious complications afterwards (6, 8, 9, 11, 64, 85).

Based on the literatures reviewed and the charts appreciated (3, 11–13, 66, 78, 82, 86, 87), we improved and perfected the specific flowchart for the multistep diagnostic procedures of ECS from Deldycke et al. (66). The flowchart is displayed in Figure 2.

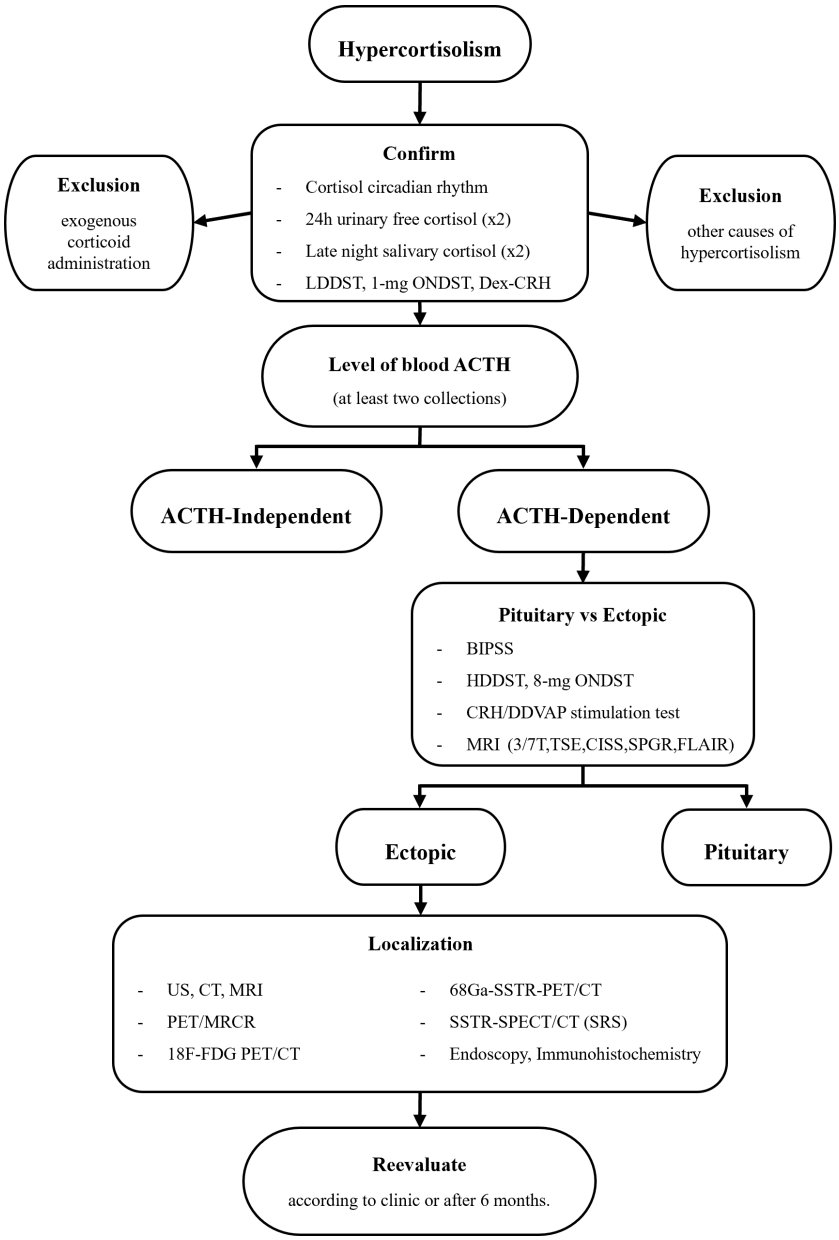

FIGURE 2
Flow diagram for multistep diagnostic procedures of ECS. Based on the figure in the review of Deldycke et al. ECS, ectopic Cushing's syndrome; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; DDAVP, desmopressin or 1-deamino-8-D-arginine-vasopressin; ONDST, overnight dexamethasone suppression test; LDDST, low-dose dexamethasone suppression test; HDDST, high-dose dexamethasone suppression test; Dex-CRH, combined LDDST-CRH test; BIPSS, bilateral inferior petrosal sinus sampling; US, ultrasound; CT, computed tomography; MRI, magnetic resonance imaging; TSE, T1-weighted turbo spin echo; CISS, constructive interference in the steady state; SPGR, spoiled gradient recalled; FLAIR, fluid attenuation inversion recovery; PET, positron emission tomography; SPECT, single-photon emission computed tomography; PET/MRCR, PET coregistration with volumetric MRI; 18F-FDG, 18F-fluoro-deoxy-glucose; SSTR-PET/CT, somatostatin receptor-based positron emission tomography/computed tomography (with 68Ga-DOTATATE/DOTATOC/DOTANOC); SSTR-SPECT/CT, somatostatin receptor-based single-photon emission computed tomography/computed tomography; SRS (octreoscan), somatostatin receptor scintigraphy (with Octreotide).

4.2 Therapeutic strategy for SCLC with PCS

The treatment of SCLC with PCS patients demands two primary factors. On one hand, it is vital to manage hypercortisolism and take prophylactic measures against infections. On the other hand, some therapeutic strategies have advanced in the treatment of SCLC.

On one hand, both infection and cancer ranked highest among all causes of death, with each being mentioned in 17 (27.87%) reports. Furthermore, a significant number of patients had infections recorded before respiratory complications (indicated in 16 reports). Infection facilitated by glucocorticoid-induced immunosuppression and chemotherapy-induced agranulocytosis is a significant poor prognostic factor for SCLC with PCS patients (6, 8, 9, 11, 64, 85). Therefore, management of hypercortisolism and prophylaxis against infection is particularly important throughout the entire treatment process. Many authors emphasized the importance of controlling hypercortisolism by a specific treatment before or concurrently with chemotherapy to prevent infectious complications (6, 43, 52, 64, 75, 76). Apart from radiotherapy and surgical removal, the main pharmacological treatments for PCS include steroidogenesis inhibitors (e.g., ketoconazole, metyrapone, mitotane, etomidate, and osilodrostat), glucocorticoid receptor antagonists (e.g., mifepristone), somatostatin analogs (e.g., octreotide, lanreotide, and pasireotide), and dopamine agonists (e.g., cabergoline) (3, 4, 12, 13). According to guidelines and high-quality reviews, steroidogenesis inhibitors have been the principal treatment to control hypercortisolism while somatostatin analogs and dopamine agonists are recommended to inhibit ectopic ACTH production with limited intensity (3, 4, 12, 13). Within our included reports, steroidogenesis inhibitors were used most frequent as in 39 (63.93%) reports, especially ketoconazole recorded in 24 (39.34%) reports, while somatostatin analogs and mifepristone were used little and no dopamine agonists were recorded.

On the other hand, the ultimate cause of hypercortisolism is ectopic hormone secretion by tumor tissues, meaning that the treatment for cancer is fundamental to the management of hypercortisolism and effective anti-cancer treatment could alleviate PCS symptoms. All 61 reports we examined recorded chemotherapy and radiotherapy as anti-tumor therapeutic strategies apart from surgery. In fact, there had been no substantial progress in treatment of SCLC for over 30 years until ICIs updated the treatment pattern and modestly improved its overall survival (1, 2, 14, 15). Formerly, platinum plus etoposide combination chemotherapy was the preferred regimen for both limited and extensive SCLC. Nowadays, the new standard of care in first-line setting for SCLC is immuno-chemotherapy that combines atezolizumab or durvalumab with platinum-etoposide (14, 15, 88, 89). Considering significant improvement in medical care over recent years and the introduction of immunotherapy into therapeutic strategy for SCLC, we look forward to seeing some reports, especially high-quality large-sample studies conducted by proficient teams, evaluating the efficacy and safety of some novel medical approaches, particularly the immunotherapy, for the treatment of SCLC with PCS in the future.

We retrieved all drug approval notifications for SCLC in the *Oncology (Cancer)/Hematologic Malignancies Approval Notifications*) section on the official website of USA Food and Drug Administration, which revealed a slow progression in treatment of SCLC compared to NSCLC, as summarized in Supplementary Materials.

4.3 Limitations

This systematic review has some limitations that need to be acknowledged in order to contextualize the conclusions that have been drawn or will be drawn from it. Firstly, the literature available on the subject is relatively limited, and there exists a significant degree of heterogeneity among the reports included in this review. This review includes several types of studies, and there are significant differences in outlines, focuses, and details of reported management of the disease, even within the same study type. Secondly, the evidence grade of case reports or case series is low. Among the 61 included reports, a significant proportion is composed of 44 case reports and 7 case series. As a result, the complete data in Table 2 were derived from the number of reports that had records of corresponding details, rather than from the number of patients, which makes the statistical analysis sketchy and generalized. Thirdly, the included reports stretch over a period from 1985 to 2022, during which medical care rapidly evolved, which could be the inherent limitation for systematic reviews with too wide a temporal scope. Ultimately, it is essential to acknowledge that this systematic review serves only as a preliminary exploration for the management of SCLC with PCS patients, and further validation is eagerly awaited from future high-quality studies covering significant sample sizes.

5 Conclusions

This systematic review indicated that the majority of PCS complications in SCLC patients were caused by ectopic hormone secretion. Furthermore, it is recommended to enhance the employment of IHC staining for distinctive hormones (ACTH or CRH) in clinical practice for early differential diagnosis of PCS. Moreover, effective management of hypercortisolism and potent safeguarding against infections could form the foundation of comprehensive treatment of SCLC with PCS patients.

Data availability statement

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

Author contributions

YL designed the study. YL and CL collected the data. CL and XQ analyzed the data. YL prepared tables and figures. YL, CL and XQ drafted the manuscript. LY and LL supervised the study and revised the manuscript. All authors read and approved the submitted version of the manuscript.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1177125/full#supplementary-material

References

1. Wang Q, Gümüş ZH, Colarossi C, Memeo L, Wang X, Kong CY, et al. SCLC: epidemiology, risk factors, genetic susceptibility, molecular pathology, screening, and early detection. *J Thorac Oncol* (2022) 18(1):31–46. doi: 10.1016/j.jtho.2022.10.002

2. Rudin CM, Brambilla E, Faivre-Finn C, Sage J. Small-cell lung cancer. *Nat Rev Dis Primers* (2021) 7:3. doi: 10.1038/s41572-020-00235-0

3. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. *Lancet* (2015) 386:913–27. doi: 10.1016/S0140-6736(14)61375-1

4. Alexandraki KI, Grossman AB. The ectopic ACTH syndrome. *Rev Endocr Metab Disord* (2010) 11:117–26. doi: 10.1007/s11154-010-9139-z

5. Valassi E, Franz H, Brue T, Feelders RA, Netea-Maier R, Tsagarakis S, et al. Diagnostic tests for Cushing's syndrome differ from published guidelines: data from ERCUSYN. *Eur J Endocrinol* (2017) 176:613–24. doi: 10.1530/EJE-16-0967

6. Nagy-Mignotte H, Shestaeva O, Vignoud L, Guillem P, Ruckly S, Chabre O, et al. Prognostic impact of paraneoplastic cushing's syndrome in small-cell lung cancer. *J Thorac Oncol* (2014) 9:497–505. doi: 10.1097/JTO.0000000000000116

7. Delisle L, Boyer MJ, Warr D, Killinger D, Payne D, Yeoh JL, et al. Ectopic corticotropin syndrome and small-cell carcinoma of the lung. Clinical features, outcome, and complications. *Arch Intern Med* (1993) 153:746–52. doi: 10.1001/archinte.1993.00410060054009

8. Shepherd FA, Laskey J, Evans WK, Goss PE, Johansen E, Khamsi F. Cushing's syndrome associated with ectopic corticotropin production and small-cell lung cancer. *J Clin Oncol* (1992) 10:21. doi: 10.1200/JCO.1992.10.1.21

9. Dimopoulos MA, Fernandez JF, Samaan NA, Holoye PY, Vassilopoulou-Sellin R. Paraneoplastic Cushing's syndrome as an adverse prognostic factor in patients who die early with small cell lung cancer. *Cancer-Am Cancer Soc* (1992) 69:66–71. doi: 10.1002/1097-0142(19920101)69:1<66::aid-cncr2820690113>3.0.co;2-2

10. Winquist EW, Laskey J, Crump M, Khamsi F, Shepherd FA. Ketoconazole in the management of paraneoplastic Cushing's syndrome secondary to ectopic adrenocorticotropin production. *J Clin Oncol* (1995) 13:157–64. doi: 10.1200/JCO.1995.13.1.157

11. Ejaz S, Vassilopoulou-Sellin R, Busaidy NL, Hu MI, Waguespack SG, Jimenez C, et al. Cushing syndrome secondary to ectopic adrenocorticotropic hormone secretion. *Cancer-Am Cancer Soc* (2011) 117:4381–89. doi: 10.1002/cncr.26029

12. Hayes AR, Grossman AB. The ectopic adrenocorticotropic hormone syndrome. *Endocrin Metab Clin* (2018) 47:409–25. doi: 10.1016/j.ecl.2018.01.005

13. Nieman LK, Biller BMK, Findling JW, Murad MH, Newell-Price J, Savage MO, et al. Treatment of cushing's syndrome: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2015) 100:2807–31. doi: 10.1210/jc.2015-1818

14. Barrows ED, Blackburn MJ, Liu SV. Evolving role of immunotherapy in small cell lung cancer. *Semin Cancer Biol* (2022) 86:868–74. doi: 10.1016/j.semcancer.2022.02.021

15. Remon J, Aldea M, Besse B, Planchard D, Reck M, Giaccone G, et al. Small cell lung cancer: a slightly less orphan disease after immunotherapy. *Ann Oncol* (2021) 32:698–709. doi: 10.1016/j.annonc.2021.02.025

16. Dingemans AC, Früh M, Ardizzoni A, Besse B, Faivre-Finn C, Hendriks LE, et al. Small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up(☆). *Ann Oncol* (2021) 32:839–53. doi: 10.1016/j.annonc.2021.03.207

17. Atkinson M, Lansdown AJ. Endocrine immune-related adverse events: Adrenal, parathyroid, diabetes insipidus, and lipoatrophy. *Best Pract Res Clin Endocrinol Metab* (2022) 36:101635. doi: 10.1016/j.beem.2022.101635

18. Lupu J, Pages C, Laly P, Delyon J, Laloi M, Petit A, et al. Transient pituitary ACTH-dependent Cushing syndrome caused by an immune checkpoint inhibitor combination. *Melanoma Res* (2017) 27:649–52. doi: 10.1097/CMR.0000000000000405

19. Paepegaey AC, Dot JM, Beauvy J, Juttet P, Le Berre JP. Pembrolizumab-induced cyclic ACTH-dependent Cushing's syndrome treated by a block-and-replace approach with osilodrostat. *Ann Endocrinol (Paris)* (2022) 83:73–5. doi: 10.1016/j.ando.2021.11.007

20. Tan MH, Iyengar R, Mizokami-Stout K, Yentz S, MacEachern MP, Shen LY, et al. Spectrum of immune checkpoint inhibitors-induced endocrinopathies in cancer patients: a scoping review of case reports. *Clin Diabetes Endocrinol* (2019) 5:1. doi: 10.1186/s40842-018-0073-4

21. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *PloS Med* (2021) 18:e1003583. doi: 10.1371/journal.pmed.1003583

22. Shepherd FA. Ketoconazole. Use in the treatment of ectopic adrenocorticotropic hormone production and Cushing's syndrome in small-cell lung cancer. *Arch Internal Med* (1985) 145:863–64. doi: 10.1001/archinte.145.5.863

23. Hoffman DM, Brigham B. The use of ketoconazole in ectopic adrenocorticotropic hormone syndrome. *Cancer-Am Cancer Soc* (1991) 67:1447–49. doi: 10.1002/1097-0142(19910301)67:5<1447::AID-CNCR2820670531>3.0.CO;2-I

24. Tabata M, Ohnoshi T, Ueoka H, Kiura K, Segawa Y, Shibayama T, et al. [A case of small cell lung cancer associated with diabetes insipidus and Cushing's syndrome]. *Nihon Kyobu Shikkan Gakkai Zasshi* (1993) 31:235–39.

25. Auchus RJ, Mastorakos G, Friedman TC, Chrousos GP. Corticotropin-releasing hormone production by a small cell carcinoma in a patient with ACTH-dependent Cushing's syndrome. *J Endocrinol Invest* (1994) 17:447–52. doi: 10.1007/BF03347737

26. Huang TP, Wang PW, Liu RT, Tung SC, Jean WY, Lu YC, et al. Ectopic ACTH syndrome with nocardiosis–a case report. *Changgeng Yi Xue Za Zhi* (1994) 17:371–77.

27. Takano K, Takayama K, Nakano H, Hagimoto N, Nakanishi Y, Hara N. Small cell lung cancer associated with ectopic ACTH syndrome. *Nihon Kyōbu Shikkan Gakkai zasshi* (1996) 34:220–25.

28. Sato S, Yokoyama A, Ohtsuka T, Nomoto T, Abe M, Kohno N, et al. Cushing's syndrome due to small cell lung cancer with ectopic production of adrenocorticotropic and parathyroid hormone. *Nippon Ronen Igakkai Zasshi. Japanese J Geriatrics* (1997) 34:215–20. doi: 10.3143/geriatrics.34.215

29. Castro Cabezas M, Vrinten DH, Burgers JA, Croughs RJ. Central diabetes insipidus and Cushing's syndrome due to ectopic ACTH production by disseminated small cell lung cancer: a case report. *Neth J Med* (1998) 53:32. doi: 10.1016/S0300-2977(98)00051-5

30. Dube L, Daenen S, Kouatchet A, Soltner C, Alquier P. [Severe metabolic alkalosis following hypokalemia from a paraneoplastic Cushing syndrome]. *Ann Fr Anesth Reanim* (2001) 20:860–64. doi: 10.1016/s0750-7658(01)00518-4

31. Bodvarsson S, Burlingham W, Kusaka S, Hafez GR, Becker BN, Pintar T, et al. Donor-derived small cell lung carcinoma in a kidney transplant recipient. *Cancer-Am Cancer Soc* (2001) 92:2429–34. doi: 10.1002/1097-0142(20011101)92:9<2429::aid-cncr1592>3.0.co;2-g

32. Agha A, Brennan S, Moore KB, Grogan L, Thompson CJ. Small-cell lung cancer presenting as diabetes insipidus and Cushing's syndrome. *Pituitary* (2005) 8:105–07. doi: 10.1007/s11102-005-3308-1

33. Tanaka H, Kobayashi A, Bando M, Hosono T, Tsujita A, Yamasawa H, et al. [Case of small cell lung cancer complicated with diabetes insipidus and Cushing syndrome due to ectopic adrenocorticotropic hormone secretion]. *Nihon Kokyuki Gakkai Zasshi* (2007) 45:793–98.

34. Hadem J, Cornberg M, Länger F, Schedel I, Kirchhoff T, Niedermeyer J, et al. Making sense of muscle fatigue and liver lesions. *Z für Gastroenterologie* (2007) 45:609. doi: 10.1055/s-2006-927284

35. Yang HJ, Sung HJ, Kim JE, Lee HJ, Park JM, Park CK, et al. A case of ectopic ACTH syndrome associated with small cell lung cancer presented with hypokalemia. *Endocrinology and Metabolism* (2007) 22:359–64. doi: 10.3803/jkes.2007.22.5.359

36. Müssig K, Maser-Gluth C, Hartmann M, Wehrmann M, Horger M, Kanz L, et al. 68-year-old female patient with dyspnea and hypokalemic hypertension. *Der Internist* (2007) 48:1145–50. doi: 10.1007/s00108-007-1930-x

37. Servonnet A, Delacour H, Roux X, Dehan C, Gardet V, Morand C. Ectopic ACTH syndrome and severe hypokalaemia. *Annales biologie clinique (Paris)* (2007) 65:425. doi: 10.1684/abc.2007.0142

38. Guabello G, Brunetti L, Palladini G, Musumeci S, Lovati E, Perfetti V. Paraneoplastic cushing's syndrome and nephrotic syndrome in a patient with disseminated small cell lung cancer. *Am J Clin Oncol* (2008) 31:102–03. doi: 10.1097/01.coc.0000203741.06225.8a

39. Fernandez-Rodriguez E, Villar-Taibo R, Pinal-Osorio I, Cabezas-Agricola JM, Anido-Herranz U, Prieto A, et al. Severe hypertension and hypokalemia as first clinical manifestations in ectopic Cushing's syndrome. *Arq Bras Endocrinol Metabol* (2008) 52:1066–70. doi: 10.1590/s0004-27302008000600019

40. Vadlamudi RS, Van Dort M, Barklow T, Byrd RJ, Moorman JP. Strongyloides hyperinfection syndrome complicating (ectopic) Cushing syndrome. *South Med J* (2008) 101:750–52. doi: 10.1097/SMJ.0b013e31817a836e

41. Bindi M, Moruzzo D, Pinelli M, Rosada J, Castiglioni M. [Hypokalemia from ectopic ACTH secretion and hypothiroidism in patient affected by small cell lung cancer]. *Recenti Prog Med* (2009) 100:137–39.

42. Martínez-Valles MA, Palafox-Cazarez A, Paredes-Avina JA. Severe hypokalemia, metabolic alkalosis and hypertension in a 54 year old male with ectopic ACTH syndrome: a case report. *cases J* (2009) 2:6174. doi: 10.4076/1757-1626-2-6174

43. Cicin I, Uzunoglu S, Ermantas N, Usta U, Temizoz O, Karagol H. A destroyer immunologic cause in small cell lung carcinoma: ecthopic cushing's syndrome. *Med J Trakya Univ* (2010) 27:312–14. doi: 10.5174/tutfd.2008.00718.1

44. Suyama K, Naito Y, Yoh K, Niho S, Goto K, Ohmatsu H, et al. Development of Cushing's syndrome during effective chemotherapy for small cell lung cancer. *Intern Med* (2011) 50:335–38. doi: 10.2169/internalmedicine.50.4127

45. von Stempel C, Perks C, Corcoran J, Grayez J. Cardio-respiratory failure secondary to ectopic Cushing's syndrome as the index presentation of small-cell lung cancer. *Case Rep* (2013) 2013):r2013009974. doi: 10.1136/bcr-2013-009974

46. Akinosoglou K, Siagris D, Geropoulou E, Kosmopoulou O, Velissaris D, Kyriazopoulou V, et al. Hyperamylasaemia and dual paraneoplastic syndromes in small cell lung cancer. *Ann Clin Biochem: Int J Lab Med* (2014) 51:101–05. doi: 10.1177/0004563213500658

47. Pérez Vega C. Panhipopituitarismo reversible en un paciente con síndrome de Cushing por secreción ectópica de hormona adrenocorticotropa secundaria a un carcinoma microcítico de pulmón. *Rev Clínica Española* (2014) 214:e5–08. doi: 10.1016/j.rce.2013.10.007

48. Nandagopal L, Arias C, Pillai U, Osman-Malik Y. Ectopic ACTH and cisplatin toxicity—A diagnostic dilemma. *Am J Ther* (2014) 21:e154–56. doi: 10.1097/MJT.0b013e3182691b03

49. Cekerevac I, Petrović M, Novković L, Bubanja D, Bubanja I, Djokić B, et al. ECTOPIC ACTH SECRETION WITH CONCOMITANT HYPERAMYLASEMIA IN A PATIENT WITH SMALL CELL LUNG CARCINOMA: CASE REPORT. *Acta clinica Croatica (Tisak)* (2015) 54:536–40.

50. Jeong C, Lee J, Ryu S, Lee HY, Shin AY, Kim JS, et al. A case of ectopic adrenocorticotropic hormone syndrome in small cell lung cancer. *Tuberculosis Respir Dis* (2015) 78:436–39. doi: 10.4046/trd.2015.78.4.436

51. Kaya T. Severe hypokalaemia, hypertension, and intestinal perforation in ectopic adrenocorticotropic hormone syndrome. *J OF Clin AND Diagn Res* (2016) 10(1):OD09–11. doi: 10.7860/JCDR/2016/17198.7127

52. Aoki M, Fujisaka Y, Tokioka S, Hirai A, Henmi Y, Inoue Y, et al. Small-cell lung cancer in a young adult nonsmoking patient with ectopic adrenocorticotropin (ACTH) production. *Internal Med (Tokyo 1992)* (2016) 55:1337–39. doi: 10.2169/internalmedicine.55.6139

53. Ohara N, Kaneko M, Sato K, Usuda H, Tanaka J, Maekawa T, et al. Acute exacerbation of idiopathic pulmonary fibrosis following treatment for cushing's syndrome. *Internal Med* (2016) 55:389–94. doi: 10.2169/internalmedicine.55.5566

54. Hine J, Schwell A, Kairys N. An unlikely cause of hypokalemia. *J Emergency Med* (2017) 52:e187–91. doi: 10.1016/j.jemermed.2016.12.011

55. Zhang HY, Zhao J. Ectopic Cushing syndrome in small cell lung cancer: A case report and literature review. *Thorac Cancer* (2017) 8:114–17. doi: 10.1111/1759-7714.12403

56. Wilkins CM, Johnson VL, Fargason RE, Birur B. Psychosis as a sequelae of paraneoplastic syndrome in Small- Cell Lung Carcinoma: A psycho-neuroendocrine interface. *Clin Schizophr Related Psychoses* (2017). doi: 10.3371/CSRP.CWVJ.111717

57. Lobo Ferreira T, Nunes Da Silva T, Canário D, Francisca Delerue M. Hypertension and severe hypokalaemia associated with ectopic ACTH production. *BMJ Case Rep* (2018) 2018:bcr2017223406. doi: 10.1136/bcr-2017-223406

58. Foray N, Stone T, Johnson A, Ali M, Kulkarni S, Gao J, et al. Severe metabolic alkalosis–a diagnostic dilemma. *Respir Med Case Rep* (2018) 25:177–80. doi: 10.1016/j.rmcr.2018.08.019

59. Kamijo S, Hasuike S, Nakamura K, Takaishi Y, Yamada Y, Ozono Y, et al. Acute liver failure due to severe hepatic metastasis of small-cell lung cancer producing adrenocorticotropic hormone complicating ectopic cushing syndrome. *Internal Med* (2019) 58:2977–82. doi: 10.2169/internalmedicine.1976-18

60. Pingle SR, Shah T, Mosleh W, Kim AS. Cushing syndrome cardiomyopathy: an unusual manifestation of small-cell lung cancer. *ESC Heart Failure* (2020) 7:3189–92. doi: 10.1002/ehf2.12860

61. Kosuda A, Shirahata T, Kudo N, Uehara Y, Miyawaki M, Hagiwara A, et al. Long-term survival of a patient with small cell lung cancer secreting ADH and ACTH simultaneously, following the prolonged use of amrubicin. *Internal Med* (2020) 59:107–12. doi: 10.2169/internalmedicine.2838-19

62. Gerhardt LMS, Sabath L, Müller B, Capraro J, Borm K. Paraneoplastisches Cushing-Syndrom als Ursache von therapierefraktärer Hypokaliämie. *DMW - Deutsche Medizinische Wochenschrift* (2020) 145:783–86. doi: 10.1055/a-1163-9873

63. Lemos CS, Deveza N, Baptista JP, Martins P. Disseminated strongyloides stercoralis infection associated with endogenous hypercortisolism - A case report. *Eur J Case Rep Intern Med* (2020) 7:1509. doi: 10.12890/2020_001509

64. Senarathne UD, Dayanath BKTP, Punchihewa R, Gunasena B. Patient with respiratory distress, facial oedema and refractory hypokalaemia. *BMJ Case Rep* (2021) 14:e240330. doi: 10.1136/bcr-2020-240330

65. Qiang W, Song S, Chen T, Wang Z, Feng J, Zhang J, et al. A rare case of ectopic ACTH syndrome with rhabdomyolysis. *BMC Endocr Disord* (2021) 21:98. doi: 10.1186/s12902-021-00755-0

66. Deldycke A, Haenebalcke C, Taes Y. Paraneoplastic Cushing syndrome, case-series and review of the literature. *Acta clinica belgica (English Ed Online)* (2018) 73:298–304. doi: 10.1080/17843286.2017.1373927

67. Rieu M, Rosilio M, Richard A, Vannetzel J, Kuhn J. Paradoxical effect of somatostatin analogues on the ectopic secretion of corticotropin in two cases of small cell lung carcinoma. *Hormone Res* (1993) 39:207–12. doi: 10.1159/000182737

68. Sakuraba M, Murasugi M, Oyama K, Adachi T, Ikeda T, Onuki T. Diagnosis and surgical treatment of ectopic adrenocorticotropic hormone-producing pulmonary tumors accompanied by Cushing syndrome. *Japanese J Thorac Cardiovasc Surg* (2003) 51:656–59. doi: 10.1007/s11748-003-0004-9

69. Richa CG, Saad KJ, Halabi GH, Gharios EM, Nasr FL, Merheb MT. Case-series of paraneoplastic Cushing syndrome in small-cell lung cancer. *Endocrinol Diabetes Metab Case Rep* (2018) 2018:18–0004. doi: 10.1530/EDM-18-0004

70. Zhou T, Wang Y, Zhao X, Liu Y, Wang YX, Gang XK, et al. Small cell lung cancer starting with diabetes mellitus: Two case reports and literature review. *World J Clin cases* (2019) 7:1213–20. doi: 10.12998/wjcc.v7.i10.1213

71. Rosales-Castillo A, Bustos-Merlo A. Arterial hypertension of infrequent cause. *Hipertensión y Riesgo Vasc* (2022) 39:92–4. doi: 10.1016/j.hipert.2021.09.002

72. Piasecka M, Larsson M, Papakokkinou E, Olsson L, Ragnarsson O. Is ectopic Cushing's syndrome underdiagnosed in patients with small cell lung cancer? *Front Med* (2022) 9:954033. doi: 10.3389/fmed.2022.954033

73. Ilias I, Torpy DJ, Pacak K, Mullen N, Wesley RA, Nieman LK. Cushing's syndrome due to ectopic corticotropin secretion: twenty years' Experience at the national institutes of health. *J Clin Endocrinol Metab* (2005) 90:4955–62. doi: 10.1210/jc.2004-2527

74. Doi M, Sugiyama T, Izumiyama H, Yoshimoto T, Hirata Y. Clinical features and management of ectopic ACTH syndrome at a single institute in Japan. *Endocr J* (2010) 57:1061–69. doi: 10.1507/endocrj.K10E-265

75. Lopez-Montoya V, Gutierrez-Restrepo J, Grajales JLT, Aristizabal N, Pantoja D, Roman-Gonzalez A, et al. Ectopic cushing syndrome in Colombia. *Arch Endocrinol Metab* (2020) 64(6):687–94. doi: 10.20945/2359-3997000000271

76. Ghazi AA, Abbasi Dezfooli A, Amirbaigloo A, Daneshvar Kakhki A, Mohammadi F, Tirgari F, et al. Ektopowy zespół Cushinga u pacjentów z nowotworem śródpiersia lub płuc — doniesienie z ośrodka trzeciego stopnia referencyjności w Iranie. *Endokrynol Pol* (2015) 66:2–09. doi: 10.5603/EP.2015.0002

77. Fleseriu M, Auchus R, Bancos I, Ben-Shlomo A, Bertherat J, Biermasz NR, et al. Consensus on diagnosis and management of Cushing's disease: a guideline update. *Lancet Diabetes Endocrinol* (2021) 9:847–75. doi: 10.1016/S2213-8587(21)00235-7

78. Nieman LK, Biller BMK, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al. The diagnosis of cushing's syndrome: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2008) 93:1526–40. doi: 10.1210/jc.2008-0125

79. Ejaz S, Vassilopoulou-Sellin R, Busaidy NL, Hu MI, Waguespack SG, Jimenez C, et al. Cushing syndrome secondary to ectopic adrenocorticotropic hormone secretion: the University of Texas MD Anderson Cancer Center Experience. *Cancer-Am Cancer Soc* (2011) 117:4381–89. doi: 10.1002/cncr.26029

80. Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, et al. Diagnosis and complications of Cushing's syndrome: a consensus statement. *J Clin Endocrinol Metab* (2003) 88:5593–602. doi: 10.1210/jc.2003-030871

81. Witek P, Witek J, Zieliński G, Podgajny Z, Kamiński G. Ectopic Cushing's syndrome in light of modern diagnostic techniques and treatment options. *Neuro Endocrinol Lett* (2015) 36:201–08.

82. Pinelli S, Barbot M, Scaroni C, Ceccato F. Second-line tests in the diagnosis of adrenocorticotropic hormone-dependent hypercortisolism. *Ann Lab Med* (2021) 41:521–31. doi: 10.3343/alm.2021.41.6.521

83. Frete C, Corcuff JB, Kuhn E, Salenave S, Gaye D, Young J, et al. Non-invasive diagnostic strategy in ACTH-dependent cushing's syndrome. *J Clin Endocrinol Metab* (2020) 105(10):dgaa409. doi: 10.1210/clinem/dgaa409

84. Guignat L, Bertherat J. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline: commentary from a European perspective. *Eur J Endocrinol* (2010) 163:9–13. doi: 10.1530/EJE-09-0627

85. Collichio FA, Woolf PD, Brower M. Management of patients with small cell carcinoma and the syndrome of ectopic corticotropin secretion. *Cancer-Am Cancer Soc* (1994) 73:1361–67. doi: 10.1002/1097-0142(19940301)73:5<1361::aid-cncr2820730509>3.0.co;2-j

86. Wagner-Bartak NA, Baiomy A, Habra MA, Mukhi SV, Morani AC, Korivi BR, et al. Cushing syndrome: diagnostic workup and imaging features, with clinical and pathologic correlation. *Am J roentgenol (1976)* (2017) 209:19. doi: 10.2214/AJR.16.17290

87. Isidori AM, Kaltsas GA, Pozza C, Frajese V, Newell-Price J, Reznek RH, et al. The ectopic adrenocorticotropin syndrome: clinical features, diagnosis, management, and long-term follow-up. *J Clin Endocrinol Metab* (2006) 91:371–77. doi: 10.1210/jc.2005-1542

88. Wong SK, Iams WT. Front line applications and future directions of immunotherapy in small-cell lung cancer. *Cancers (Basel)* (2021) 13(3):506. doi: 10.3390/cancers13030506

89. Esposito G, Palumbo G, Carillio G, Manzo A, Montanino A, Sforza V, et al. Immunotherapy in small cell lung cancer. *Cancers (Basel)* (2020) 12(9):2522. doi: 10.3390/cancers12092522

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Glossary

18F-FDG	18F-fluoro-deoxy-glucose
ACTH	Corticotropin, adrenocorticotropic hormone
BIPSS	Bilateral inferior petrosal sinus sampling
CD	Cushing's disease (from pituitary)
CISS	Constructive interference in the steady state
CRH	Corticotropin-releasing hormone
CS	Cushing's syndrome
CT	Computed tomography
DDVAP	Desmopressin, 1-deamino-8-D-arginine-vasopressin
Dex-CRH	Combined LDDST-CRH test
EC	Etoposide plus carboplatin
ECS	Ectopic Cushing's syndrome
EP	Etoposide plus cisplatin
FLAIR	Fluid attenuation inversion recovery
HDDST	High-dose dexamethasone suppression test
ICI	Immune checkpoint inhibitor
IHC staining	Immunohistochemical staining
JBI	Joanna Briggs Institute
LDDST	Low-dose dexamethasone suppression test
MRI	Magnetic resonance imaging
NSCLC	Non-small cell lung cancer
ONDST	Overnight dexamethasone suppression test
PCS	Paraneoplastic Cushing's syndrome
PET	Positron emission tomography
PET/MRCR	PET coregistration with volumetric MRI
SCLC	Small cell lung cancer
SIADH	Syndrome of inappropriate antidiuretic hormone secretion
SPECT	Single-photon emission computed tomography
SPGR	Spoiled gradient recalled
SRS (octreoscan)	Somatostatin receptor scintigraphy (with Octreotide)
SSTR-PET/CT	Somatostain receptor-based positron emission tomography/computed tomography (with 68Ga-DOTATATE/DOTATOC/DOTANOC)
SSTR-SPECT/CT	Somatostain receptor-based single-photon emission computed tomography/computed tomography
TSE	T1-weighted turbo spin echo
US	Ultrasound

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Nivolumab-induced diabetes mellitus—a case report with literature review of the treatment options

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Background: Immune checkpoint inhibitor (ICI) treatment has become important for treating various cancer types, including metastatic renal cell carcinoma. However, ICI treatment can lead to endocrine immune-related adverse events (irAEs) by overstimulating the patient's immune system. Here, we report a rare case of a new onset of diabetes mellitus (DM), caused by nivolumab, and we discuss the feasible treatment options with a focus on TNF antagonism.

Case presentation: A 50-year-old man was diagnosed with metastatic renal cell carcinoma. Due to systemic progression, a combined immunotherapy with ipilimumab and nivolumab was initiated, according to the current study protocol (SAKK 07/17). The administration of ipilimumab was stopped after 10 months, due to partial response as seen in the computer tomography (CT), and nivolumab was continued as monotherapy. Fourteen months after the start of the treatment, the patient was admitted to the emergency department with lethargy, vomiting, blurred vision, polydipsia, and polyuria. The diagnosis of DM with diabetic ketoacidosis was established, although autoantibodies to β-cells were not detectable. Intravenous fluids and insulin infusion treatment were immediately initiated with switching to a subcutaneous administration after 1 day. In addition, the patient received an infusion of the TNF inhibitor infliximab 4 days and 2 weeks after the initial diagnosis of DM. However, the C-peptide values remained low, indicating a sustained insulin deficiency, and the patient remained on basal bolus insulin treatment. Two months later, nivolumab treatment was restarted without destabilization of the diabetic situation.

Conclusions: In contrast to the treatment of other irAEs, the administration of corticosteroids is not recommended in ICI-induced DM. The options for further treatment are mainly based on the low numbers of case series and case reports. In our case, the administration of infliximab—in an attempt to salvage the function of β-cells—was not successful, and this is in contrast to some

previous reports. This apparent discrepancy may be explained by the absence of insulin resistance in our case. There is so far no evidence for immunosuppressive treatment in this situation. Prompt recognition and immediate start of insulin treatment are most important in its management.

KEYWORDS

immune checkpoint inhibitor (ICI), immune-related adverse event (irAE), diabetes mellitus, TNF blockade, case report

Introduction

Immune checkpoint inhibitor (ICI) treatment has become an important therapeutic option in the first-line treatment of metastatic renal cell carcinoma (1–4). While being effective, ICI treatment is associated with a broad spectrum of autoimmune complications, known as immune-related adverse events (irAEs) (5–7). Thus, ICI-induced diabetes mellitus (DM) is a rare side effect but develops with a rapid loss of insulin production (5–7). Accordingly, it often presents clinically with an acute onset with severe and persistent insulin deficiency (8, 9). In this report, we present a case of nivolumab-induced DM, presenting with diabetic ketoacidosis (DKA).

Case presentation

A 50-year-old man was diagnosed with metastatic renal cell carcinoma in 2016 which was initially treated by local pulmonary surgery (2016, 2017) and 3 years later with local excision of a metastatic lesion of the right thigh (2019). Four years after the initial diagnosis, the disease progressed at multiple sites (2020). Therefore, the patient was enrolled in the study SAKK 07/17 (10), including a combined immunotherapy with ipilimumab, a CTLA-4 inhibitor, and nivolumab, a PD-1 inhibitor. Ipilimumab was administered every 6 weeks with a dosage of 1 mg/kg body weight intravenously, beginning 2 weeks after the start of nivolumab. Nivolumab was administered every 4 weeks with a dosage of 480 mg intravenously. The administration of ipilimumab could be stopped 10 months after the beginning of the treatment according to the study protocol, as a result of partial response in the CT, and nivolumab was continued. The treatment was well tolerated, and no abnormalities of blood glucose were noted so far in a normal-weight patient (BMI 24.9 kg/m^2).

Abbreviations: anti-GAD, acid decarboxylase antibodies; anti-IA2, anti-tyrosine phosphatase antibodies; ASCO, American Society of Clinical Oncology; CT, computer (computed) tomography; DM, diabetes mellitus; DKA, diabetic ketoacidosis; ESE, European Society of Endocrinology; ESMO, European Society for Medical Oncology; ICI, immune checkpoint inhibitor; ICI-induced DM, immune checkpoint inhibitor-induced diabetes mellitus; ICU, intensive care unit; irAE(s), immune-related adverse events(s); LADA, latent autoimmune DM in adult; N/A, not applicable; SITC, Society for Immunotherapy of Cancer.

However, 17 days after the last cycle of nivolumab and 14 months after the start of the whole treatment, the patient presented to the emergency department with lethargy, vomiting, blurred vision, polydipsia, and polyuria.

Diagnostic assessment

Laboratory testing revealed DKA with a venous blood gas pH of 7.058 and serum glucose of 29.0 mmol/L (Table 1). The urine analysis was positive for glucose, ketones, and proteins. The level of HbA1c was elevated to a value of 8.7% and C-peptide was low (<50 pmol/L), indicating an insulinopenic DM. The serum titers of anti-glutamic acid decarboxylase (anti-GAD) and anti-tyrosine phosphatase (anti-IA2) antibodies were not detectable. The values of amylase and lipase were remarkedly elevated without clinical signs of pancreatitis. The patient had no symptoms of exocrine pancreas insufficiency; therefore, the pancreatic elastase in the feces

TABLE 1 Laboratory results on day 1 of admission (**in blue** = values below the threshold value, **in red** = values above the threshold value, in black = values in the normal range).

Analysis	Value	Reference range
Venous blood gas Ph	7.058	7.38–7.42
Serum glucose (mmol/L)	29.0	4.3–6.4
Sodium (mmol/L)	131	135–145
Potassium (mmol/L)	5.3	3.6–4.8
Bicarbonate (mmol/L)	8.3	21–26
Anion gap (mmol/L)	29	8–16
Pancreatic amylase (U/L)	318	13–53
Lipase (U/L)	402	21–67
CRP (mg/L)	<5	<5
Interleukin 6 (ng/L)	15.2	<7.0
HbA1c (%)	8.7	4.8–5.9
Anti-GAD IgG (IU/ml)	<10	<10
Anti-IA-2 IgG (U/ml)	<15	<15
C-peptide (pmol/L)	<50	370–1,470

was not measured. Interleukin 6 was elevated in the context of a normal CRP. There were no signs of hypophysitis or other irAEs. In magnetic resonance imaging of the pancreas, there were no signs of pancreatitis or tumor progression in the pancreas. The findings were interpreted in the context of a new ICI-induced DM with the main symptom of a DKA.

Therapeutic intervention

The patient was referred to the intensive care unit (ICU), and intravenous fluids and continuous insulin infusion treatment were immediately initiated. After 1 day, ketoacidosis was resolved and glucose levels were improved; thus, insulin therapy was switched to a subcutaneous administration of insulin glargine (Lantus) and insulin lispro (Humalog). In an attempt to preserve the remaining β-cell function, infliximab (5 mg/kg body weight) was additionally administered on the fourth day of hospitalization with a repetition in the same dosage after 2 weeks. These applications were well tolerated by the patient. The C-peptide values remained low after the second infliximab infusion. Accordingly, the treatment with infliximab was not continued. The patient could be discharged with satisfactory glucose levels with insulin glargine (28 units per day) and insulin lispro (sliding scale). Over the following year, the patient remained on multiple daily insulin injections with unchanged insulin requirement. He received a flash glucose monitoring system and instructions from the local diabetes department resulting in an improvement of HbA1c and fasting glucose levels (Figure 1). Nevertheless, the patient still showed significant glucose fluctuations similar to type 1 DM. Additionally, the C-peptide values remained low (Figure 1).

Follow-up and outcome

Two months after the diagnosis of an ICI-induced DM, nivolumab was restarted without destabilizing the diabetic situation. In the recent CT scan, the patient showed persistent partial response. The treatment with nivolumab could be stopped after 2 years of treatment according to the study protocol within a still ongoing partial response in the CT.

Discussion

DM is a rare irAE after ICI treatment. It occurs in approximately 0.1%–1.0% of patients treated with ICI (8, 9). In a large case series, most cases occurred after the application of monotherapy with a PD-1 inhibitor (76%), followed by a combination therapy of CTLA-4 with either PD-1 or PD-L1 (17%) (11). ICI-induced DM is diagnosed at a median of 7–25 weeks after initiation of ICI treatment. Risk factors for an early manifestation of this irAE are described in patients with DKA (9, 12), documentation of positive islet cell antibodies (9, 12), and with a combined PD-1 and CTLA-4 inhibitor treatment.

To date, only limited data are available regarding pathophysiology and disease definition. In general, ICI-induced DM is defined as severe and persistent insulin deficiency presenting as i) DKA or with decreased to absent C-peptide and ii) persistent insulin dependence for at least weeks to months after acute illness (8, 13). However, its differentiation from an initial presentation of type 1 DM can be particularly difficult.

ICI-induced DM presents acutely in 50%–75% of cases in the setting of DKA (8, 9, 13, 14) as also shown in our case report.

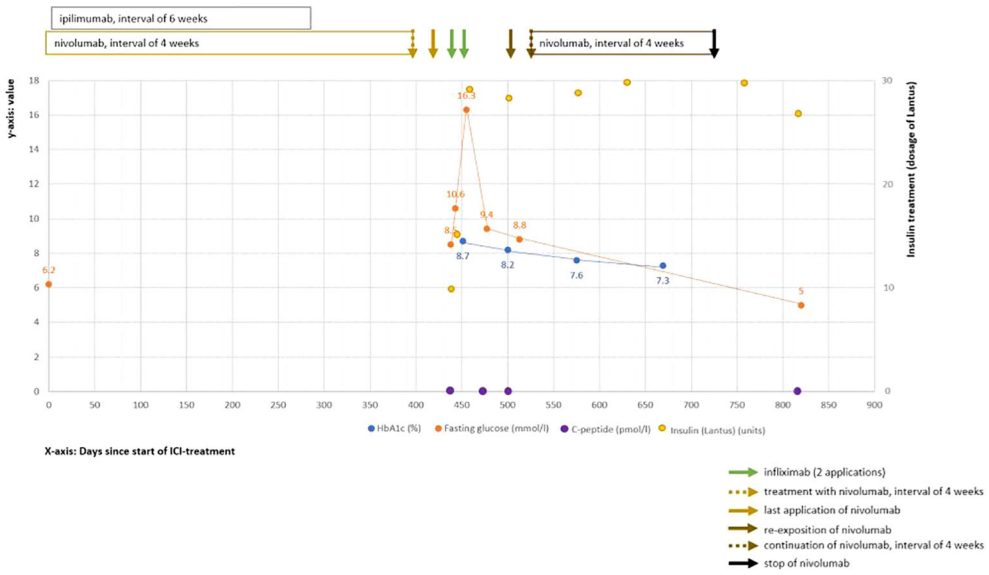

FIGURE 1
Clinical course of ICI-induced diabetes mellitus. Shown are values over the time course after starting ICI treatment (time point 0). The *Y*-axis (left side) shows the laboratory values of HbA1c (in blue color, %), fasting glucose (in orange color, mmol/L), and C-peptide (in violet color, pmol/L). On the right *Y*-axis, the insulin dosages (in yellow color) are displayed. The *X*-axis shows the time interval since the start of the ICI treatment in days.

However, a decreased incidence of DKA over the last years could be observed mainly due to increased awareness leading to an early detection and initiation of treatment (14). As the HbA1c is often elevated to 7.6%–9.7% at diagnosis, subacute severe hyperglycemia must be assumed (9, 13, 14). However, due to the commonly rapid onset of hyperglycemia in ICI-induced DM, HbA1c is not a reliable screening parameter in this patient population (15). In the case series of Stamatouli et al. (9), in 85% of patients, it was shown that acute progressive hyperglycemia is due to rapid β-cell loss, evident from low or even absent C-peptide values at diagnosis. This hypothesis is supported by the case series of Tsang et al. (14), in which 6 out of 10 patients who developed ICI-induced DM had detectable C-peptide levels shortly before diagnosis, with a marked decrease or complete absence of detectability shortly after diagnosis. These results contrast with the observations in the Type 1 Diabetes TrialNet, where 93% of patients with type 1 DM still showed detectable C-peptide levels 2 years after initial diagnosis (16).

Additional markers in DM are DM-specific antibodies. A relevant proportion (40%–70%) of patients with ICI-induced DM have at least one positive DM-specific antibody (most anti-GAD) (9, 13, 17). However, their clinical and diagnostic relevance has not yet been fully clarified. In comparison, the autoantibody positivity in type 1 DM is 70% for anti-GAD (18), 58% for anti-IA2 (12), and 60%–80% for anti-zinc transporters, respectively. As in spontaneous type 1 DM, ICI-induced DM may be based on a genetic predisposition, with HLA genes being of particular importance as they are significantly associated with the occurrence of many autoimmune diseases. In several case series (8, 9), a predominance of DR4 and DR3 alleles could be shown in patients with ICI-induced DM, known also as susceptible alleles for the development of type 1 DM. On the other hand, the presence of protective HLA genes did not prevent the occurrence of ICI-induced DM (14).

Interestingly, elevated pancreatic enzymes can be additionally detected in 32%–57% at diagnosis, with imaging demonstrating pancreatic lesions in some cases (9, 11, 13). This suggests that the diagnosis may be preceded by exocrine pancreas inflammation. Case series support this hypothesis and show the presence of acute pancreatitis in 20% of cases at diagnosis.

Even though ICI-induced DM seems to be pathophysiological based on an insulin deficiency and thus has similarities with type 1 DM, the median age of disease manifestation (62 to 68 years) is significantly higher than in patients with type 1 DM or even LADA (latent autoimmune diabetes in adult) (9, 13, 14). This can be seen in the context of ICI exposure at older ages. As the median BMI (26 to 32.2 kg/m^2) is higher than would be expected in type 1 DM (9, 13), the differentiation from exacerbation of underlying type 2 DM may also be difficult unless there was a documented, well-controlled type 2 DM without insulin administration before the initiation of ICI treatment.

Due to the frequently acute manifestation of the disease with associated high morbidity for this already at-risk population and the rising incidence with increasingly widespread use of ICIs, thus, early detection of ICI-induced DM is of particular importance. Therefore, the ASCO Guidelines (7) recommend regular monitoring of plasma glucose before and at the beginning of each therapy cycle as well as during follow-up for at least 6 months. If an ICI-induced DM is suspected, the international guidelines (ASCO, ESMO, SITC) recommend a diagnostic workup including the examination of plasma glucose, HbA1c, diabetes-specific antibodies, C-peptide, anions gap in the metabolic panel, and urinary ketones (5–7, 11). After diagnostics, therapeutic measures should be initiated promptly and not be delayed pending the results.

For the management of ICI-induced DM, the current international guidelines (ASCO, ESMO, SITC, ESE) recommend treatment with insulin. If DKA is present, hydration, the use of insulin perfusion, correction of the electrolyte abnormalities, and ICU monitoring are mandatory (5–7, 15, 19).

In almost all reported cases, insulin dependency in ICI-induced DM was permanent. To our knowledge, only in three cases with ICI-induced DM could insulin treatment be stopped in the further course of the disease. Trinh et al. reported a case of ICI-induced DM with positive autoantibodies against islet cells, impaired insulin secretion, and insulin resistance where insulin treatment could be stopped after infliximab and intra-articular corticosteroid injections administered due to an oligoarthritis (20). In the second case, β-cell function could be regained after stopping pembrolizumab therapy, resulting in an improvement of glycemic control and detectable C-peptide values. Unfortunately, no baseline C-peptide was measured at the time of diagnosis, and therefore, it is not clear whether this patient was insulinopenic and fulfilled the diagnostic criteria of ICI-induced DM (21). The third case is a patient with BMI 26 kg/m2 (2), pre-existing hypertension, and dyslipidemia, with a detectable C-peptide at the time of diagnosis (1.0 nmol/L, normal value >0.37), a high HbA1c of 11.4%, and without DKA at the time of presentation (22). In all three cases, an insulin resistance was identified or may be suspected which might explain the different course of these cases compared with our case. Additionally, the lack of islet autoantibodies in our case may point to a difference in etiology.

In addition, there have been various attempts to treat ICI-induced DM with glucocorticoids since this treatment is well established for other irAEs. None of these attempts resulted in the resolution of the DM or reduction of insulin dosage (9, 13, 19, 23–27). Therefore, ESMO (5), ASCO (7), and ESE Guidelines (15) do not recommend the use of glucocorticoids in ICI-induced DM (Table 2).

Other immunosuppressive agents such as infliximab have been considered for the treatment of ICI-induced DM. This is based on the fact that TNF-α plays an important role in insulin resistance in rodents and that TNF-α blockers had a beneficial effect in limited cases of type 1 DM (28–31). Motivated by this and the case report by Trinh et al. (20), we opted for an early infliximab therapy in our case which unfortunately did not result in the preservation of the remaining β-cells. A possible explanation for the treatment failure of infliximab may be the absence of insulin resistance in our case, which was present in the case of Trinh et al. (20)

Additionally, other immunosuppressive treatments have been tested in ICI-induced DM. Hereby, some immunosuppressive agents (e.g., abatacept, CTLA-4-Ig) that are tested in type 1 DM are not suitable for ICI-induced DM as they interfere with the T-cell reaction necessary for the antitumor reaction. Other agents, such as

TABLE 2 Evaluated systemic therapeutic options for ICI-induced DM in addition to insulin treatment.

Case series and case reports			
Treatment	Glucocorticoids	Glucocorticoids + GLP-1 agonist	Infliximab + intra-articular corticosteroid infiltration
Author (number of patients)[reference]	1. Aleksova et al. (n = 1) (23) 2. Stamatouli et al. (n = 4) (9) 3. Kapke et al. (n = 1) (24) 4. Chae et al. (n = 1) (25) 5. Porntharukchareon et al. (n = 1) (26)	Fukui et al. (n = 1) (27)	Trinh et al. (n = 1) (20)
Dosage	1. Prednisone 2 mg/kg body weight 2. Prednisone 50 mg daily (n = 1) 10 mg daily (n = 3) 3. Prednisone 60 mg daily 4. Prednisone 10 mg daily 5. Prednisone 7.5 mg daily	Prednisone 1 g daily Exenatide 10 µg daily	No information
Insulin treatment	Persistent insulin treatment	Persistent insulin treatment	Insulin stop
Effect on β-cell function	All studies: no effect	No effect	Reversal of β-cell dysfunction Remark: partial insulin resistance
International guidelines			
ASCO Guidelines 2021 (7)	(X) Not indicated	(Not applicable) No statement	(X) Not indicated
ESMO Guidelines 2022 (5)	(X) Not recommended	(Not applicable) No statement	(Not applicable) No statement
SITC Guidelines 2021 (6)	(Not applicable) No statement	(Not applicable) No statement	(Not applicable) No statement
ESE Guidelines 2022 (15)	(X) Not recommended	(Not applicable) No statement	(Not applicable) No statement

tocilizumab (anti-IL-6) or rituximab (anti-CD20), have been described as prolonging C-peptide production without interference in antitumor immunity and therefore might influence β-cell dysfunction in early ICI-induced DM (8, 32, 33). To our knowledge, there are no data regarding these immunosuppressants in ICI-induced DM (Table 2).

Moreover, it needs to be considered that—by the time of a manifest hyperglycemia in type 1 DM—already 40%–95% of the pancreatic β-cells are irreversibly lost with possibly an even greater loss in ICI-induced DM due to its rapid occurrence (34–36). Therefore, it is questionable whether any immunosuppressive treatment may preserve β-cell function, even if administered early. Due to this fact, the current international guidelines do not indicate the use of any immunosuppressive treatment (7).

Regarding the ICI treatment itself, international guidelines recommend pausing it until glucose levels are controlled or at least until DKA is resolved (5–7). After stabilization of DM, current literature advocates the resumption of therapy, particularly in patients with clinical response (11, 19).

Ultimately, the treatment of ICI-induced DM consists of prolonged insulin treatment and patient education about DM management (7, 15). Assisting measures such as flash glucose monitoring may contribute to a better glucose control and HbA1c (37) as was also demonstrated by our case.

In a few studies, oral antidiabetic agents were added to the insulin regime with an improvement of glucose control despite the insulinopenic character of the DM (38, 39). Administration of a GLP-1 agonist (glucagon-like peptide-1 receptor agonist) had no influence on the endogenous insulin secretion or the insulin dosage in one case report (27) (Table 2).

Conclusion

We report a case with the new onset of DM due to PD-1 blockade. The administration of infliximab did not lead to an improvement of β-cell function, which was shown in persistent low C-peptide values and the necessity of insulin injections. There is little evidence for the administration of an immunosuppressive treatment in this situation. Therefore, the mainstay of treatment remains the administration of insulin. We emphasize the need for prompt recognition, the involvement of endocrinologists, and the necessity of urgent treatment as a fatal outcome could be possible.

Data availability statement

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

Ethics statement

Ethical approval was not required for the studies involving humans because it is not necessary (case report, retrospective). The

studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

ED, AZ, MD, and LG participated in the care of the patient. ED, SD, and LG drafted the manuscript. AZ and MD have revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

AZ received consulting/advisor fees from Bristol-Myers Squibb, Merck Sharp & Dohme, Hoffmann–La Roche, NBE Therapeutics, Secarna, ACM Pharma, and Hookipa and maintains further non-commercial research agreements with Secarna, Roche, Vectorbiopharma, T3 Pharma, and Bright Peak Therapeutics.

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

Publisher's note

References

1. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, et al. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. *N Engl J Med* (2018) 378(14):1277–90. doi: 10.1056/NEJMoa1712126

2. Motzer R, Alekseev B, Rha SY, Porta C, Eto M, Powles T, et al. Lenvatinib plus pembrolizumab or everolimus for advanced renal cell carcinoma. *N Engl J Med* (2021) 384(14):1289–300. doi: 10.1056/NEJMoa2035716

3. Choueiri TK, Powles T, Burotto M, Escudier B, Bourlon MT, Zurawski B, et al. Nivolumab plus Cabozantinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med* (2021) 384(9):829–41. doi: 10.1056/NEJMoa2026982

4. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med* (2019) 380(12):1116–27. doi: 10.1056/NEJMoa1816714

5. Haanen J, Obeid M, Spain L, Carbonnel F, Wang Y, Robert C, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up☆. *Ann Oncol* (2022) 33(12):1217–38. doi: 10.1016/j.annonc.2022.10.001

6. Brahmer JR, Abu-Sbeih H, Ascierto PA, Cappelli LC, Cortazar FB, Gerber DE, et al. Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immune checkpoint inhibitor-related adverse events. *J Immunother Cancer* (2021) 9(6): e002435. doi: 10.1136/jitc-2021-002435

7. Schneider BJ, Naidoo J, Santomasso BD, Lacchetti C, Adkins S, Anadkat M, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: ASCO guideline update. *J Clin Orthod* (2021) 39 (36):4073–126. doi: 10.1200/JCO.21.01440

8. Quandt Z, Young A, Anderson M. Immune checkpoint inhibitor diabetes mellitus: a novel form of autoimmune diabetes. *Clin Exp Immunol* (2020) 200 (2):131–40. doi: 10.1111/cei.13424

9. Stamatouli AM, Quandt Z, Perdigoto AL, Clark PL, Kluger H, Weiss SA, et al. Collateral damage: insulin-dependent diabetes induced with checkpoint inhibitors. *Diabetes.* (2018) 67(8):1471–80. doi: 10.2337/dbi18-0002

10. Stenner-Liewen F, Cathomas R, Rothermundt CA, Schardt J, Patrikidou A, Zihler D, et al. 716P Optimizing ipilimumab in metastatic renal cell carcinoma: SAKK 07/17 study. *Ann Oncol* (2020) 31:S563. doi: 10.1016/j.annonc.2020.08.788

11. de Filette JMK, Pen JJ, Decoster L, Vissers T, Bravenboer B, Van der Auwera BJ, et al. Immune checkpoint inhibitors and type 1 diabetes mellitus: a case report and systematic review. *Eur J Endocrinol* (2019) 181(3):363–74. doi: 10.1530/EJE-19-0291

12. Ellis TM, Schatz DA, Ottendorfer EW, Lan MS, Wasserfall C, Salisbury PJ, et al. The relationship between humoral and cellular immunity to IA-2 in IDDM. *Diabetes.* (1998) 47(4):566–9. doi: 10.2337/diabetes.47.4.566

13. Kotwal A, Haddox C, Block M, Kudva YC. Immune checkpoint inhibitors: an emerging cause of insulin-dependent diabetes. *BMJ Open Diabetes Res Care* (2019) 7 (1):e000591. doi: 10.1136/bmjdrc-2018-000591

14. Tsang VHM, McGrath RT, Clifton-Bligh RJ, Scolyer RA, Jakrot V, Guminski AD, et al. Checkpoint inhibitor-associated autoimmune diabetes is distinct from type 1 diabetes. *J Clin Endocrinol Metab* (2019) 104(11):5499–506. doi: 10.1210/jc.2019-00423

15. Husebye ES, Castinetti F, Criseno S, Curigliano G, Decallonne B, Fleseriu M, et al. Endocrine-related adverse conditions in patients receiving immune checkpoint inhibition: an ESE clinical practice guideline. *Eur J Endocrinol* (2022) 187(6):G1–G21. doi: 10.1530/EJE-22-0689

16. Greenbaum CJ, Beam CA, Boulware D, Gitelman SE, Gottlieb PA, Herold KC, et al. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. *Diabetes.* (2012) 61 (8):2066–73. doi: 10.2337/db11-1538

17. Barroso-Sousa R, Barry WT, Garrido-Castro AC, Hodi FS, Min L, Krop IE, et al. Incidence of endocrine dysfunction following the use of different immune checkpoint inhibitor regimens: A systematic review and meta-analysis. *JAMA Oncol* (2018) 4 (2):173–82. doi: 10.1001/jamaoncol.2017.3064

18. Pietropaolo M, Eisenbarth GS. Autoantibodies in human diabetes. *Curr Dir Autoimmun* (2001) 4:252–82. doi: 10.1159/000060541

19. Zand Irani A, Almuwais A, Gibbons H. Immune checkpoint inhibitor-induced diabetes mellitus with pembrolizumab. *BMJ Case Rep* (2022) 15(1). doi: 10.1136/bcr-2021-245846

20. Trinh B, Donath MY, Läubli H. Successful treatment of immune checkpoint inhibitor-induced diabetes with infliximab. *Diabetes Care* (2019) 42(9):e153–4. doi: 10.2337/dc19-0908

21. Hansen E, Sahasrabudhe D, Sievert L. A case report of insulin-dependent diabetes as immune-related toxicity of pembrolizumab: presentation, management and outcome. *Cancer Immunol Immunother.* (2016) 65(6):765–7. doi: 10.1007/s00262-016-1835-4

22. Marchand L, Thivolet A, Dalle S, Chikh K, Reffet S, Vouillarmet J, et al. Diabetes mellitus induced by PD-1 and PD-L1 inhibitors: description of pancreatic endocrine and exocrine phenotype. *Acta Diabetol* (2019) 56(4):441–8. doi: 10.1007/s00592-018-1234-8

23. Aleksova J, Lau PKH, Soldatos G, McArthur G. Glucocorticoids did not reverse type 1 diabetes mellitus secondary to pembrolizumab in a patient with metastatic melanoma. *BMJ Case Rep* (2016) 2016. doi: 10.1136/bcr-2016-217454

24. Kapke J, Shaheen Z, Kilari D, Knudson P, Wong S. Immune checkpoint inhibitor-associated type 1 diabetes mellitus: case series, review of the literature, and optimal management. *Case Rep Oncol* (2017) 10(3):897–909. doi: 10.1159/000480634

25. Chae YK, Chiec L, Mohindra N, Gentzler R, Patel J, Giles F. A case of pembrolizumab-induced type-1 diabetes mellitus and discussion of immune checkpoint inhibitor-induced type 1 diabetes. *Cancer Immunol Immunother.* (2017) 66(1):25–32. doi: 10.1007/s00262-016-1913-7

26. Porntharukchareon T, Tontivuthikul B, Sintawichai N, Srichomkwun P. Pembrolizumab- and ipilimumab-induced diabetic ketoacidosis and isolated adrenocorticotropic hormone deficiency: a case report. *J Med Case Rep* (2020) 14 (1):171. doi: 10.1186/s13256-020-02502-w

27. Fukui A, Sugiyama K. Yamada T. A case of nivolumab-induced fulminant type 1 diabetes with steroids and glucagon-like peptide 1 administration during the early onset. *J Clin Case Rep* (2016) 6(11). doi: 10.4172/2165-7920.1000883

28. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* (1993) 259(5091):87–91. doi: 10.1126/science.7678183

29. Timper K, Hruz P, Beglinger C, Donath MY. Infliximab in the treatment of Crohn disease and type 1 diabetes. *Diabetes Care* (2013) 36(7):e90–1. doi: 10.2337/dc13-0199

30. Mastrandrea L, Yu J, Behrens T, Buchlis J, Albini C, Fourtner S, et al. Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. *Diabetes Care* (2009) 32(7):1244–9. doi: 10.2337/dc09-0054

31. Myśliwska J, Ryba-Stanisławowska M, Smardzewski M, Słomiński B, Myśliwiec M, Siebert J. Enhanced apoptosis of monocytes from complication-free juvenile-onset diabetes mellitus type 1 may be ameliorated by TNF-α inhibitors. *Mediators Inflamm* (2014) 2014:946209. doi: 10.1155/2014/946209

32. Matthews JB, Staeva TP, Bernstein PL, Peakman M, von Herrath MITN-JDRF Type 1 Diabetes Combination Therapy Assessment Group. Developing combination immunotherapies for type 1 diabetes: recommendations from the ITN-JDRF Type 1 Diabetes Combination Therapy Assessment Group. *Clin Exp Immunol* (2010) 160 (2):176–84. doi: 10.1111/j.1365-2249.2010.04153.x

33. Jacobsen LM, Newby BN, Perry DJ, Posgai AL, Haller MJ, Brusko TM. Immune mechanisms and pathways targeted in type 1 diabetes. *Curr Diabetes Rep* (2018) 18 (10):90. doi: 10.1007/s11892-018-1066-5

34. Klinke DJ 2nd. Extent of beta cell destruction is important but insufficient to predict the onset of type 1 diabetes mellitus. *PloS One* (2008) 3(1):e1374. doi: 10.1371/journal.pone.0001374

35. Gale EAM. Can we change the course of beta-cell destruction in type 1 diabetes? *N Engl J Med* (2002) 346(22):1740–2. doi: 10.1056/NEJM200205303462211

36. Kyriacou A, Melson E, Chen W, Kempegowda P. Is immune checkpoint inhibitor-associated diabetes the same as fulminant type 1 diabetes mellitus? *Clin Med* (2020) 20(4):417–23. doi: 10.7861/clinmed.2020-0054

37. Rodríguez de Vera-Gómez P, Piñar-Gutiérrez A, Guerrero-Vázquez R, Bellido V, Morales-Portillo C, Sancho-Márquez MP, et al. Flash glucose monitoring and diabetes mellitus induced by immune checkpoint inhibitors: an approach to clinical practice. *J Diabetes Res* (2022) 2022:4508633. doi: 10.1155/2022/4508633

38. Alrifai T, Ali FS, Saleem S, Ruiz DCM, Rifai D, Younas S, et al. Immune checkpoint inhibitor induced diabetes mellitus treated with insulin and metformin: evolution of diabetes management in the era of immunotherapy. *Case Rep Oncol Med* (2019) 2019:8781347. doi: 10.1155/2019/8781347

39. Tittel SR, Laubner K, Schmid SM, Kress S, Merger S, Karges W, et al. Immune-checkpoint inhibitor-associated diabetes compared to other diabetes types - A prospective, matched control study. *J Diabetes.* (2021) 13(12):1007–14. doi: 10.1111/1753-0407.13215

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Causal relationship between rheumatoid arthritis and hypothyroidism or hyperthyroidism: a bidirectional two-sample univariable and multivariable Mendelian randomization study

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Objective: The causal relationship between Rheumatoid arthritis (RA) and hypothyroidism/hyperthyroidism remains controversial due to the limitations of conventional observational research, such as confounding variables and reverse causality. We aimed to examine the potential causal relationship between RA and hypothyroidism/hyperthyroidism using Mendelian randomization (MR).

Method: We conducted a bidirectional two-sample univariable analysis to investigate the potential causal relationship between hypothyroidism/hyperthyroidism and RA. Furthermore, we performed a multivariate analysis to account for the impact of body mass index (BMI), smoking quantity, and alcohol intake frequency.

Results: The univariable analysis indicated that RA has a causative influence on hypothyroidism (odds ratio [OR]=1.07, 95% confidence interval [CI]=1.01–1.14, P=0.02) and hyperthyroidism (OR=1.32, 95% CI=1.15–1.52, P<0.001). When hypothyroidism/hyperthyroidism was considered as an exposure variable, we only observed a causal relationship between hypothyroidism (OR=1.21, 95% CI=1.05–1.40, P=0.01) and RA, whereas no such connection was found between hyperthyroidism (OR=0.91, 95% CI=0.83–1.01, P=0.07) and RA. In the multivariate MR analyses, after separately and jointly adjusting for the effects of daily smoking quantity, alcohol intake frequency, and BMI, the causal impact of RA on hypothyroidism/hyperthyroidism and hypothyroidism on RA remained robust. However, there is no evidence to suggest a causal effect of hyperthyroidism on the risk of RA (P >0.05).

Conclusion: Univariate and multivariate MR analyses have validated the causal association between RA and hypothyroidism/hyperthyroidism. Hypothyroidism confirmed a causal relationship with RA when employed as an exposure variable, whereas no such relationship was found between hyperthyroidism and RA.

KEYWORDS

Mendelian randomization, rheumatoid arthritis, hypothyroidism, hyperthyroidism, causal relationship

1 Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by persistent joint pain and the degradation of joint cartilage and bone. Moreover, it causes varying degrees of harm to various extra-articular systems. In the general population, the prevalence of RA ranges from 0.5% to 1%, with a higher incidence in women than in men (1). RA leads to functional impairment, reduced work capacity, and a lower quality of life, substantially burdening individuals and society (2, 3). Furthermore, RA is associated with a significantly higher mortality rate than the general population, with approximately 40% of patients with RA succumbing to cardiovascular disease (4, 5). Thyroid dysfunction, encompassing hyperthyroidism, hypothyroidism, subclinical hyperthyroidism, and subclinical hypothyroidism, is a common endocrine disorder diagnosed primarily through biochemical indicators such as thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), free triiodothyronine (FT3), and free thyroxine (FT4). The prevalence of hyperthyroidism ranges from 0.2% to 1.3% (6, 7), while that of hypothyroidism varies from 0.2 to 5.3% (6, 8). Hyperthyroidism and hypothyroidism could impact various bodily systems, including the integumentary, muscular, skeletal, cardiovascular, nervous, digestive, endocrine, and circulatory systems. While most studies have suggested a link between RA and thyroid dysfunction (9), one study found no significant difference in the incidence and prevalence of hypothyroidism between patients with and without RA (10).

Therefore, the potential causal relationship between RA and hypothyroidism/hyperthyroidism requires further investigation. Most of our insights into the relationship between hypothyroidism/hyperthyroidism and RA are derived from observational studies, which are susceptible to reverse causality, selective bias, and confounding variables. Therefore, further research employing innovative methodologies is warranted.

Mendelian randomization (MR) is one such technique that utilizes genetic variation as an instrumental variable to assess causal relationships between exposures and specific outcomes (11).

2 Method

2.1 Data sources

We acquired summary statistics for RA from the MRCIEU GWAS database available at https://gwas.mrcieu.ac.uk/. The pooled GWAS data, involving individuals of European ancestry, comprised a population of 58,284 for subsequent analysis (GWAS ID: ieu-a-832), with 14,361 cases and 42,923 control participants (12). We obtained summary data from the publicly available FinnGen Biobank for the GWAS datasets associated with hypothyroidism and hyperthyroidism. The dataset for hypothyroidism (GWAS ID: finn-b-E4_HYTHYNAS) comprised 26,306 cases and 187,684 controls, while hyperthyroidism (GWAS ID: finn-b-AUTOIMMUNE_HYPERTHYROIDISM) comprised 962 cases and 172,976 controls. In these datasets, hypothyroidism was defined as "Hypothyroidism, other/unspecified," and hyperthyroidism as "Autoimmune hyperthyroidism." Data regarding smoking quantity were extracted from the GWAS and Sequencing Consortium of Alcohol and Nicotine use (GSCAN) (13) (cigarettes per day: GWAS ID: ieu-b-25, sample size: 337,334). Summary-level data for alcohol intake frequency were obtained from the UK BioBank (GWAS ID: ukb-a-25, sample size: 336,965). Genetic instruments associated with BMI were sourced from a previously published GWAS study (14). We obtained these data from two consortia: the Genetic Investigation of Anthropometric Traits (GIANT) consortium and the Genetic Epidemiology of Adult Health and Aging Study (GERA) consortium (GWAS ID: ebi-a-GCST006368, sample size: 315,347).

To minimize bias due to population stratification, we focused exclusively on individuals of European ancestry. All datasets are available for download from the IEU GWAS database at this link: https://gwas.mrcieu.ac.uk/datasets/.

A detailed description of the data source is provided in Supplementary Table 1.

Since all the data were derived from publicly accessible studies, our research did not require patient consent or ethical clearance.

2.2 Study design

Our study design comprised two primary steps. First, we performed a two-sample univariate analysis, using RA as the exposure variable, and examined its association with hypothyroidism/hyperthyroidism as the respective outcomes. Subsequently, we performed another two-sample univariate analysis, using hypothyroidism/hyperthyroidism as the exposure variables, and assessed their relationships with RA as the outcomes. In a second step, to ensure that any causal effects were not influenced by factors such as BMI, smoking quantity, and alcohol intake frequency, a multivariate analysis was conducted to account for the impact of these variables.

2.3 Instrumental variable selection

In MR studies, genetic variants frequently serve as instrumental variables (IVs). Three critical assumptions must be met to obtain reliable casual estimates in MR studies: 1) IVs should exhibit a strong association with the exposure; 2) IVs should not be associated with any potential confounders that might affect the relationship between the exposure and outcome; 3) IVs should exclusively influence the outcome through the exposure (15, 16). Figure 1 depicts a detailed description.

Firstly, we identified independent single nucleotide polymorphisms (SNPs) significantly associated with the exposure ($P<5\times10^{-8}$). We conducted SNP clustering with a window size of 10,000 kb and an R2<0.001 threshold to remove linkage disequilibrium (LD). Subsequently, we checked all the exposure-related SNPs in the PhenoScanner database (http://www.phenoscanner.medschl.cam.ac.uk/) to identify any SNPs associated with potential confounders (BMI, smoking, alcohol consumption) and the outcome ($P<5\times10^{-8}$). We extracted SNP effects from the outcome GWAS dataset and harmonized the impact of the exposure and outcome. We excluded palindromic SNPs with ambiguous results (EAF >0.42). We employed the MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) method to identify and remove potential outliers (17). Finally, we assessed instrumental strength using the F-statistic ($F = \frac{N-k-1}{k} \times \frac{R^2}{1-R^2}$), following the procedures outlined by Burgess and Thompson (18).

3 Mendelian randomization analyses

For our MR analyses, we employed R (version 4.2.2) packages, namely "TwoSampleMR" (version 0.5.6) and "MR-PRESSO" (version 1.0).

We assessed the association between hypothyroidism/hyperthyroidism and RA using three methods: inverse variance weighted (IVW), weighted median (WM), and Mendelian randomization-Egger (MR-Egger) methods. IVW method offers consistent estimates when all genetic variants are valid IVs (19). Conversely, MR-Egger regression provides consistent estimates, notably when all considered genetic variants are incorrect IVs. The weighted median approach generates consistent appraisals, requiring at least half of the weights to be derived from accurate IVs (20). Our primary result was based on the IVW method, while MR-Egger and WM approaches were used to assess the reliability and stability of the results.

We used the MR-Egger intercept test to detect horizontal pleiotropy, with a P-intercept >0.05 indicating the absence of such pleiotropy. We further employed the IVW method and Egger regression to evaluate heterogeneity, with P<0.05 indicating its presence. Cochran's Q statistic was used to assess heterogeneity (21). Moreover, we conducted a leave-one-out analysis to investigate whether a single SNP was driving the causal association.

3.1 Univariable MR estimates

A rigorous screening process included removing specific SNPs, including rs6679677, rs13426947, rs3087243, rs34046593, rs2561477, rs2844456, rs6936656, rs1571878, rs12764378, rs706778, rs8032939, and rs34536443 for their associations with confounders or outcomes. Furthermore, rs1042169, rs13330176, rs225433, rs2661798, rs3799963, and rs4452313 were excluded as they failed to harmonize. Subsequently, we employed 26 valid IVs for MR estimation of RA's impact on hypothyroidism/hyperthyroidism (Supplementary Tables 2, 3). For the MR estimation of hypothyroidism's impact on RA, we considered and

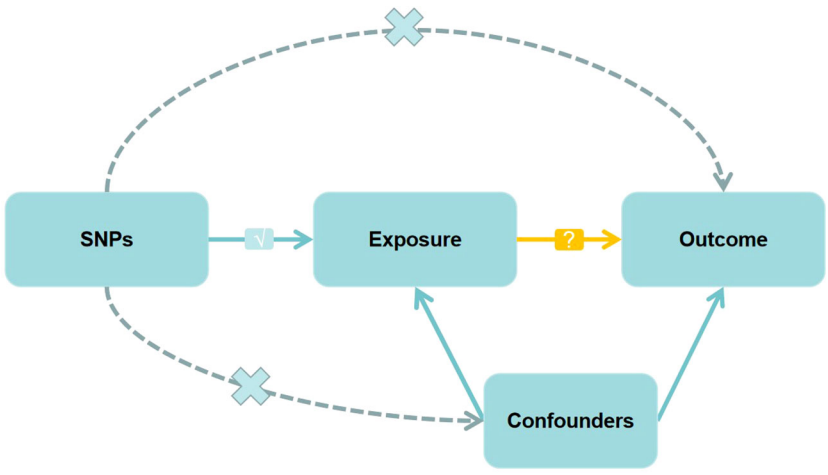

FIGURE 1
Directed acyclic graph of the Mendelian randomization (MR) framework investigating the causal relationship between exposure and outcome. The 'x' means that genetic variants are not associated with confounders or cannot be directly involved in outcome but via the exposure pathway. The '√' means that genetic variants are highly correlated with exposure. SNPs, single-nucleotide polymorphisms.

utilized 31 valid IVs (Supplementary Table 5), following the removal of specific SNPs (rs6679677, rs4853458, rs11571297, rs932036, rs9277542, rs707937, rs7902146, rs4409785, and rs7310615) due to their associations with confounders or outcomes, and rs9265890, which failed to harmonize. We considered and utilized three valid IVs for the MR estimation of hyperthyroidism's impact on RA (Supplementary Table 5). These were retained after excluding specific SNPs (rs6679677 and rs9275576) due to their associations with confounders or outcomes and rs9265890, which failed to harmonize.

In the initial step of the univariable MR analysis, we found a significant causal effect of RA on hypothyroidism (IVW: odds ratio [OR] = 1.07, 95% confidence interval [CI] = 1.01–1.14, P = 0.02). The results obtained from the WM method aligned with those from the IVW method (P = 0.02). However, the MR-Egger regression method indicated a similar causal effect direction; however, they did not reach significance (P = 0.63). A significant causal effect of RA on hyperthyroidism was observed (IVW OR = 1.32, 95% CI = 1.15–1.52, P< 0.001). The WM method results were consistent with the IVW method (P = 0.03); however, the MR-Egger regression method results were insignificant (P = 0.21). Conversely, in the inverse analysis, hypothyroidism exhibited a significant causal effect on RA (IVW OR = 1.21, 95% CI = 1.05–1.40, P = 0.01). However, neither reached significance, while the WM method (P = 0.06) and MR-Egger regression (P = 0.89) indicated causal effects in the same direction. However, there was no estimated causal effect of hyperthyroidism on RA in the IVW (P = 0.07) and MR-Egger regression methods (P = 0.27). Although, the WM method (P = 0.02) showed a causal effect of hyperthyroidism on RA, the focus was on the results of the IVW method, as shown in Table 1 and Figure 2A. All instrumental variables used in this study exhibited F-statistic values exceeding 10, indicating the robustness of the selected IVs (Supplementary Tables 2-5).

In the sensitivity analysis, Cochran's Q test (P<0.05) revealed heterogeneity in the valid IVs used to estimate the effect of RA on hypothyroidism and hypothyroidism on RA. Therefore, a random effects model was employed. Conversely, the valid IVs used to assess the impact of RA on hyperthyroidism and hyperthyroidism on RA did not exhibit heterogeneity in Cochran's Q test (P >0.05). Furthermore, there was no evidence of horizontal pleiotropy (P >0.05 for MR-Egger intercept) in any of the univariate MR analyses (Supplementary Table 6). The leave-one-out analysis results (Supplementary Figure 3) indicated no individual SNP significantly affected the causal effects. Scatter and funnel plots are presented in Supplementary Figures 1 and 2.

3.2 Multivariable MR estimates

In multivariable MR analysis, we adjusted individually for smoking quantity, alcohol intake frequency, and BMI; strong evidence supported a direct causal effect of RA on the risk of hypothyroidism. This was observed when adjusting for smoking quantity (IVW: OR = 1.19, P<0.001), alcohol intake frequency (IVW: OR = 1.19, P<0.001), and BMI (IVW: OR = 1.21, P<0.001). In multivariable MR analysis jointly adjusted for these factors, the effect size of the association slightly increased (IVW: OR = 1.21, P<0.001).

Similarly, in multivariable MR analysis, when individually adjusting for smoking quantity, alcohol intake frequency, and BMI, strong evidence supported a direct causal effect of RA on the risk of hyperthyroidism. This was observed when adjusting for smoking quantity (IVW: OR = 1.34, P<0.001), alcohol intake frequency (IVW: OR = 1.32, P<0.001), and BMI (IVW: OR = 1.27, P<0.001). In multivariable MR analysis jointly adjusted for these factors, the effect size of the association was slightly reduced (IVW: OR = 1.27, P<0.001).

TABLE 1 MR Results of RA on Risk of hypothyroidism/hyperthyroidism, and hypothyroidism/hyperthyroidism on Risk of RA.

Exposures	Outcomes	nSNPs	Method	OR (95%CI)	P
RA	Hypothyroidism	26	MR-Egger	1.03(0.90-1.19)	0.63
			WM	1.07(1.01-1.14)	0.02
			IVW	1.07(1.01-1.14)	0.02
RA	Hyperthyroidism	26	MR-Egger	1.20(0.91-1.58)	0.21
			WM	1.25(1.02-1.54)	0.03
			IVW	1.32(1.15-1.52)	<0.001
Hypothyroidism	RA	31	MR-Egger	1.03(0.70-1.51)	0.89
			WM	1.14(1.00-1.31)	0.06
			IVW	1.21(1.05-1.40)	0.01
Hyperthyroidism	RA	3	MR-Egger	0.55(0.32-0.94)	0.27
			WM	0.92(0.85-0.99)	0.02
			IVW	0.91(0.83-1.01)	0.07

RA, Rheumatoid arthritis; IVW, inverse variance weighted; WM, weighted median; nSNPs, number of SNPs used in MR; OR, odds ratio; CI, confidence interval; a Statistically significant (p< 0.05).

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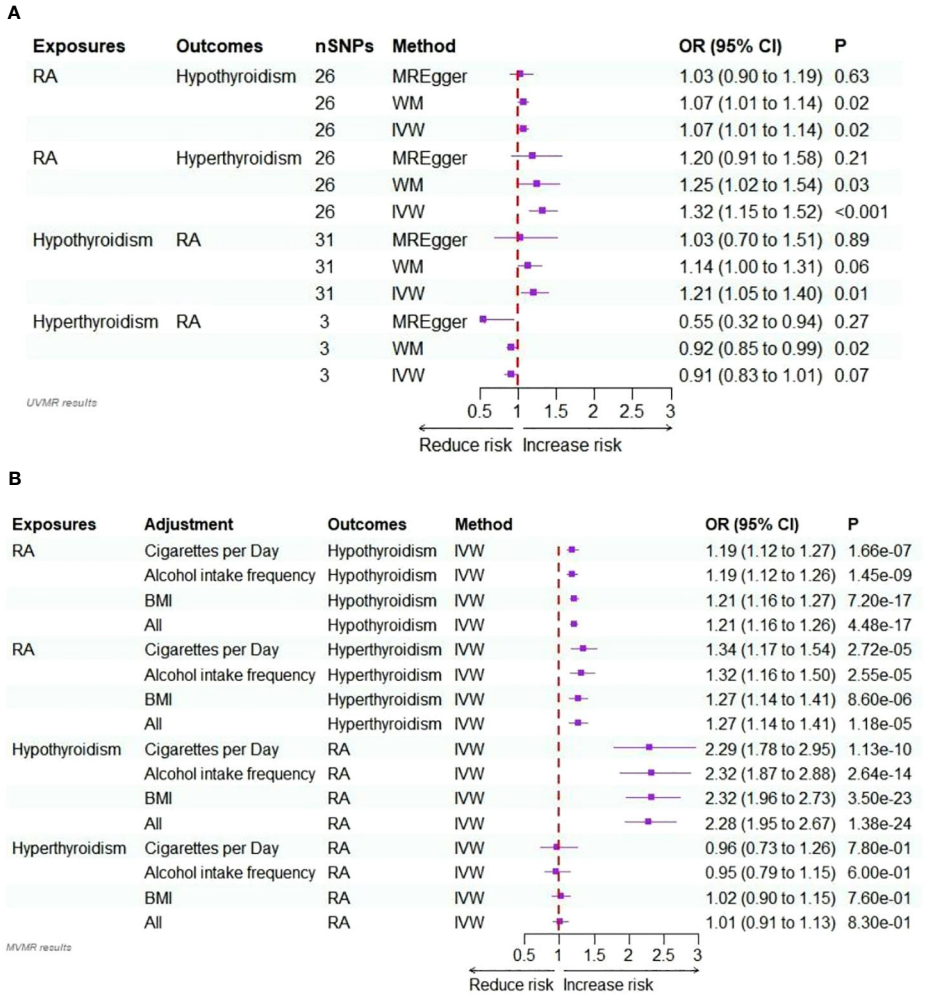

FIGURE 2
(A) Forest plots of the bidirectional two-sample univariable Mendelian randomization analysis of the relationship for RA on hypothyroidism/hyperthyroidism, and hypothyroidism/hyperthyroidism on RA. **(B)** Forest plots of the bidirectional two-sample multivariable Mendelian randomization analysis of the relationship for RA on hypothyroidism/hyperthyroidism, and hypothyroidism/hyperthyroidism on RA.

In the multivariable MR analysis, where we individually adjusted for smoking quantity, alcohol intake frequency, and BMI, there was compelling evidence of a direct causal effect of hypothyroidism on the risk of RA. Specifically, when adjusted for smoking quantity, the IVW OR was 2.29 with P<0.001. Similarly, the IVW OR was 2.32 (P<0.001) when adjusted for alcohol intake frequency and 2.32 (P<0.001) when adjusted for BMI. The association remained strong even in the multivariable MR analysis, simultaneously adjusted for all these factors (IVW: OR = 2.28, P<0.001).

However, there was no discernible evidence of a causal effect of hyperthyroidism on the risk of RA in the multivariate MR analyses, whether adjusted for smoking quantity, alcohol intake frequency, and BMI individually or collectively (P >0.05). Detailed results are provided in Supplementary Table 7, Figure 2B.

4 Discussion

In our study, we performed bidirectional two-sample univariable and multivariable MR analyses to investigate the potential causal relationship between RA and hypothyroidism/hyperthyroidism. The results of the univariable analysis indicated a causal relationship between RA and hypothyroidism/hyperthyroidism. However, when we considered hypothyroidism/hyperthyroidism as exposure factors, we only identified a causal relationship between hypothyroidism and RA and not between hyperthyroidism and RA. In the multivariate MR analyses, after adjusting for smoking quantity, alcohol intake frequency, and BMI, the causal association between RA and hypothyroidism/hyperthyroidism and that between hypothyroidism and RA remained robust.

RA is an autoimmune disease characterized by symmetrical synovial inflammation resulting from a complex interplay of genetic and environmental factors that disrupt immune tolerance. Extra-articular clinical manifestations occur in approximately 40% of patients with RA (22). Thyroid dysfunction represents one of the most common chronic endocrine disorders. Patients with thyroid dysfunction frequently exhibit clinical manifestations similar to those seen in patients with RA, such as fatigue, muscle weakness,

joint pain, and swelling. Furthermore, both conditions share similar pathogenic mechanisms involving autoimmune, inflammatory, genetic, and environmental factors. This study focuses explicitly on hyperthyroidism and hypothyroidism in thyroid dysfunction. Thyroid dysfunction, with or without autoimmune thyroid disease (AITD), is observed in 6% to 33.8% of patients with RA (23). The results of a meta-analysis highlight an increased risk of thyroid dysfunction in patients with RA, with a more significant association with hypothyroidism (OR= 2.25, 95% CI=1.78–2.84) than hyperthyroidism (OR 1.65, 95% CI 1.24–2.19), consistent with our findings (9). The predominant causes of hyperthyroidism and hypothyroidism are Graves' disease and Hashimoto's thyroiditis, respectively. In a study involving 2791 cases of Graves' disease and 495 cases of Hashimoto's thyroiditis, 3.15% of individuals with Graves' disease and 4.24% of individuals with Hashimoto's thyroiditis had RA (24). Furthermore, research by Nisihara et al. revealed positive antinuclear antibodies (ANA) in 17.5% of patients with AITD (excluding rheumatic diseases), with rheumatoid factor (RF) detected in 7.7% of such patients (25). In a study by Elnady et al., significant differences were observed with ANA (50.8%), RF (34.4%), and anti-cyclic citrullinated peptide (anti-CCP) (19.7%) (26). Conversely, in a cohort study of 800 patients with RA, 9.8% had AITD, 37.8% tested positive for anti-thyroid peroxidase (TPO) antibodies, and 20.8% were positive for anti-thyroglobulin (TG) antibodies (27). This evidence suggests an association between RA and hypothyroidism/hyperthyroidism, warranting further investigation into the underlying mechanisms that link these conditions.

Autoimmune thyroid disease significantly contributes to thyroid dysfunction. Shared physiopathologic mechanisms exist among autoimmune diseases (AD), including RA and AITD (28, 29). These conditions share common susceptibility genes and environmental factors, which could account for the higher incidence of autoimmune diseases in affected individuals. Some susceptibility genes associated with the development of RA include HLA-DRB1, PTPN22, AFF3, CD28, CD40, CTLA4, IL2RA, IL2, IL21, PRKCQ, STAT4, TAGAP, REL, TNFAIP3, TRAF1, BLK, CCL21, FCGR2A, PADI4, and PRDM1 (30). Furthermore, susceptibility genes for AITD include TSHR, TG, HLA, CTLA4, PTPN22, CD40, CD25, ARID 5B, BT61, FCRL3, IL2RA, and FOXP3 (31). Among these, PTPN22, CTLA4, HLA_DRB1, FCRL3, and IL2RA are common susceptibility genes between both conditions, while CD40 is exclusively associated with Graves' disease and RA (32). Regarding environmental factors, smoking significantly increases the risk of RA (33) and Graves' disease (34). Surprisingly, smoking has been found to reduce the risk of hypothyroidism, although this protective effect diminishes in the years following smoking cessation (35–37). Alcohol intake frequency is protective against RA (38, 39). Meanwhile, Carlé demonstrated that moderate alcohol intake frequency has diminished the risk of developing Hashimoto's thyroiditis and Graves' disease (40). Furthermore, gut flora dysbiosis has been proposed as an essential environmental factor in developing RA and hypothyroidism/hyperthyroidism (41–43). Chen et al. found that anti-tumor necrosis factor treatment (anti-TNFα) in mice reduced the expression of pro-inflammatory cytokines in the thyroid gland, thereby reducing inflammation (44). Hennie et al. showed that in individuals with RA and hypothyroidism, anti-TNFα therapy was associated with improved thyroid function (45). Moreover, patients receiving anti-TNFα treatment had a lower incidence of thyroid disease than those who did not (46). Elevated levels of interleukin-1 (IL-1) and IL-6 have been associated with the severity of RA and joint damage (47, 48). In summary, our study associates RA with hypothyroidism/hyperthyroidism, confirming that RA increases the risk of hypothyroidism/hyperthyroidism, and conversely, hypothyroidism elevates the risk of RA. However, we did not identify a causal relationship between hyperthyroidism and the risk of developing RA. We propose that hyperthyroidism might have a protective effect against RA by potentially increasing T regulatory cells, countering the adverse effects of hyperthyroidism on autoimmunity. However, further experimental validation with larger sample sizes is essential.

The strengths of our study include the utilization of bidirectional two-sample univariable and multivariable MR analyses to explore the potential causal relationship between RA and hypothyroidism/hyperthyroidism. Furthermore, using exposure and outcome datasets from distinct consortiums minimizes the impact of sample overlap.

However, this research has several limitations. Firstly, despite employing an MR design and excluding known confounders, the unaccounted potential confounders could still affect the results. Secondly, this research exclusively focused on individuals of European ancestry, which could limit the generalizability of the findings to individuals of other ancestral backgrounds. Therefore, caution is warranted when interpreting the implications of our findings for broader populations. Thirdly, autoimmune diseases generally exhibit a higher susceptibility in women than men. However, due to limitations in the available data from the original GWAS, we could not stratify the study by sex. Furthermore, our respective indicators for smoking and alcohol consumption, namely " cigarettes per day" and "alcohol intake frequency" provide a limited perspective as they only reflect the quantity and frequency of smoking and drinking without capturing the full biological effects. Comprehensive indicators are available, such as the duration, frequency, and type of smoking, and the description of drinking includes the number of units per drink, the total weekly alcohol consumption, and the type of alcohol consumed. Therefore, further analysis is needed to comprehensively explore the effects of smoking and drinking. Finally, hypothyroidism and hyperthyroidism have complex etiologies with multiple subtypes, and the lack of consideration for these subtypes is a limitation in our MR analysis.

5 Conclusion

Our study utilized bidirectional two-sample univariable and multivariable MR analytical techniques to investigate a potential causal relationship between RA and hypothyroidism/hyperthyroidism. We confirmed the causal relationship between

RA and hypothyroidism/hyperthyroidism. When hypothyroidism/hyperthyroidism were considered as exposure factors, we identified a causal relationship between hypothyroidism and RA; however, no such association was identified between hyperthyroidism and RA.

Data availability statement

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

Ethics statement

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participant's legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and institutional requirements.

Author contributions

RL: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. XD: Data curation, Visualization, Writing – original draft. XL: Visualization, Writing – original draft. QL: Data curation, Visualization, Writing – original draft. KZ: Data curation, Visualization, Writing – original draft. DP: Supervision, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1256208/full#supplementary-material

References

1. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res* (2002) 4 Suppl 3(Suppl 3):S265–72. doi: 10.1186/ar578

2. Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. *Ann Rheumatic Dis* (2014) 73(7):1316–22. doi: 10.1136/annrheumdis-2013-204627

3. Sokka T, Kautiainen H, Pincus T, Verstappen SMM, Aggarwal A, Alten R, et al. Work disability remains a major problem in rheumatoid arthritis in the 2000s: data from 32 countries in the QUEST-RA study. *Arthritis Res Ther* (2010) 12(2):R42. doi: 10.1186/ar2951

4. Symmons DPM, Gabriel SE. Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE. *Nat Rev Rheumatol* (2011) 7(7):399–408. doi: 10.1038/nrrheum.2011.75

5. Holmqvist M, Gränsmark E, Mantel A, Alfredsson L, Jacobsson LTH, Wallberg-Jonsson S, et al. Occurrence and relative risk of stroke in incident and prevalent contemporary rheumatoid arthritis. *Ann Rheumatic Dis* (2013) 72(4):541–6. doi: 10.1136/annrheumdis-2012-201387

6. Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré JC. The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. *J Clin Endocrinol Metab* (2014) 99(3):923–31. doi: 10.1210/jc.2013-2409

7. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* (2002) 87(2):489–99. doi: 10.1210/jcem.87.2.8182

8. Rosario PW. Subclinical hypothyroidism. *N Engl J Med* (2017) 377(14):1404. doi: 10.1056/NEJMc1709853

9. Liu Y-J, Miao H-B, Lin S, Chen Z. Association between rheumatoid arthritis and thyroid dysfunction: A meta-analysis and systematic review. *Front Endocrinol* (2022) 13:1015516. doi: 10.3389/fendo.2022.1015516

10. McCoy SS, Crowson CS, Gabriel SE, Matteson EL. Hypothyroidism as a risk factor for development of cardiovascular disease in patients with rheumatoid arthritis. *J Rheumatol* (2012) 39(5):954–8. doi: 10.3899/jrheum.111076

11. Davey Smith G, Ebrahim S. What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? *BMJ (Clinical Res ed.)* (2005) 330(7499):1076–9. doi: 10.1136/bmj.330.7499.1076

12. Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* (2014) 506(7488):376–81. doi: 10.1038/nature12873

13. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nat Genet* (2019) 51(2):237–44. doi: 10.1038/s41588-018-0307-5

14. Hoffmann TJ, Choquet H, Yin J, Banda Y, Kvale MN, Glymour M, et al. A large multiethnic genome-wide association study of adult body mass index identifies novel loci. *Genetics* (2018) 210(2):499–515. doi: 10.1534/genetics.118.301479

15. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* (2003) 32(1):1-22. doi: 10.1093/ije/dyg070

16. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet* (2014) 23(R1):R89–98. doi: 10.1093/hmg/ddu328

17. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet* (2018) 50(5):693–8. doi: 10.1038/s41588-018-0099-7

18. Burgess S, Thompson SG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol* (2011) 40(3):755–64. doi: 10.1093/ije/dyr036

19. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* (2013) 37 (7):658–65. doi: 10.1002/gepi.21758

20. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. *Int J Epidemiol* (2016) 45(6):1961–74. doi: 10.1093/ije/dyw220

21. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* (2016) 40(4):304–14. doi: 10.1002/gepi.21965

22. Guellec D, Cozien S, Ruyssen-Witrand A, Dieudé P, Saraux A. Prevalence and clinical significance of extra-articular manifestations at diagnosis in the ESPOIR cohort with recent-onset arthritis. *Semin In Arthritis Rheumatism* (2020) 50(3):409–13. doi: 10.1016/j.semarthrit.2020.01.004

23. Przygodzka M, Filipowicz-Sosnowska A. Prevalence of thyroid diseases and antithyroid antibodies in women with rheumatoid arthritis. *Polskie Archiwum Medycyny Wewnetrznej* (2009) 119(1-2):39–43. doi: 10.20452/pamw.600

24. Boelaert K, Newby PR, Simmonds MJ, Holder RL, Carr-Smith JD, Heward JM, et al. Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease. *Am J Med* (2010) 123(2):183.e1–9. doi: 10.1016/j.amjmed.2009.06.030

25. Nisihara R, Pigosso YG, Prado N, Utiyama SRR, De Carvalho GA, Skare TL, et al. Rheumatic disease autoantibodies in patients with autoimmune thyroid diseases. *Med Principles Pract Int J Kuwait University Health Sci Centre* (2018) 27(4):332–6. doi: 10.1159/000490569

26. Elnady BM, Kamal NM, Shaker RHM, Soliman AF, Hasan WA, Alghamdi HA, et al. Prevalence and clinical significance of nonorgan specific antibodies in patients with autoimmune thyroiditis as predictor markers for rheumatic diseases. *Medicine* (2016) 95(38):e4336. doi: 10.1097/MD.0000000000004336

27. Cárdenas Roldán J, Amaya-Amaya J, Castellanos-de la Hoz J, Giraldo-Villamil J, Montoya-Ortiz G, Cruz-Tapias P, et al. Autoimmune thyroid disease in rheumatoid arthritis: a global perspective. *Arthritis* (2012) 2012:864907. doi: 10.1155/2012/864907

28. Deshmukh HA, Maiti AK, Kim-Howard XR, Rojas-Villarraga A, Guthridge JM, Anaya J-M, et al. Evaluation of 19 autoimmune disease-associated loci with rheumatoid arthritis in a Colombian population: evidence for replication and gene-gene interaction. *J Rheumatol* (2011) 38(9):1866–70. doi: 10.3899/jrheum.110199

29. Anaya J-M, Rojas-Villarraga A, García-Carrasco M. The autoimmune tautology: from polyautoimmunity and familial autoimmunity to the autoimmune genes. *Autoimmune Dis 2012* (2012) p:297193. doi: 10.1155/2012/297193

30. Zhernakova A, Withoff S, Wijmenga C. Clinical implications of shared genetics and pathogenesis in autoimmune diseases. *Nat Rev Endocrinol* (2013) 9(11):646–59. doi: 10.1038/nrendo.2013.161

31. Effraimidis G, Wiersinga WM. Mechanisms in endocrinology: autoimmune thyroid disease: old and new players. *Eur J Endocrinol* (2014) 170(6):R241–52. doi: 10.1530/EJE-14-0047

32. Huber A, Menconi F, Corathers S, Jacobson EM, Tomer Y. Joint genetic susceptibility to type 1 diabetes and autoimmune thyroiditis: from epidemiology to mechanisms. *Endocrine Rev* (2008) 29(6):697–725. doi: 10.1210/er.2008-0015

33. Meng W, Zhu Z, Jiang X, Too CL, Uebe S, Jagodic M, et al. DNA methylation mediates genotype and smoking interaction in the development of anti-citrullinated peptide antibody-positive rheumatoid arthritis. *Arthritis Res Ther* (2017) 19(1):71. doi: 10.1186/s13075-017-1276-2

34. Ferrari SM, Fallahi P, Antonelli A, Benvenga S. Environmental issues in thyroid diseases. *Front In Endocrinol* (2017) 8:50. doi: 10.3389/fendo.2017.00050

35. Belin RM, Astor BC, Powe NR, Ladenson PW. Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* (2004) 89(12):6077–86. doi: 10.1210/jc.2004-0431

36. Andersen SL, Olsen J, Wu CS, Laurberg P. Smoking reduces the risk of hypothyroidism and increases the risk of hyperthyroidism: evidence from 450,842 mothers giving birth in Denmark. *Clin Endocrinol* (2014) 80(2):307–14. doi: 10.1111/cen.12279

37. Carlé A, Bülow Pedersen I, Knudsen N, Perrild H, Ovesen L, Banke Rasmussen L, et al. Smoking cessation is followed by a sharp but transient rise in the incidence of overt autoimmune hypothyroidism - a population-based, case-control study. *Clin Endocrinol* (2012) 77(5):764–72. doi: 10.1111/j.1365-2265.2012.04455.x

38. Azizov V, Dietel K, Steffen F, Dürholz K, Meidenbauer J, Lucas S, et al. Ethanol consumption inhibits TFH cell responses and the development of autoimmune arthritis. *Nat Commun* (2020) 11(1):1998. doi: 10.1038/s41467-020-15855-z

39. Scott IC, Tan R, Stahl D, Steer S, Lewis CM, Cope AP. The protective effect of alcohol on developing rheumatoid arthritis: a systematic review and meta-analysis. *Rheumatol (Oxford England)* (2013) 52(5):856–67. doi: 10.1093/rheumatology/kes376

40. Carlé A, Bülow Pedersen I, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, et al. Graves' hyperthyroidism and moderate alcohol consumption: evidence for disease prevention. *Clin Endocrinol* (2013) 79(1):111–9. doi: 10.1111/cen.12106

41. Yan H-X, An W-C, Chen F, An B, Pan Y, Jin J, et al. Intestinal microbiota changes in Graves' disease: a prospective clinical study. *Bioscience Rep* (2020) 40(9): BSR20191242. doi: 10.1042/BSR20191242

42. Knezevic J, Starchl C, Tmava Berisha A, Amrein K. Thyroid-gut-axis: how does the microbiota influence thyroid function? *Nutrients* (2020) 12(6):1769. doi: 10.3390/nu12061769

43. Chen Y, Ma C, Liu L, He J, Zhu C, Zheng F, et al. Analysis of gut microbiota and metabolites in patients with rheumatoid arthritis and identification of potential biomarkers. *Aging* (2021) 13(20):23689–701. doi: 10.18632/aging.203641

44. Chen K, Wei Y, Sharp GC, Braley-Mullen H. Decreasing TNF-alpha results in less fibrosis and earlier resolution of granulomatous experimental autoimmune thyroiditis. *J Leukocyte Biol* (2007) 81(1):306–14. doi: 10.1189/jlb.0606402

45. Raterman HG, Jamnitski A, Lems WF, Voskuyl AE, Dijkmans BAC, Bos WH, et al. Improvement of thyroid function in hypothyroid patients with rheumatoid arthritis after 6 months of adalimumab treatment: a pilot study. *J Rheumatol* (2011) 38 (2):247–51. doi: 10.3899/jrheum.100488

46. Tarhan F, Orük G, Niflioğlu O, Ozer S. Thyroid involvement in ankylosing spondylitis and relationship of thyroid dysfunction with anti-TNF α treatment. *Rheumatol Int* (2013) 33(4):853–7. doi: 10.1007/s00296-012-2438-9

47. Srirangan S, Choy EH. The role of interleukin 6 in the pathophysiology of rheumatoid arthritis. *Ther Adv In Musculoskeletal Dis* (2010) 2(5):247–56. doi: 10.1177/1759720X10378372

48. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* (2011) 117(14):3720–32. doi: 10.1182/blood-2010-07-273417

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Thyroid dysfunction and risk of cutaneous malignant melanoma: a bidirectional two-sample Mendelian randomization study

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Background: Epidemiologic and observational data have found a risk association between thyroid dysfunction and cutaneous malignant melanoma (CMM), however, the cause and direction of these effects are yet unknown. By using a bidirectional two-sample Mendelian randomization (MR) methodology, we hoped to further investigate the causal link between thyroid dysfunction and CMM in this work.

Methods: A genome-wide association study (GWAS) of 9,851,867 single nucleotide polymorphisms (SNPs) in a European population was used to develop genetic tools for thyroid dysfunction. Hypothyroidism was linked to 22,687 cases and 440,246 controls. For hyperthyroidism, there were 3545 cases and 459,388 controls. A total of 3751 cases and 372016 controls were included in the genetic data for CMM from UK Biobank (http://www.nealelab.is/uk-biobank) (the Dataset: ieu - b - 4969). Among them, inverse variance weighting (IVW) is the main MR Analysis method for causality assessment. MR-Egger method, MR Pleiotropic residual and outlier test (MR-PRESSO), and simple and weighted median (VM) were used to supplement the IVW method. Sensitivity analyses, mainly Cochran's Q test, leave-one-out analysis, and MR Egger intercept test were performed to assess the robustness of the outcomes.

Results: The two-sample MR Analysis results revealed a negative correlation between genetically predicted hypothyroidism and the probability of CMM (OR=0.987, 95%CI =0.075-0.999, p=0.041). The supplemental MR Analysis did not reveal any statistically significant differences, although the direction of the effect sizes for the other approaches was consistent with the IVW effect sizes. The results of the causal analysis were relatively robust, according to a sensitivity analysis. The risk of CMM was unaffected by hyperthyroidism (p>0.05). No correlation between CMM and thyroid dysfunction was seen in the reverse MR analysis.

Conclusion: Although the magnitude of the causal association is weak and further investigation of the mechanism of this putative causal relationship is required, our findings imply that hypothyroidism may be a protective factor for CMM.

KEYWORDS

thyroid dysfunction, hypothyroidism, hyperthyroidism, cutaneous malignant melanoma (CMM), Mendelian randomization study

Introduction

Hypothyroidism and hyperthyroidism are common endocrine disorders with potentially damaging health consequences that affect all populations worldwide. Among them, hypothyroidism affects 0.6%-12% of women and 1.3%-4% of men (1, 2); In the general population, the frequency of hyperthyroidism ranges from 0.2% to 1.3% (3–5), is 1.48 times more prevalent in women than in males (6), and rises with age. Thyroid hormones are essential for growth, neuronal development, reproduction, and regulation of energy metabolism (7). Increased blood thyroid-stimulating hormone (TSH) levels in an iodine-rich environment cause hypothyroidism, which is most typically caused by Hashimoto's thyroiditis. Hypothyroidism is widely established to be related to comorbidities such as diabetes, and cardiovascular and cerebrovascular illness (8). Graves' disease accounts for 70%-80% of hyperthyroidism in countries with adequate iodine and around 50% of hyperthyroidism in places with insufficient iodine (5, 9).

Since thyroid hormone is crucial for physiological processes like growth, maturation, and human metabolism, it has been suggested previously that thyroid function may influence the development of cancer (10, 11). With inconsistent findings from past research, the precise relationship between thyroid function and cancer has been a matter of controversy for more than 200 years (12, 13). CMM, the most dangerous type of skin cancer, is becoming more common everywhere. According to recent statistics, men and women in the United States are more likely to develop CMM than any other type of cancer (14). For a long time, scholars have recognized the "thyroid-skin connection", but there are few studies on the role of THs in the occurrence and/or progression of CMM. Early observations found that both abnormally low and excessive thyroid hormones (THs) can change the appearance and function of human skin and its appendages, leading to pretibial myxoedema and alopecia at rest (15, 16). Thyroid-stimulating hormone receptor mRNA was found to be highly expressed in cultured keratinocytes, epidermal melanocytes, and melanoma cells by Andrzej (16). Genes associated with the hypothalamic-pituitary-thyroid axis are also expressed in the skin but are selective in both cell type and gene type. Giacomo et al. described a case of melanoma onset after utilizing hormone, thyroid and growth hormone replacement therapy (17). Shah Monica et al. showed in a retrospective study that male melanoma patients had a substantially higher frequency of hypothyroidism than the overall population (18). Although the results of current observational studies are useful for researching thyroid function and CMM they are prone to be influenced by several confounding variables, making it difficult to draw reliable conclusions about the cause of an event. Therefore, more research into the causes of thyroid illness and malignant cutaneous melanoma is required.

Mendelian randomization (MR) is a data analysis method applied to the etiology inference of epidemics, which uses genetic variation to assess the causal relationship between exposure and outcome. It serves as a valuable tool, especially when randomized controlled trials are not feasible to examine causal relationships and observational studies deviate due to confounding factors or reverse causal relationships, which can be addressed by using genetic variation as a tool variable for testing exposure. Since alleles follow the principle of random allocation during gametogenesis, genotypes can be used as instrumental variables of intermediate phenotypes to be studied to infer their causal relationship with disease states, and the estimated effect value is not affected by confounding factors and reverse causality (19, 20).

Genome-wide association studies (GWAS) that have been widely disseminated in recent years have made it possible for researchers to examine complicated disorders, and the findings of many features have paved the path for rigorous and well-researched MR Analysis. Numerous MR studies have examined the link between cancer and thyroid function level in recent years. According to Lu et al.'s research, hypothyroidism has a protective causative association that is inversely correlated with the likelihood of developing hepatocellular carcinoma (HCC) (21). TSH levels were found to be inversely correlated with thyroid cancer by Yuan et al.'s MR Analysis, and thyroid dysfunction was found to be related to breast cancer (22).

However, more MR analyses are required to further examine more reliable results as the GWAS database grows. This investigation used a two-sample MR analysis to determine the possible causative link between thyroid illness and CMM.

Materials and methods

Study design

Hypothyroidism and hyperthyroidism summary data are from genome-wide association studies (GWASs) from ieu open gwas

project database (https://gwas.mrcieu.ac.uk/) retrieved. The data of hypothyroidism and hyperthyroidism were obtained from Dataset: ukb-b-20289 and Dataset: ukb-b-19732 with 9,851,867 single nucleotide polymorphisms (SNPS) in 462,933 Europeans. Hypothyroidism was associated with 22,687 cases and 440,246 controls. There were 3545 cases and 459,388 controls for hyperthyroidism. CMM of the data from the UK Biobank (http://www.nealelab.is/uk-biobank) (the Dataset: ieu-b-4969) with 3751 cases and 372,016 controls. A total of 11396019 SNPs were covered. All cases met the diagnostic criteria of hyperthyroidism/hypothyroidism and CMM (The diagnostic criteria are available in Supplementary File 1). Table 1 shows the sources of data for the analysis. The exposure and outcome samples covered in this study were all human and were secondary analyses of previously published data. Therefore, ethical approval was not required. Figure 1 shows three key assumptions of this bidirectional MR study.

Data sources and SNPs selection

We selected genome wide significant ($p< 5 \times 10^{-8}$) single nucleotide polymorphism loci (SNPs) associated with CMM from large GWAS meta-analyses as instrumental variables (IV). Using thyroid dysfunction as the exposure factor and CMM as the outcome index, we found that there were 13 SNPs in the MR analysis of hyperthyroidism and CMM and 107 SNPs in the MR analysis of hypothyroidism and CMM. Using CMM as the exposure factor and thyroid disease as the outcome index, there were 8 SNPS in the MR Analysis of cutaneous melanoma and hyperthyroidism, and 10 SNPS in the MR Analysis of hypothyroidism and cutaneous melanoma. To ensure SNP independence, we performed LD-pruned tests (r^2<0.001 and kb=10000) using R software.

TABLE 1 Sources of data for the analysis.

Phenotype		Source of Genetic Variants	
	Consortium	Participants	
Hyperthyroidism	MRC-IEU	Case	3545
		Control	459388
		Sample size	462933
		Number of SNPs	9851867
Hypothyroidism	MRC-IEU	Case	22687
		Control	440246
		Sample size	462933
		Number of SNPs	9851867
Cutaneous Malignant Melanoma	UK Biobank	Case	3751
		Control	372016
		Sample size	375767
		Number of SNPs	11396019

Statistical analysis

In this two-sample MR analysis, the bidirectional causality between thyroid dysfunction and CMM was estimated using the inverse-variance weighted (IVW), MR-Egger, simple and weighted median (VM), and MR Multiple effects residual and outlier test (MR-PRESSO).

IVW analysis is the main method of our MR research, which is characterized by regression without considering the existence of intercept term and using outcome variance because it has the most convincing estimation when directional pleiotropy of IVs is missing (23). MR-Egger is an alternative robust method for Mendelian randomization using summary data (24). At least 50% of the weight comes from valid IVs, then the weighted median will provide consistent estimates (25). MR-Egger and VM as complements should both be considered as sensitivity analyses for Mendelian randomization studies with multiple genetic variants. The MR-PRESSO method was used to identify horizontal pleiotropy outliers in multi-instrument summary-level MR testing and to reassess causal effects after removing pleiotropy IV (26).

To further account for possible horizontal pleiotropy, sensitivity analyses were performed to determine whether the results were robust or the data were heterogeneous. We used Cochran's Q value to test heterogeneity (27), p<0.05 was considered to indicate the presence of heterogeneity, and IVW random effect method was used as the main effect size. The deviation of the intercept from zero in MR Egger regression is a valid indicator of response level pleiotropy. Leave-one-out analysis was performed by deleting an SNP in the analysis and estimating the causal effect (23). The flowchart for the selection of IVs and MR Analysis is shown in Figure 2.

We used the R packages "TwoSampleMR" and "MR-PRESSO" to do the MR analysis, and R software version 4.2.1 was used for all statistical analyses. R created a graphic depiction of the data.

Results

Positive MR relationship between thyroid dysfunction and CMM

A total of 107 SNPs were included as valid IVs for this analysis. IVW is MR's method of meta-summarizing the effects of multiple loci when analyzing multiple SNPs. MR Analysis showed that hypothyroidism may increase the risk of CMM (IVW: OR = 0.987, 95%CI =0.075-0.999, P=0.041). IVW Cochran's Q analysis of p < 0.05, indicates that there is heterogeneity between SNPs, therefore IVW random effect model was used as a main analysis method. MR Egger regression is used to detect the presence of horizontal pleiotropy in IVs. When the intercept test of MR Egger

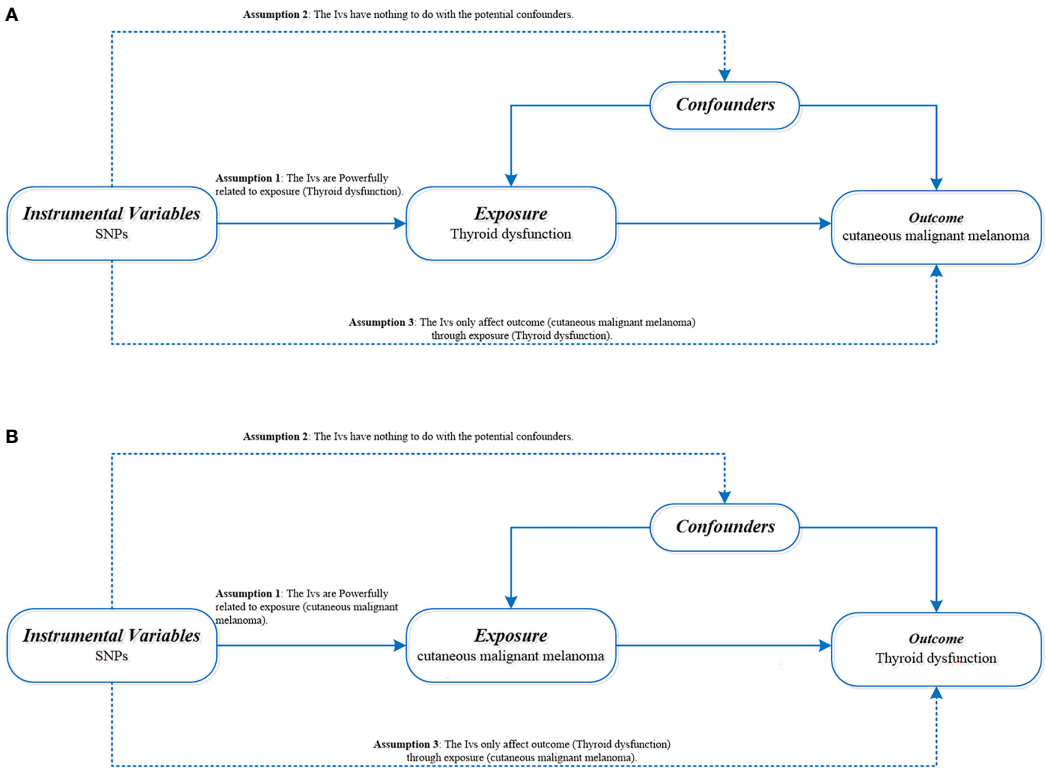

FIGURE 1
(A) Directed acyclic diagram of MR Framework to explore the causal relationship between thyroid dysfunction and CMM. **(B)** Directed acyclic graph of MR Framework to explore the causal relationship between CMM and thyroid dysfunction. SNPs, single nucleotide polymorphism; CMM, cutaneous malignant melanoma; iv, Instrumental variables.

shows $p<0.05$, it indicates that the difference is statistically significant and there is horizontal pleiotropy. However, the results of MR Egger regression here show that $p>0.05$, the difference is not statistically significant, indicating that genetic level pleiotropy does not cause bias in the results (intercept=-0.000064, $p=0.948$).

MR analysis showed that thyroid function hyperfunction and there is no causation between its CMM (IVW: OR = 0.989, 95% CI = 0.922 1.061, $p=0.753$). When hyperthyroidism was used as the exposure factor, Cochran's Q analysis of IVW was $p>0.05$, so the IVW fixed effect model was used for MR Analysis. In addition, MR – the Egger intercept method ($p=0.145$) not detected the genetic level pleiotropic; No abnormal values were detected by the MR-PRESSO method ($p=0.759$).The specific results of the above MR Analysis and the results of the sensitivity analysis are shown in Table 2 and Figure 3.

The relationship between CMM and thyroid dysfunction was examined by inverse MR

There was no evidence to support that CMM was a risk factor OR protective factor for hyperthyroidism and hypothyroidism (IVW: OR=1.016, 95%CI =0.955-1.080, $p=0.623$; OR=0.811, 95% CI= 0.647-1.016, $p=0.068$). Cochran's Q test showed that there was heterogeneity among the 10 SNPs in the MR analysis of malignant cutaneous melanoma and hypothyroidism ($p<0.05$). Therefore, the IVW random effects model was chosen. Cochran's Q test showed no significant heterogeneity of the 8 SNPs in the MR Analysis of CMM and hyperthyroidism ($p=0.607$), so we selected the IVW fixed effect model. We ultimately found no evidence to support a causal relationship between CMM and the risk of thyroid dysfunction.

Additionally, the outcomes of the IVW approach and the MR-Egger method, simple median method, weighted median method, and MR-PRESSO method were comparable (Table 2 and Figure 4). The leave-one-out strategy added to the evidence of the reliability of the effect estimates from the MR Analysis (Supplementary Materials-Figure 1).

Discussion

GWAS data from two distinct consortiums with populations of European ancestry were included in this two-sample MR Study. By using two-sample MR analysis, we methodically evaluated the bidirectional causality between thyroid insufficiency and CMM. Although there is a causal link between hypothyroidism and a lower risk of CMM, there is no causal link between hyperthyroidism and an increased risk of the disease. Similarly to this, there is no conclusive proof that there is a hereditary link between the risk of cutaneous melanoma and the occurrence of thyroid dysfunction, according to additional sensitivity analysis, and the effect estimates were reliable.

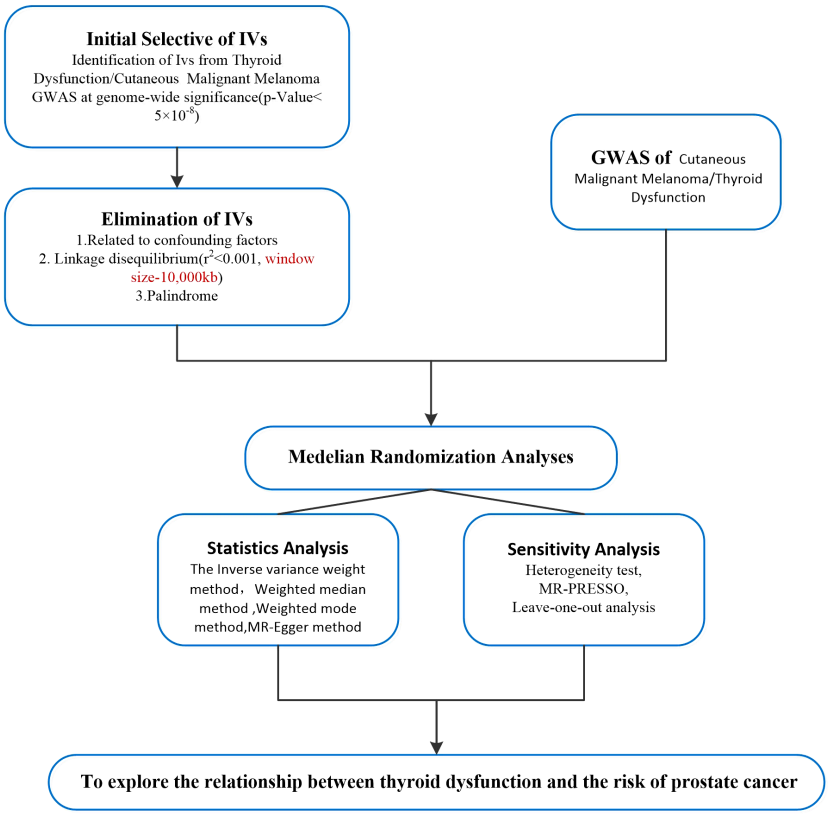

FIGURE 2
Flow chart of Mendelian randomization analysis of the study. iv, instrumental variable; GWAS, genome-wide association study; IVW, inverse variance weighted method; MR-PRESSO, Mendelian randomization pleiotropy residual sum, and outlier test; CMM, cutaneous malignant melanoma; iv, Instrumental variables.

An autoimmune condition known as thyroid dysfunction includes both hyperthyroidism and hypothyroidism. Although the pathophysiology of the two diseases is distinct, clinical work makes it simpler to diagnose and treat the disease by enhancing the serological assessment of thyroid function in conjunction with clinical symptoms. However, growing clinical evidence in recent years has demonstrated that even small changes in thyroid function, such as subclinical dysfunction and changes within the reference range, can have a significant effect on clinical endpoint outcomes like bone mineral density, depression, metabolic syndrome, and cardiovascular disease (28). Cancer is one of the common diseases with the highest incidence rate and mortality in the world. The International Agency for Research on Cancer (IARC) predicts that there will be 18.1 million new cancer cases worldwide in 2020 (29). In addition, the American Cancer Society predicts that 1.95 million new cancer cases and 600,000 cancer deaths are expected in the

TABLE 2 MR analysis results.

MR estimates for the causal effect of thyroid dysfunction on Cutaneous Malignant Melanoma(CMM)										
	Outcome	*nSNP*	*IVW*		*MR-Egger*		*Weighted Median*		*Weighted Mode*	
			OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Hyper	CMM	13	0.989 (0.922,1.061)	0.753	1.093 (0.978,1.221)	0.145	1.018 (0.939,1.104)	0.658	1.024 (0.942,1.113)	0.588
Hypo	CMM	107	0.987 (0.975,1.000)	0.04	0.999 (0.973,1.026)	0.948	0.989 (0.971,1.007)	0.215	0.989 (0.969,1.007)	0.227
MR estimates for the causal effect of Cutaneous Malignant Melanoma(CMM) on thyroid dysfunction										
	Outcome	*nSNP*	*IVW*		*MR-Egger*		*Weighted Median*		*Weighted Mode*	
			OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
CMM	Hyper	8	1.016 (0.955,1.080)	0.623	1.091 (0.945,1.259)	0.281	1.032 (0.954,1.116)	0.429	1.045 (0.956,1.143)	0.363
CMM	Hypo	10	0.811 (0.647,1.016)	0.068	0.838 (0.484,1.450)	0.545	0.832 (0.689,1.004)	0.055	0.808 (0.645,1.011)	0.096

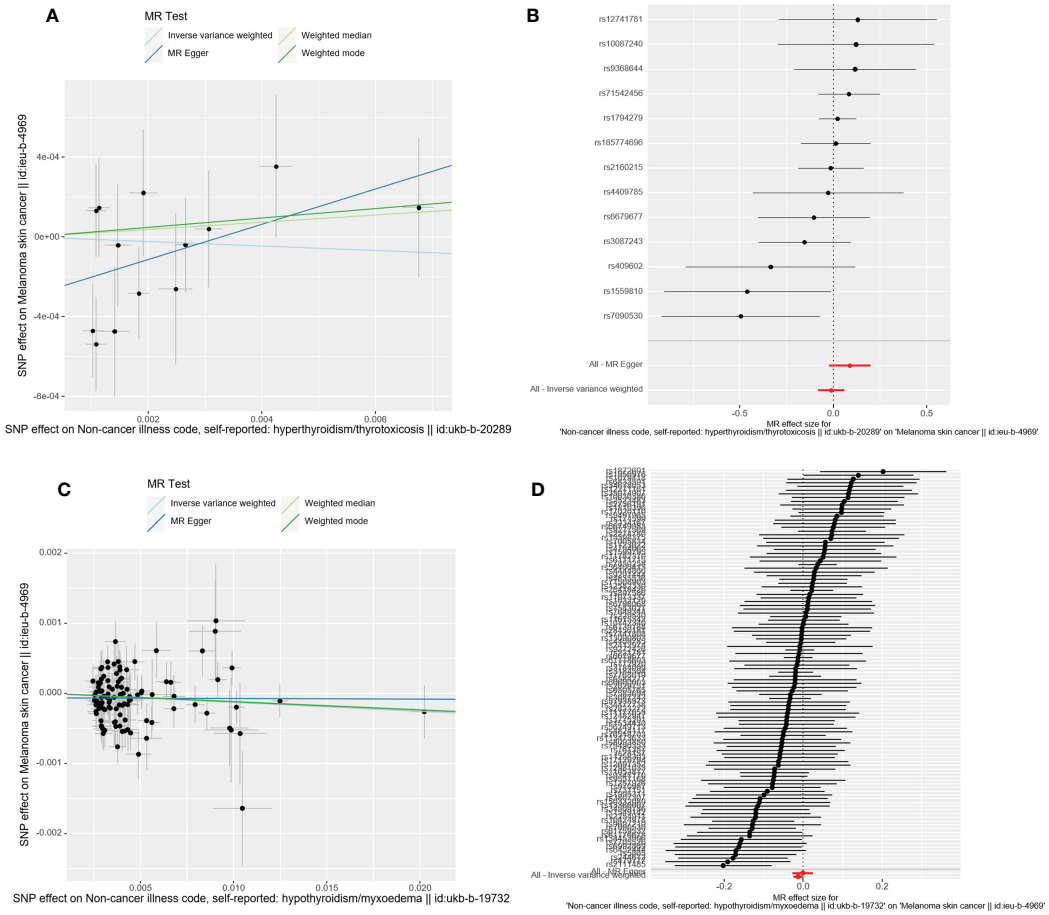

FIGURE 3
Scatter plot and forest plot of the causal relationship between thyroid dysfunction and CMM. CMM, cutaneous malignant melanoma. **(A)** Scatter plot of causality between hyperthyroidism and malignant cutaneous melanoma. **(B)** the susceptibility of hyperthyroidism to the risk of CMM; Red dots indicate the combined causal estimates for all SNPs using both MR-Egger and IVW) methods. **(C)** Scatter plot of causality between hypothyroidism and CMM. **(D)** the susceptibility of hypothyroidism to the risk of CMM; Red dots indicate the combined causal estimates for all SNPs using both MR-Egger and IVW) methods.

United States in 2023 (30). Current research on the connection between thyroid health and cancer is contradictory. According to studies, there is no connection between SCH and breast or prostate cancer (12). The relative risk (OR) for cancer in 2,414,165 adults with diagnosed hypothyroidism compared to those without hypothyroidism was 1.73(1.72-1.74) based on the 2019 Spanish population-based statistics (p<0.0001). In addition, patients aged 65 years or older with hypothyroidism have a reduced risk of bladder, colorectal, gastric, pancreatic, and prostate cancers (31). Although existing studies have analyzed the causal relationship between thyroid dysfunction and other cancers, there has been no MR analysis of the causal relationship between thyroid dysfunction and CMM, and the exact relationship between the two has not been clarified by existing clinical and basic research.

Studies using epidemiological data have demonstrated a favorable correlation between hypothyroidism and the advancement of CMM (31–33). In line with the findings of the aforementioned epidemiological data, a retrospective analysis from the Israeli National Cancer Registry indicated that elevated Log-TSH was linked to a higher risk of CMM (HR: 1.11) (34). In contrast to the epidemiological data of the Israeli population mentioned above, our MR analysis, which was focused on a European population, revealed the opposite conclusion from a genetic point of view, which may have skewed the statistical results due to the Israeli population's small size. In contrast to these investigations, our MR analysis comprised a considerably bigger population. Our work is more trustworthy because of our more stringent genetic instrument inclusion criteria and the removal of potential confounding variables. Furthermore, the aforementioned Israeli research discovered a link between hyperthyroidism and melanoma mortality (adjusted HR: 2.20) (35). Our MR analysis, however, could not identify a genetic link between hyperthyroidism and CMM. Since drug therapy alters the body's initial immune status, more studies have recently focused on thyroid dysfunction in people with CMM caused by the use of immune checkpoint inhibitors (36), which is distinct from the causal relationship between thyroid dysfunction and CMM investigated in this study.

The advantage of our study is that we are the first to use a two-sample MR analysis to explore the bidirectional causal link between

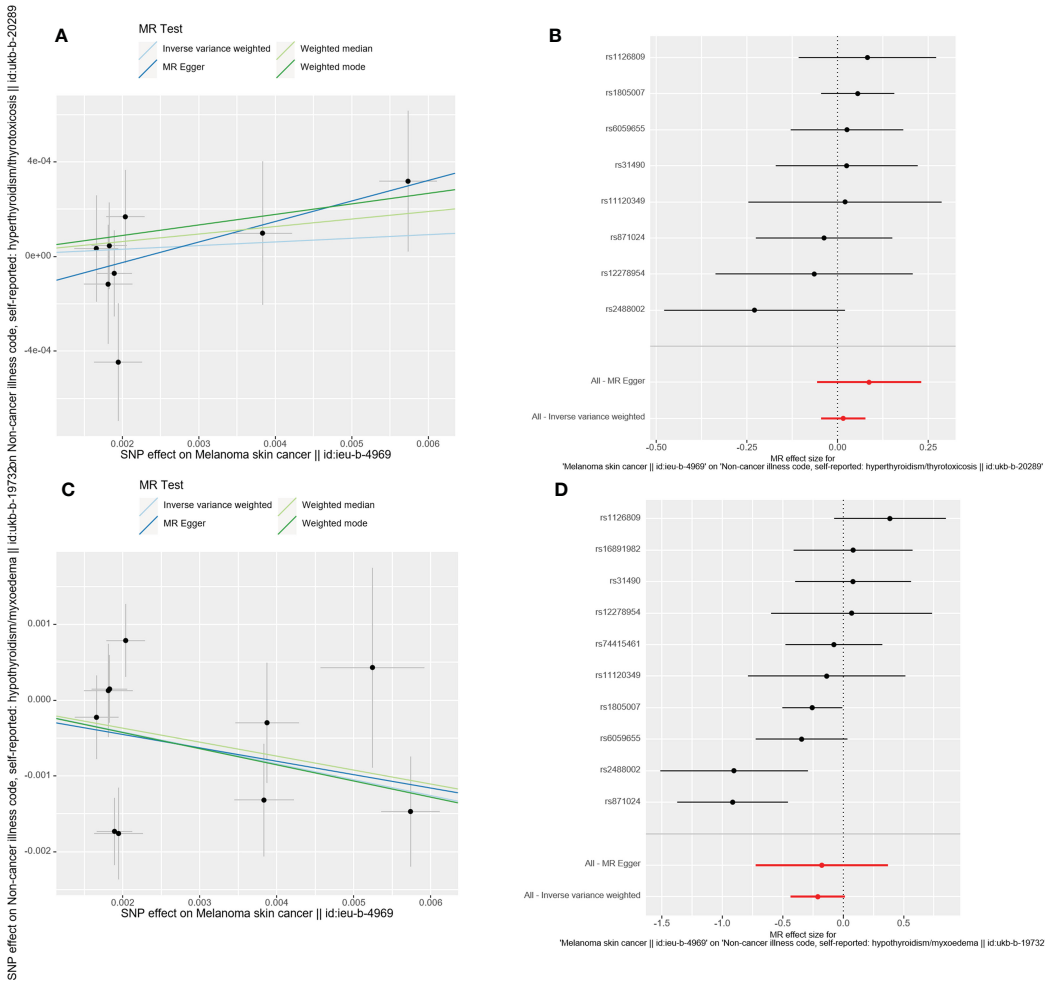

FIGURE 4
Scatter plot and forest plot of the causal relationship between CMM and thyroid dysfunction. CMM, cutaneous malignant melanoma. **(A)** Scatter plot of causality between malignant cutaneous melanoma and hyperthyroidism. **(B)** the susceptibility of CMM to the risk of hyperthyroidism; Red dots indicate the combined causal estimates for all SNPs using both MR-Egger and IVW) methods. **(C)** Scatter plot of causality between malignant cutaneous melanoma and hypothyroidism. **(D)** the susceptibility of CMM to the risk of hypothyroidism; Red dots indicate the combined causal estimates for all SNPs using both MR-Egger and IVW) methods.

thyroid dysfunction and CMM. MR analysis is able to minimize the interference of confounding factors. Additionally, the genetic techniques that we developed by combining numerous separate data sets allowed us to reduce the possibility of biased outcomes as a result of under-enrollment. This MR analysis still has several limitations, such as the fact that the research population was chosen because it was European, which may have led to outcome bias. To generalize this analysis to other populations, more research is required.

We could have been unable to identify variations in thyroid dysfunction in the initial data set due to the heterogeneity of the included data sets.

Conclusion

Our research demonstrates that hyperthyroidism does not raise the risk of CMM, whereas hypothyroidism is causally linked to a decreased risk of the disease. In addition, we found no clear evidence of genetic causality between CMM and the risk of thyroid dysfunction.

Data availability statement

The Supplementary Materials section of this paper contains the original data and photographs used in this research. Please get in touch with the authors of this manuscript if you require any further original information.

Ethics statement

The studies involving humans were approved by Medical Ethics Committee of the First People's Hospital of Jiashan. The studies were conducted in accordance with the local legislation and institutional requirements. This study's exposure and result samples were human individuals, and it was a secondary analysis of already published data, thus no ethical approval was required. The Ethics Committee of the First People's Hospital of Jiashan granted us an exemption because all datasets utilized in this study were public domain.

Author contributions

YF: Participating in the topic selection and writing of the study. HD: Writing the Manuscript. LP: Participating in the interpretation of the results of the MR Analysis. YS: Data Collection. JH and QX, ZH: Drawing the Figures and Tables. HD1†, LP2†, these authors contributed equally to this work and share first authorship. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1239883/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Leave-one-out plot of the risk of bidirectional causality between thyroid dysfunction and CMM. CMM, cutaneous malignant melanoma. **(A)** Hyperthyroidism and CMM; **(B)** Hypothyroidism and CMM; **(C)** CMM and hyperthyroidism; **(D)** CMM and hypothyroidism.

SUPPLEMENTARY FIGURE 2
Funnel plot of the risk of bidirectional causality between thyroid dysfunction and CMM. CMM, malignant cutaneous melanoma. **(A)** Hyperthyroidism and CMM; **(B)** Hypothyroidism and CMM; **(C)** CMM and hyperthyroidism; **(D)** CMM and hypothyroidism.

References

1. Chaker L, Razvi S, Bensenor IM, Azizi F, Pearce EN, Peeters RP. Hypothyroidism. *Nat Rev Dis Primers* (2022) 8(1):30. doi: 10.1038/s41572-022-00357-7

2. Wilson SA, Stem LA, Bruehlman RD. Hypothyroidism: diagnosis and treatment. *Am Fam Physician* (2021) 103(10):605–13. Available at: https://www.aafp.org/pubs/afp/issues/2021/0515/p605.html

3. Walsh JP. Managing thyroid disease in general practice. *Med J Aust* (2021) 205:179–84. doi: 10.5694/mja16.00545

4. Abdel-Moneim A, Gaber AM, Gouda S, Osama A, Othman SI, Allam G. Relationship of thyroid dysfunction with cardiovascular diseases: updated review on heart failure progression. *Hormones (Athens)* (2020) 19(3):301–9. doi: 10.1007/s42000-020-00208-8

5. Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré JC. The incidence and prevalence of thyroid dysfunction in europe: A meta-analysis. *J Clin Endocrinol Metab* (2014) 99:923–31. doi: 10.1210/jc.2013-2409

6. Iglesias P, Díez JJ. Hyperthyroidism and cardiovascular risk factors: A nationwide study. *Endocrinol Diabetes Nutr (Engl Ed)* (2023) 70(4):255–61. doi: 10.1016/j.endien.2023.03.015

7. Taylor PN, Albrecht D, Scholz A, Gutierrez-Buey G, Lazarus JH, Dayan CM, et al. Global epidemiology of hyperthyroidism and hypothyroidism. *Nat Rev Endocrinol* (2018) 14(5):301–16. doi: 10.1038/nrendo.2018.18

8. Tian L, Shao F, Qin Y, Guo Q, Gao C. Hypothyroidism and related diseases: a methodologic quality assessment of meta-analysis. *BMJ Open* (2019) 9(3):e024111. doi: 10.1136/bmjopen-2018-024111

9. Laurberg P, Pedersen KM, Vestergaard H, Sigurdsson G. Sigurdsson High incidence of multinodular toxic goiter in the elderly population in a low iodine intake area vs. high incidence of Graves' disease in the young in a high iodine intake area: comparative surveys of thyrotoxicosis epidemiology in East-Jutland Denmark and Iceland J. Intern. *J Intern Med* (1991) 229(5):415–20. doi: 10.1111/j.1365-2796.1991.tb00368.x

10. Hellevik AI, Asvold BO, Bjøro T, Romundstad PR, Nilsen TIL, Vatten LJ. Thyroid function and cancer risk: A prospective population study. *Cancer Epidemiol Biomarkers Prev* (2009) 18(2):570–4. doi: 10.1158/1055-9965.EPI-08-0911

11. Hercbergs A. The thyroid gland as an intrinsic biologic response modifier in advanced neoplasia—a novel paradigm. *In Vivo* (1996) 10:245–7.

12. Angelousi AG, Anagnostou VK, Stamatakos MK, Georgiopoulos GA, Kontzoglou KC. Mechanisms in endocrinology: primary HT and risk for breast cancer: a systematic review and meta-analysis. *Eur J Endocrinol* (2012) 166(3):373–81. doi: 10.1530/EJE-11-0838

13. Shi XZ, Jin X, Xu P, Shen HM. Relationship between breast cancer and levels of serum thyroid hormones and antibodies: a meta-analysis. *Asian Pac J Cancer Prev* (2014) 15(16):6643–7. doi: 10.7314/apjcp.2014.15.16.6643

14. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin* (2023) 73(1):17–48. doi: 10.3322/caac.21763

15. Messenger AG. Thyroid hormone and hair growth. *Br J Dermatol* (2000) 142 (4):633–4. doi: 10.1046/j.1365-2133.2000.03521.x

16. Slominski A, Wortsman J, Kohn L, Ain KB, Venkataraman GM, Pisarchik A, et al. Expression of hypothalamic–pituitary–thyroid axis related genes in the human skin. *J Invest Dermatol* (2002) 119:1449–55. doi: 10.1046/j.1523-1747.2002.19617.x

17. Caldarola G, Battista C, Pellicano R. Melanoma onset after estrogen, thyroid, and growth hormone replacement therapy. *Clin Ther* (2010) 32(1):57–9. doi: 10.1016/j.clinthera.2010.01.011

18. Monica S, Orengo IF, Rosen T. High prevalence of hypothyroidism in male patients with cutaneous melanoma. *Dermatol Online J* (2006) 12(2):1. doi: 10.5070/D33RN7K0D8

19. Burgess S, Foley CN, Allara E, Staley JR, Howson JMM. A Robust and efficient method for mendelian randomization with hundreds of genetic variants. *Nat Commun* (2020) 11:376. doi: 10.1038/s41467-019-14156-4

20. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol.* (2016) 27 (11):3253–65. doi: 10.1681/ASN.2016010098

21. Lu L, Wan B, Li L, Sun M. Hypothyroidism has a protective causal association with hepatocellular carcinoma: A two-sample Mendelian randomization study. *Front Endocrinol (Lausanne)* (2022) 13:987401. doi: 10.3389/fendo.2022.987401

22. Yuan S, Kar S, Vithayathil M, Carter P, Mason AM, Burgess S, et al. Causal associations of thyroid function and dysfunction with overall, breast and thyroid cancer: A two-sample Mendelian randomization study. *Int J Cancer* (2020) 147 (7):1895–903. doi: 10.1002/ijc.32988

23. Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing mendelian randomization investigations. *Wellcome Open Res* (2019) 4:186. doi: 10.12688/wellcomeopenres.15555.1

24. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* (2015) 44(2):512–25. doi: 10.1093/ije/dyv080

25. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* (2016) 40:304–14. doi: 10.1002/gepi.21965

26. Verbanck M, Che CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet* (2018) 50(5):693–8. doi: 10.1038/s41588-018-0099-7

27. Greco MFD, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomization studies with summary data and a continuous outcome. *Stat Med* (2015) 34(21):2926–40. doi: 10.1002/sim.6522

28. Medici M, Visser WE, Visser TJ, Peeters RP. Genetic determination of the hypothalamic-pituitary-thyroid axis: where do we stand. *Endocr Rev* (2015) 36(2):214–44. doi: 10.1210/er.2014-1081

29. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

30. Díez JJ, Iglesias P. Malignant neoplasms in people with hypothyroidism in Spain: A population-based analysis. *PloS One* (2022) 17(10):e0275568. doi: 10.1371/journal.pone.0275568

31. Shah M, Orengo IF, Ted Rosen T. High prevalence of hypothyroidism in male patients with cutaneous melanoma. Dermatol. *Online J* (2006) 12(2):1. doi: 10.5070/D33RN7K0D8

32. Ferrari SM, Fallahi P, Galetta F, Citi E, Benvenga S, Antonelli A. Thyroid disorders induced by checkpoint inhibitors. *Rev Endocr. Metab Disord* (2018) 19 (4):325–33. doi: 10.1007/s11154-018-9463-2

33. Ulisse S, Baldini E, Pironi D, Gagliardi F, Tripodi D, Lauro A. Is melanoma progression affected by thyroid diseases. *Int J Mol Sci* (2022) 23(17):10036. doi: 10.3390/ijms231710036

34. Krashin E, Silverman B, Steinberg DM, Yekutieli D, Giveon S, Fabian O, et al. Opposing effects of thyroid hormones on cancer risk: a population-based study. *Eur J Endocrinol* (2021) 184(3):477–86. doi: 10.1530/EJE-20-1123

35. Krashin E, Silverman B, Steinberg DM, Yekutieli D, Giveon S, Fabian O, et al. Pre-diagnosis thyroid hormone dysfunction is associated with cancer mortality. *Endocr Relat Cancer* (2021) 28(11):705–13. doi: 10.1530/ERC-21-0187

36. Lomax AJ, Lim J, Cheng R, Sweeting A, Lowe P, McGill N, et al. Immune toxicity with checkpoint inhibition for metastatic melanoma: case series and clinical management. *J Skin Cancer* (2018) 2018:9602540. doi: 10.1155/2018/9602540

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Impact of the diagnosis of metabolic dysfunction-associated fatty liver disease and non-alcoholic fatty liver disease in patients undergoing liver transplantation for hepatocellular carcinoma

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Purpose: Whether the diagnosis of non-alcoholic fatty liver disease or metabolic dysfunction-associated fatty disease has a different impact on liver transplant recipients with hepatocellular carcinoma is not yet clear.

Methods: Data from a two-center retrospective cohort study were collected to compare and investigate the differences between non-alcoholic fatty liver disease and metabolic dysfunction-associated fatty liver disease in clinicopathologic parameters and prognosis among liver transplant recipients with hepatocellular carcinoma.

Results: A total of 268 liver transplant recipients with hepatocellular carcinoma were included. The prevalence among pre- and post-transplant metabolic dysfunction-associated fatty liver disease was 10.82% and 30.22%, while for non-alcoholic fatty liver disease, it was 7.09% and 26.87%, respectively. The clinicopathological parameters were similar between the two pre-transplant groups. In contrast, the post-transplant group with metabolic dysfunction-associated fatty liver disease exhibited a higher prevalence of diabetes mellitus and a greater body mass index. However, the other parameters were similar between the two post-transplant groups ($p > 0.05$). Factors such as the largest tumor size > 4 cm, microvascular invasion, lack of tumor capsule, post-transplant metabolic dysfunction-associated fatty liver disease, and decreased post-transplant lymphocyte percentage were related to an increased risk of recurrence.

Conclusion: In patients undergone liver transplantation for hepatocellular carcinoma, the diagnosis of metabolic dysfunction-associated fatty disease is more strongly associated with metabolic abnormalities than the diagnosis of non-alcoholic fatty liver disease and is an independent predictor of hepatocellular carcinoma recurrence.

KEYWORDS

hepatocellular carcinoma, liver transplantation, non-alcoholic fatty liver disease, metabolic dysfunction-associated fatty liver disease, prognosis

Introduction

Hepatocellular carcinoma (HCC), currently the sixth most common tumor worldwide, ranks fourth in terms of tumor mortality (1). Major risk factors contributing to the occurrence and development of HCC have been well established, including infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), excessive alcohol consumption, and exposure to fungal metabolite (2, 3). Moreover, growing evidence suggests that metabolic risk factors, such as steatosis, obesity, and metabolic syndrome, can collectively contribute to the development of HCC, with a rising prevalence of HCC related to metabolic dysfunction (4, 5). Additionally, metabolic dysfunction can lead to concurrent diseases such as type 2 diabetes mellitus (T2DM), chronic kidney disease, cardiovascular disease (CVD), certain extrahepatic cancers, and severe liver-related complications (6). Non-alcoholic fatty liver disease (NAFLD), which is associated with metabolic dysfunction (7, 8), has emerged as a significant contributor to liver-related morbidity and mortality globally, with a prevalence of approximately 25% (9, 10). Moreover, NAFLD is one of the major indications for liver transplantation (10). However, NAFLD stands out in its diagnostic approach, often failing to account for the influence of coexisting metabolic dysfunction and various liver disease etiologies (11).

Recently, the concept of metabolic dysfunction-associated fatty liver disease (MAFLD) has been introduced by international consensus, focusing on its relevance to the underlying conditions of systemic metabolic dysfunction (12, 13). The new criteria define MAFLD as hepatic steatosis together with the presence of metabolic conditions (T2DM, obesity/overweight, or at least two metabolic abnormalities). Although MAFLD is presumed to have a stronger association with metabolic syndrome than NAFLD (14), understanding the application of this new terminology in liver transplantation remains limited. Moreover, the differential impacts of MAFLD and NAFLD on the pathological characteristics and outcomes of liver transplant recipients (LTR) with HCC have not been thoroughly explored in the existing literature.

Hence, we conducted a two-center retrospective study on LTR with HCC to investigate their clinicopathological data and prognosis, aiming to assess the respective influences of MAFLD and NAFLD on these patients.

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease; LTR, liver transplant recipients; BMI, body mass index.

Materials and methods

Study design and participants

This retrospective cohort study was conducted at Beijing Chaoyang Hospital (March 2011 and December 2021) and China-Japan Friendship Hospital (February 2018 and December 2021) involving LTR with HCC who underwent liver transplantation. The study was approved by the Institutional Review Board of both hospitals (No.2022-D-115) in accordance with the 1964 Helsinki Declaration and its later amendments. Informed consent was not required, given the retrospective nature of the study.

Inclusion criteria and exclusion criteria

Inclusion criteria for LTR encompassed histopathologically confirmed HCC without distant metastases, undergoing liver transplantation, and a follow-up period of at least 6 months. Exclusion criteria consisted of combined-organ transplantation, liver retransplantation, presence of any other type of tumor, and missing data for a MAFLD diagnosis.

Definitions

MAFLD diagnosis was based on hepatic steatosis in conjunction with one of the following three conditions: a body mass index (BMI) $\geq$ 23 kg/m^2 in Asians, T2DM, or metabolic dysregulation (12, 13). T2DM was defined as a history of diabetes, and/or fasting plasma

glucose ≥ 7.0 mmol/L, and/or two-hour post-load plasma glucose ≥ 11.1 mmol/L, and/or HbA1c ≥ 6.5% (15). Metabolic dysregulation was characterized by having at least two of the following metabolic conditions: waist circumference ≥ 90 in Asian men and ≥ 80 cm in Asian women; blood pressure ≥ 130/85 mmHg or receiving drug treatment; plasma triglycerides ≥ 1.70 mmol/L or receiving drug treatment; plasma high-density lipoprotein (HDL)-cholesterol < 1.0 mmol/L for males and < 1.3 mmol/L for females or receiving drug treatment; prediabetes (fasting plasma glucose levels 5.6-6.9 mmol/L, or two-hour post-load plasma glucose levels 7.8-11.0 mmol/L or HbA1c 5.7% to 6.4%).

NAFLD was diagnosed when hepatic steatosis was present, and secondary causes, such as excessive alcohol intake, viral hepatitis, autoimmune liver disease, parenteral nutrition, genetic disorders, hepatic malignancies, hepatobiliary infections, biliary tract diseases, medications, and starvation, were excluded (16). Alcohol intake was limited to ≤ 20 g/d for males and ≤ 10 g/d for females to define non-alcohol related liver conditions.

Hepatic steatosis was assessed through histopathology post-surgery for the recipient's liver and via abdominal ultrasonography during follow-up for LTR. The diagnosis was conducted by two experienced pathologists and sonologists, who were blinded to each other's evaluation.

Data collection

Data collection was carried out through medical records and follow-up visits until December 2022. The following clinical variables were obtained: age, sex, alcohol intake, smoking (≥ 1 cigarette/d), body mass index, waist circumference, Child-Pugh grade, model of end-stage liver disease score (MELD score), immunosuppressive regimen, tumor-free survival, and overall survival in addition to histories of tumor therapy, CVD, hypertension and DM. CVD was comprised of coronary heart disease and stroke (17). Histopathological parameters included the largest tumor size, number of tumors, total tumor size, and tumor differentiation. The presence of tumor capsule, hepatic cirrhosis, hepatic capsule invasion, microvascular invasion, macrovascular invasion, and tumor within Milan criteria (18) was also noted. Laboratory measurements contained total bilirubin levels, aspartate aminotransferase levels, alanine transaminase levels, albumin levels, triglyceride levels, high-density lipoprotein cholesterol levels, fasting plasma glucose levels, alpha-fetoprotein levels, creatinine levels, neutrophil count, neutrophil percentage, lymphocyte count, lymphocyte percentage, neutrophil-to-lymphocyte count ratio.

Follow-up

Patient follow-up visits were scheduled at three-month intervals during the first postoperative year, semi-annually during the second postoperative year, and annually thereafter. The follow-up period began on the day of patient discharge and ended either on the date of tumor recurrence or the closing date of follow-up. The primary objective of the study was to investigate the occurrence of MAFLD and NAFLD post-transplantation, with the secondary aim being to evaluate tumor-free survival.

Statistical analysis

The normal distribution of continuous variables was tested using a Kolmogorov-Smirnov test. The independent samples t-test was employed for normally distributed variables, while the Wilcoxon ranksum test was selected for non-normally distributed variables. The Chi-square or Fisher's exact test was used to compare categorical variables. The Kaplan-Meier method was used for survival analysis. The Cox regression model was employed for multifactor survival analysis. Data were analyzed using SPSS 19.0 computer software (IBM Corp., Armonk, NY, USA). The figures were generated using R 4.2.1 (https://www.R-project.org/). All statistical tests were two-sided, with statistical significance set at a P-value <0.05.

Results

Characteristics of LTR

A total of 268 patients with HCC, who underwent liver transplantation, were included in this study. The majority of patients were male (89.55%, n=234), with a mean age of 53.88 ± 9.05 years (range: 28~76 years). All LTR were of Asian descent. Prior to liver transplantation, 20.90% (n=56) of patients had DM while 17.91% (n=48) had hypertension. The mean BMI of LTR was 24.26 kg/m^2. Following liver transplantation, the prevalence of DM and hypertension increased to 34.70% (n=93) and 27.61% (n=74), respectively, with a mean BMI of 23.42 kg/m^2. During the follow-up period, 63 patients experienced tumor recurrence, while 204 LTR remained alive.

Overlap between pre- and post-transplant MAFLD and NAFLD

Before liver transplantation, 10.82% (29/268) of patients were diagnosed with MAFLD and 7.09% (19/268) with NAFLD. Among the 29 LTR with MAFLD, 15 also met the criteria for NAFLD. Following liver transplantation, the overall prevalence of MAFLD and NAFLD increased to 30.22% (81/268) and 26.87% (72/268), respectively. Among the 81 LTR with MAFLD, 41 also met the criteria for NAFLD. However, only a small number of LTR (n=3) fulfilled both criteria before and after liver transplantation (Figure 1).

Comparison between pre-transplant MAFLD and NAFLD

The clinical characteristics of the pre-transplant MAFLD and NAFLD groups are described in Table 1. The two groups exhibited

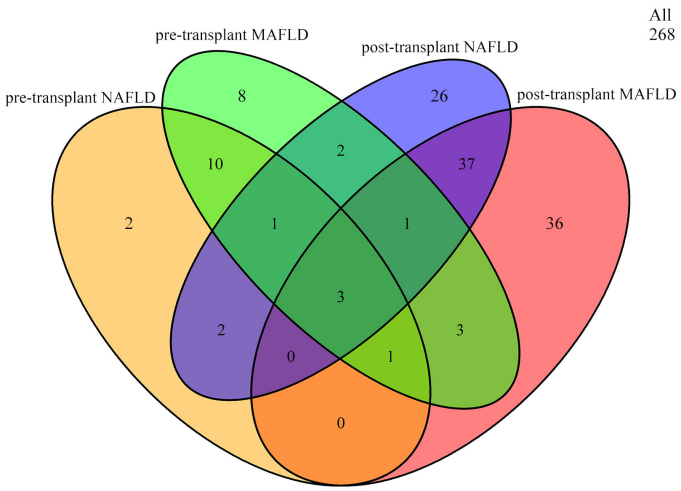

FIGURE 1
Overlap between pre- and post-transplant MAFLD and NAFLD. MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease.

similar histopathological and laboratory features, including age, sex, Child-Pugh grade, MELD score, body mass index, waist circumference, history of tumor therapy, smoking, CVD, T2DM, hypertension, cirrhosis, largest tumor size, total tumor size, number of tumors, tumor differentiation, presence of tumor capsule, hepatic capsule invasion, microvascular invasion, macrovascular invasion, HCC within Milan criteria, serum albumin levels, serum creatinine levels, serum alpha-fetoprotein levels, triglyceride levels, HDL-cholesterol levels, fasting plasma glucose levels, neutrophil count, neutrophil percentage, lymphocyte count, lymphocyte percentage and neutrophil-to-lymphocyte count ratio ($p > 0.05$).

Comparison between post-transplant MAFLD and NAFLD

A comparison of clinical parameters between the post-transplant MAFLD and NAFLD groups is presented in Table 2. The MAFLD group had a higher prevalence of DM (53% vs. 31%) and a greater BMI (25.53 ± 3.02 kg/m^2 vs. 23.51 ± 3.53 kg/m^2). Conversely, the NAFLD group displayed decreased waist circumference and fasting plasma glucose levels, although these differences did not reach statistical significance ($p > 0.05$). Other parameters, including age, sex, immunosuppressive regimen, smoking, CVD, hypertension, aspartate aminotransferase levels, alanine transaminase levels, total bilirubin levels, serum albumin levels, serum creatinine levels, alpha-fetoprotein levels, triglyceride levels, high-density lipoprotein cholesterol levels, neutrophil count, neutrophil percentage, lymphocyte count, lymphocyte percentage and neutrophil-to-lymphocyte count ratio were similar between the two groups ($p > 0.05$). In addition, the tumor-free survival and overall survival of the post-transplant MAFLD and NAFLD cohorts also showed no significant differences ($p > 0.05$) as illustrated in Figure 2.

Risk factors associated with HCC recurrence

Table 3 outlines the outcomes of the univariate analysis concerning risk factors linked with HCC recurrence. Individuals who experienced liver transplant rejection with recurrence were notably younger and demonstrated a higher incidence of tumors exceeding > 4 cm in size, total tumor size surpassing > 7 cm, and more than three tumors. Other risk factors included poor tumor differentiation, absence of tumor encapsulation, hepatic capsule invasion, microvascular invasion, macrovascular invasion, deviation from Milan criteria, post-transplant MAFLD and NAFLD, elevated levels of pre- and post-transplant alpha-fetoprotein, NLR, and decreased post-transplant lymphocyte percentage.

The stepwise Cox proportional hazard model depicted in Figure 3 summarizes the prognostic factors associated with HCC recurrence in this cohort. The largest tumor size (OR = 0.27, $p = 0.012$), microvascular invasion (OR = 3.50, $p = 0.006$), absence of tumor capsule (OR = 0.31, $p = 0.024$), post-transplant MAFLD (OR = 4.96, $p = 0.001$), and decreased post-transplant lymphocyte percentage (OR = 0.95, $p = 0.032$) were identified as factors associated with a higher risk of recurrence.

To further ascertain the impact of post-transplant MAFLD on tumor recurrence, the tumor-free survival of liver transplant recipients with and without recurrence was evaluated. The findings revealed that post-transplant MAFLD was indicative of patients at a heightened risk of recurrence, resulting in significantly decreased tumor-free survival rates ($p < 0.001$) and overall survival rates ($p < 0.001$) as depicted in Figure 4.

Discussion

We conducted a comparative analysis between LTR with MAFLD and NAFLD in terms of prevalence and characteristics.

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TABLE 1 Comparison of parameters between pre-transplant MAFLD and NAFLD.

Parameters	MAFLD (n=29)	NAFLD (n=19)	P value
Age (y)	56.03 ± 9.01	56.42 ± 7.97	0.880
Sex (male)	23	14	0.918
Child-Pugh grade			0.393
A	5	2	
B	13	6	
C	11	11	
MELD score	11.07 ± 7.59	12.79 ± 7.17	0.437
Body mass index (kg/m^2)	24.01 ± 2.75	22.97 ± 2.57	0.195
Waist circumference (cm)	90.44 ± 6.48	88.31 ± 7.04	0.287
History of tumor therapy	9	5	0.725
Smoking	11	6	0.653
Cardiovascular disease	5	1	0.435
Type 2 diabetes mellitus	4	4	0.792
Hypertension	5	1	0.435
Cirrhosis	17	6	0.067
Largest tumor size ≤ 4 cm	15	6	0.169
Total tumor size ≤ 7 cm	19	13	0.835
Number of tumors ≤ 3	24	18	0.435
Differentiation			0.911
High	6	3	
Middle	20	14	
Low	3	2	
Tumor capsule	10	11	0.110
Hepatic capsule invasion	11	7	0.939
Microvascular invasion	4	0	0.091
Macrovascular invasion	1	1	1.000
Within Milan criteria	17	13	0.493
Albumin (g/L)	35.91 ± 6.63	36.27 ± 5.23	0.841
Creatinine (umol/L)	79.99 ± 37.47	79.62 ± 22.84	0.970
Alpha-fetoprotein (ng/mL)	118.79 ± 311.40	360.87 ± 984.14	0.899
Triglyceride (mmol/L)	2.00 ± 2.15	1.48 ± 0.83	0.328
HDL-cholesterol (mmol/L)	0.78 ± 0.41	0.84 ± 0.25	0.547
FPG (mmol/L)	6.74 ± 2.19	6.13 ± 2.05	0.339
Neutrophil count (10^9/L)	3.29 ± 1.79	3.79 ± 2.13	0.389
Neutrophil percentage	65.92 ± 11.99	68.27 ± 11.34	0.502
Lymphocyte count (10^9/L)	1.26 ± 0.75	1.36 ± 0.81	0.674
Lymphocyte percentage	25.96 ± 11.06	25.63 ± 9.65	0.916
NLR	3.57 ± 2.81	3.53 ± 2.55	0.958

MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; MELD, model for end-stage liver disease; HDL-cholesterol, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; NLR, neutrophil-to-lymphocyte count ratio.

TABLE 2 Comparison of parameters between post-transplant MAFLD and NAFLD.

Parameters	MAFLD (n=81)	NAFLD (n=72)	P value
Age (y)	55.65 ± 8.24	52.93 ± 9.73	0.063
Sex (male)	70	64	0.644
Immunosuppressive regimen			0.076
CNI	55	37	
mTOR inhibitor	3	7	
CNI+mTOR inhibitor	23	28	
Smoking	10	3	0.070
Cardiovascular disease	20	13	0.319
Type 2 diabetes mellitus	43	22	0.005
Hypertension	28	22	0.597
Body mass index (kg/m^2)	25.53 ± 3.02	23.51 ± 3.53	0.000
Waist circumference (cm)	93.19 ± 8.68	90.45 ± 8.83	0.055
Aspartate aminotransferase (U/L)	37.72 ± 41.21	37.51 ± 39.14	0.523
Alanine transaminase (U/L)	31.94 ± 31.12	31.66 ± 29.83	0.921
Total bilirubin (umol/L)	22.93 ± 28.32	25.91 ± 30.54	0.223
Albumin (g/L)	40.47 ± 8.61	40.39 ± 9.21	0.828
Creatinine (umol/L)	88.65 ± 53.83	84.46 ± 38.41	0.814
Alpha-fetoprotein (ng/mL)	1100.16 ± 4386.87	607.59 ± 2832.93	0.398
Triglyceride (mmol/L)	1.97 ± 0.92	1.83 ± 0.84	0.304
HDL-cholesterol (mmol/L)	0.91 ± 0.34	0.97 ± 0.42	0.405
FPG (mmol/L)	6.77 ± 1.91	6.39 ± 1.96	0.054
Neutrophil count (10^9/L)	3.97 ± 2.14	3.90 ± 2.09	0.828
Neutrophil percentage	63.25 ± 13.91	64.60 ± 14.91	0.564
Lymphocyte count (10^9/L)	1.59 ± 1.18	1.50 ± 1.16	0.556
Lymphocyte percentage	25.92 ± 11.65	25.43 ± 11.69	0.796
NLR	3.32 ± 2.68	3.40 ± 2.26	0.390

MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; CNI, calcineurin inhibitor; mTOR inhibitor, mammalian target of rapamycin inhibitor; HDL-cholesterol, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; NLR, neutrophil-to-lymphocyte count ratio.

Our findings indicate that LTR with post-transplant MAFLD are significantly associated with metabolic risk factors such as T2DM and obesity. Additionally, we identified several independent risk factors for HCC recurrence in LTR with HCC, including post-transplant MAFLD, the largest tumor size > 4 cm, microvascular invasion, absence of tumor capsule, and decreased post-transplant lymphocyte percentage.

The term MAFLD was introduced as a replacement for NAFLD to more accurately reflect the pathology of liver disease, recognizing the limitations of the NAFLD definition (12, 13). Since then, substantial efforts have been devoted to exploring the relationship between NAFLD and MAFLD. Lim et al. found significant differences in the natural progression of MAFLD and NAFLD (19), while Lin et al. revealed that patients with MAFLD had a higher risk of disease progression (14). However, it remains unknown whether NAFLD and MAFLD can be used interchangeably to characterize LTR with HCC. This study revealed that the prevalence of MAFLD was higher than that of NAFLD before and after transplantation. Surprisingly, only a small proportion of patients with pre-transplant MAFLD (10.82%) and NAFLD (7.09%) received a diagnosis, resulting in an overlap of 52% among LTR with MAFLD. However, a meta-analysis of 379,801 patients reported a pooled prevalence of 39.22% for MAFLD and 33.86% for NAFLD, with regional variations. Interestingly, among patients with MAFLD, the pooled prevalence of those with both MAFLD and NAFLD was remarkably high at 81.59% (19). However, LTR constituted a distinct patient population with unique pathophysiologic characteristics. It is evident that the majority of LTR with HCC in this study were caused by hepatitis infection and excessive alcohol intake. Consequently, upon admission, certain patients exhibited severe cirrhosis and liver function decompensation, as determined by high MELD scores and Child-Pugh grade B or C ratios. These factors might result in malnutrition and a decrease in lymphocyte subset percentages (20), thereby significantly reducing the number of both MAFLD and NAFLD cases. Therefore, the characteristics of pre-transplant MAFLD and NAFLD in LTR with HCC were found to be similar upon comparison, suggesting the interchangeability of these two terms in a clinical setting.

Following transplantation, the prevalence of MAFLD and NAFLD increased due to the amelioration of malnutrition with the introduction of a healthier liver. Furthermore, urbanization and the use of immunosuppressant drugs can also contribute to the elevated prevalence (9, 21–23). while the routine practice at our center for LTR with HCC involved early corticosteroid withdrawal, high doses of corticosteroids were administered along with other immunosuppressant drugs in cases of acute rejection, causing insulin resistance and weight gain (24, 25). Nevertheless, concurrent recurrence of hepatitis and alcohol consumption may lead to an increase in MAFLD cases while reducing the number of NAFLD cases based on their respective definitions. Hepatitis C recurrence in some LTR can increase the risk of dyslipidemia, hepatic steatosis, and insulin resistance, whereas alcohol intake is strongly associated with hepatic steatosis and dyslipidemia, although further research is needed to investigate the link between HBV and metabolic syndrome (26–28). Moreover, compared to the NAFLD group, LTR with MAFLD were more strongly associated with metabolic disorders, including a higher proportion of individuals with T2DM and a higher BMI, which are key criteria for diagnosing MAFLD. Other characteristics did not exhibit significant differences, as MAFLD and NAFLD share a similar pathophysiology involving the metabolic functions of the liver, particularly an extended endoplasmic reticulum network (29). Consequently, there was an overlap of 41 patients (51%) among LTR with MAFLD, which is still lower than the prevalence (81.59%) reported in a meta-analysis (19).

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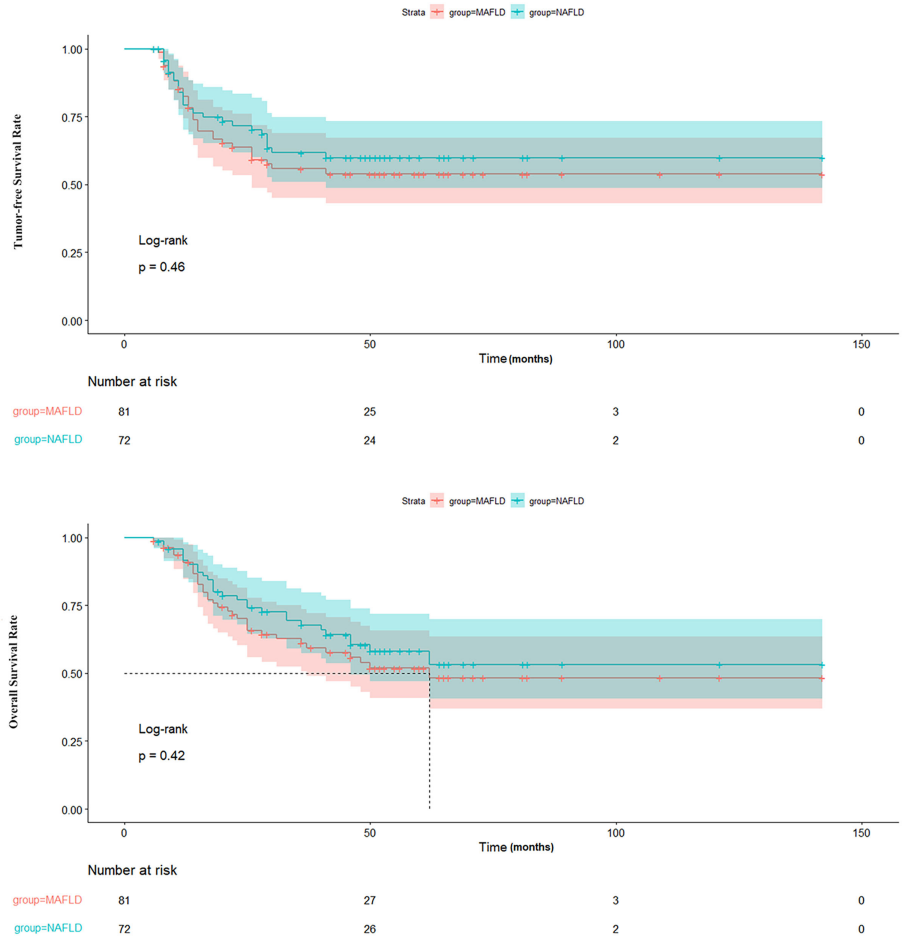

FIGURE 2
Comparison of TFS and OS between patients with MAFLD and NAFLD. TFS, tumor-free survival; OS, overall survival; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease.

TABLE 3 Risk factors for HCC recurrence.

Parameters	Univariate analysis		
	With recurrence (N=63)	Without recurrence (N=205)	P value
Pre-surgery			
Age (y)	51.44 ± 9.25	54.62 ± 8.88	0.014
Sex (male)	55	185	0.504
Smoking	21	70	0.905
Drinking	19	53	0.500
History of tumor therapy	31	108	0.629
MELD score	9.17 ± 6.02	9.80 ± 6.87	0.227
Child-Pugh grade (A/B/C)	18/34/11	60/105/40	0.912
NAFLD	3	16	0.410
MAFLD	5	24	0.399
Cirrhosis	52	157	0.318

(Continued)

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TABLE 3 Continued

Parameters	Univariate analysis		
	With recurrence (N=63)	Without recurrence (N=205)	P value
Pre-surgery			
Largest tumor size ≤ 4 cm	19	112	0.001
Total tumor size ≤ 7 cm	20	143	0.000
Number of tumors ≤ 3	38	178	0.000
Differentiation (high/moderate/low)	5/39/19	45/128/32	0.005
Tumor capsule	11	63	0.039
Hepatic capsule invasion	29	46	0.000
Microvascular invasion	36	36	0.000
Macrovascular invasion	18	12	0.000
Within Milan criteria	18	127	0.000
Neutrophil count (10^9/L)	3.72 ± 3.06	3.26 ± 2.28	0.192
Neutrophil percentage	67.07 ± 12.17	67.07 ± 13.28	0.997
Lymphocyte count (10^9/L)	1.07 ± 0.64	1.01 ± 0.68	0.537
Lymphocyte percentage	23.11 ± 11.15	22.95 ± 11.41	0.919
NLR	6.41 ± 11.50	5.56 ± 10.49	0.586
Alpha-fetoprotein (ng/mL)	3057.89 ± 6464.33	470.74 ± 1957.62	0.000
Post-surgery			
Smoking	2	19	0.115
Drinking	18	40	0.127
Hepatitis recurrence	11	25	0.284
Immunosuppressive regimen (CNI/mTOR/both)	41/7/15	107/20/78	0.113
NAFLD	26	46	0.003
MAFLD	32	49	0.000
Neutrophil count (10^9/L)	3.77 ± 1.60	3.83 ± 1.94	0.817
Neutrophil percentage	65.73 ± 13.29	63.22 ± 12.45	0.169
Lymphocyte count (10^9/L)	1.37 ± 1.02	1.57 ± 0.83	0.124
Lymphocyte percentage	23.03 ± 11.43	27.18 ± 10.10	0.006
NLR	3.92 ± 3.04	3.05 ± 2.96	0.048
Alpha-fetoprotein (ng/mL)	2417.74 ± 5994.92	56.32 ± 426.89	0.003

HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease; NLR, neutrophil-to-lymphocyte count ratio; CNI, calcineurin inhibitor; mTOR inhibitor, mammalian target of rapamycin inhibitor.

Finally, we identified five independent prognostic factors for HCC recurrence in this study. Metabolic syndrome has been found to be a significant risk factor for HCC (4, 5). Since the novel definition of MAFLD was proposed based on metabolic syndrome, post-transplant MAFLD can effectively stratify LTR at a high risk of HCC recurrence. Thus MAFLD was revealed as an independent risk factor for HCC recurrence in our study. Additionally, our study further confirmed the significant association between the largest tumor size and microvascular invasion, traditionally indicators of aggressive biology (30, 31), with HCC recurrence. Furthermore, the presence of the tumor capsule exhibited a protective effect by acting as a barrier against vascular and local invasion (32, 33). Lastly, LTR with a low post-transplant lymphocyte percentage showed a worse prognosis, as lymphocytes play a critical role in tumor surveillance (34, 35). Consequently, decreased post-transplant lymphocyte percentages weakened the antitumor response in these patients, leading to HCC recurrence.

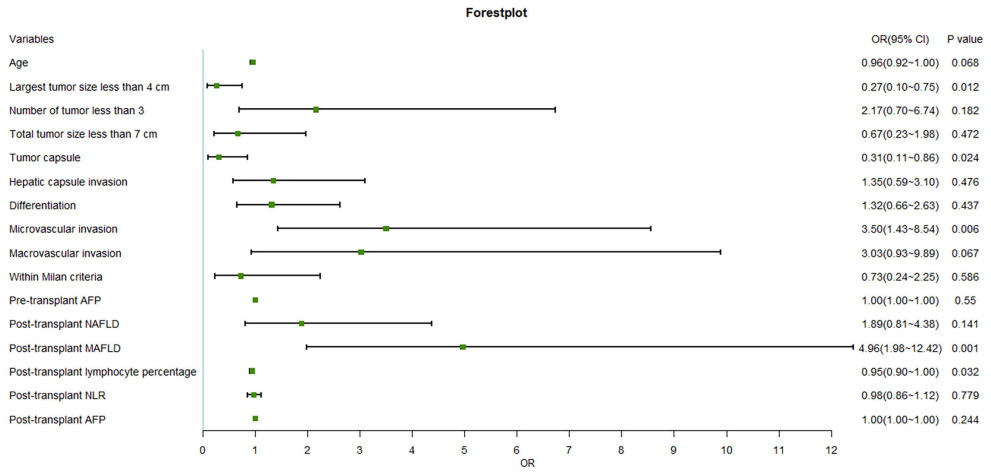

FIGURE 3
Multivariate analysis of prognostic factors related to HCC recurrence. HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease; NLR, neutrophil-to-lymphocyte count ratio.

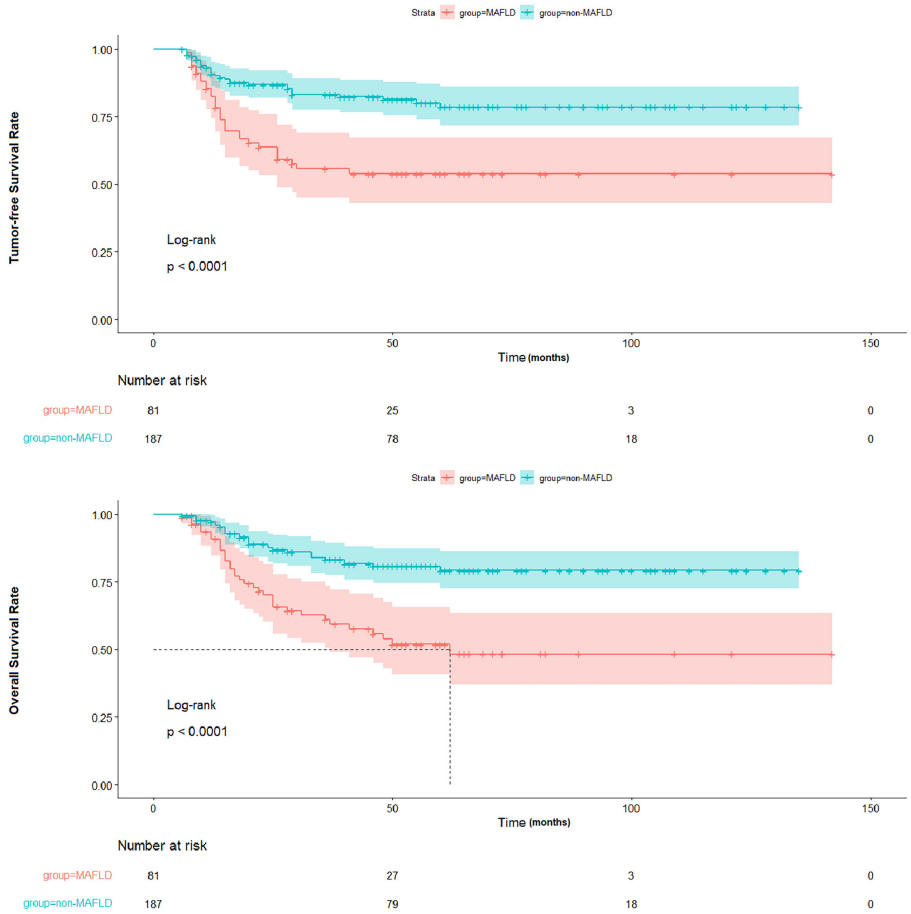

FIGURE 4
Comparison of TFS and OS between patients with and without MAFLD. TFS, tumor-free survival; OS, overall survival; MAFLD, metabolic dysfunction-associated fatty liver disease.

There are several limitations in this study. Firstly, the sample size of patients with pre- and post-transplant MAFLD and NAFLD is small, considering the substantial overlap between the two conditions. This study involved a unique population with a low prevalence of pre-transplant MAFLD and NAFLD, strongly associated with the presence of cirrhosis. Regarding post-transplant MAFLD and NAFLD, there are several conceivable explanations. LTR paid more attention to their health post-transplantation, adhering to regular follow-ups and timely treatment of emerging diseases. Besides, patients with missing data required for MAFLD classification were excluded from the study, which further decreased the prevalence of MAFLD. Hence, a two-center cohort study was conducted to increase patient enrollment. Moreover, with an increase in the number of LTR experiencing post-transplant MAFLD or NAFLD, the occurrence of LTR with overlapping diseases also escalated. Secondly, ultrasonography-based assessment was utilized instead of biopsy-based assessment for diagnosing post-transplant MAFLD and NAFLD because the latter is invasive and carries the risk of severe complications. However, the diagnosis based on ultrasound features may have certain limitations due to its dependency on operator skills. Consequently, two experienced sonologists, blinded to each other's evaluations, performed the diagnosis to minimize potential bias. Thirdly, a significant number of LTR with HCC exceeding Milan criteria had a history of tumor therapy, potentially impacting hepatic steatosis. Additionally, the MAFLD definition was based on Asian criteria. Therefore, the results of this study should be interpreted with caution for Caucasian men/women. The retrospective design of this study calls for future randomized controlled trials in multiple centers to validate these findings and assess reproducibility in Caucasian populations.

In summary, patients with MAFLD have a stronger association with fatty liver disease compared to those with NAFLD following liver transplantation. Post-transplant MAFLD has the potential to stratify patients based on tumor progression.

Data availability statement

Data analyzed in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Institutional Review Board of Beijing Chaoyang Hospital and the Institutional Review Board of China-Japan Friendship Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this was a two-center retrospective cohort study.

Author contributions

J-QZ: Data curation, Writing – original draft. J-ZL: Data curation, Writing – original draft. S-WY: Data curation, Writing – original draft. Z-YR: Formal analysis, Writing – review & editing. X-YY: Formal analysis, Writing – review & editing. ZL: Investigation, Writing – review & editing. X-LL: Investigation, Writing – review & editing. D-DH: Conceptualization, Writing – review & editing. QH: Conceptualization, Writing – review & editing.

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Conflict of interest

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

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. (2018) 68:394–424. doi: 10.3322/caac.21492

2. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology*. (2004) 127:S35–50. doi: 10.1053/j.gastro.2004.09.014

3. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. (2016) 2:16018. doi: 10.1038/nrdp.2016.18

4. White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatol*. (2012) 10:1342–59 e2. doi: 10.1016/j.cgh.2012.10.001

5. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. *Hepatology*. (2021) 73 Suppl 1:4–13. doi: 10.1002/hep.31288

6. Mantovani A, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism*. (2020) 111S:154170. doi: 10.1016/j.metabol.2020.154170

7. Muthiah MD, Cheng Han N, Sanyal AJ. A clinical overview of non-alcoholic fatty liver disease: A guide to diagnosis, the clinical features, and complications-What the non-specialist needs to know. *Diabetes Obes Metab*. (2022) 24 Suppl 2:3–14. doi: 10.1111/dom.14521

8. Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol*. (2015) 62:S47–64. doi: 10.1016/j.jhep.2014.12.012

9. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol*. (2018) 69:896–904. doi: 10.1016/j.jhep.2018.05.036

10. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. (2018) 15:11–20. doi: 10.1038/nrgastro.2017.109

11. Bedossa P. Current histological classification of NAFLD: strength and limitations. *Hepatol Int*. (2013) 7 Suppl 2:765–70. doi: 10.1007/s12072-013-9446-z

12. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol*. (2020) 73:202–9. doi: 10.1016/j.jhep.2020.03.039

13. Eslam M, Sanyal AJ, George J, International Consensus P. MAFLD: A consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology*. (2020) 158:1999–2014 e1. doi: 10.1053/j.gastro.2019.11.312

14. Lin S, Huang J, Wang M, Kumar R, Liu Y, Liu S, et al. Comparison of MAFLD and NAFLD diagnostic criteria in real world. *Liver Int*. (2020) 40:2082–9. doi: 10.1111/liv.14548

15. American Diabetes Association Professional Practice C. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2022. *Diabetes Care*. (2022) 45:S17–38. doi: 10.2337/dc22-S002

16. Farrell GC, Chitturi S, Lau GK, Sollano JDAsia-Pacific Working Party on N. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. *J Gastroenterol Hepatol*. (2007) 22:775–7. doi: 10.1111/j.1440-1746.2007.05002.x

17. Liang Y, Chen H, Liu Y, Hou X, Wei L, Bao Y, et al. Association of MAFLD with diabetes, chronic kidney disease, and cardiovascular disease: A 4.6-year cohort study in China. *J Clin Endocrinol Metab*. (2022) 107:88–97. doi: 10.1210/clinem/dgab641

18. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med*. (1996) 334:693–9. doi: 10.1056/NEJM199603143341104

19. Lim GEH, Tang A, Ng CH, Chin YH, Lim WH, Tan DJH, et al. An observational data meta-analysis on the differences in prevalence and risk factors between MAFLD vs NAFLD. *Clin Gastroenterol Hepatol*. (2023) 21:619–29 e7. doi: 10.1016/j.cgh.2021.11.038

20. Pan F, Cao S, Li XL, Jia YN, Wang RL, He Q, et al. The model for end-stage liver disease score and the follow-up period can cause the shift of circulating lymphocyte subsets in liver transplant recipients. *Front Med (Lausanne)*. (2021) 8:779443. doi: 10.3389/fmed.2021.779443

21. Reuben A. Long-term management of the liver transplant patient: diabetes, hyperlipidemia, and obesity. *Liver Transpl*. (2001) 7:S13–21. doi: 10.1053/jlts.2001.29167

22. Watt KD, Charlton MR. Metabolic syndrome and liver transplantation: a review and guide to management. *J Hepatol*. (2010) 53:199–206. doi: 10.1016/j.jhep.2010.01.040

23. Kurdi A, Martinet W, De Meyer GRY. mTOR inhibition and cardiovascular diseases: dyslipidemia and atherosclerosis. *Transplantation*. (2018) 102:S44–S6. doi: 10.1097/TP.0000000000001693

24. Xu WL, Wang RL, Liu Z, Wu Q, Li XL, He Q, et al. Granzyme B-producing B cells function as a feedback loop for T helper cells in liver transplant recipients with acute rejection. *Inflammation*. (2021) 44:2270–8. doi: 10.1007/s10753-021-01498-9

25. Zhu JQ, Wang J, Li XL, Xu WL, Lv SC, Zhao X, et al. A combination of the percentages of IFN-gamma(+)CD4(+)T cells and granzyme B(+)CD19(+)B cells is associated with acute hepatic rejection: a case control study. *J Transl Med*. (2021) 19:187. doi: 10.1186/s12967-021-02855-w

26. Wang CC, Tseng TC, Kao JH. Hepatitis B virus infection and metabolic syndrome: fact or fiction? *J Gastroenterol Hepatol*. (2015) 30:14–20. doi: 10.1111/jgh.12700

27. Shen Z, Munker S, Wang C, Xu L, Ye H, Chen H, et al. Association between alcohol intake, overweight, and serum lipid levels and the risk analysis associated with the development of dyslipidemia. *J Clin Lipidol*. (2014) 8:273–8. doi: 10.1016/j.jacl.2014.02.003

28. Serfaty L. Metabolic manifestations of hepatitis C virus: diabetes mellitus, dyslipidemia. *Clin Liver Dis*. (2017) 21:475–86. doi: 10.1016/j.cld.2017.03.004

29. Flessa CM, Kyrou I, Nasiri-Ansari N, Kaltsas G, Kassi E, Randeva HS. Endoplasmic reticulum stress in nonalcoholic (metabolic associated) fatty liver disease (NAFLD/MAFLD). *J Cell Biochem*. (2022) 123:1585–606. doi: 10.1002/jcb.30247

30. Tabrizian P, Holzner ML, Mehta N, Halazun K, Agopian VG, Yao F, et al. Ten-year outcomes of liver transplant and downstaging for hepatocellular carcinoma. *JAMA Surg*. (2022) 157:779–88. doi: 10.1001/jamasurg.2022.2800

31. Vitale A, Peck-Radosavljevic M, Giannini EG, Vibert E, Sieghart W, Van Poucke S, et al. Personalized treatment of patients with very early hepatocellular carcinoma. *J Hepatol*. (2017) 66:412–23. doi: 10.1016/j.jhep.2016.09.012

32. Arnaoutakis DJ, Mavros MN, Shen F, Alexandrescu S, Firoozmand A, Popescu I, et al. Recurrence patterns and prognostic factors in patients with hepatocellular carcinoma in noncirrhotic liver: a multi-institutional analysis. *Ann Surg Oncol*. (2014) 21:147–54. doi: 10.1245/s10434-013-3211-3

33. Ng IO, Lai EC, Ng MM, Fan ST. Tumor encapsulation in hepatocellular carcinoma. A pathologic study of 189 cases. *Cancer*. (1992) 70:45–9. doi: 10.1002/1097-0142(19920701)70:1<45::AID-CNCR2820700108>3.0.CO;2-7

34. Li C, Wen TF, Yan LN, Li B, Yang JY, Xu MQ, et al. Scoring selection criteria including total tumour volume and pretransplant percentage of lymphocytes to predict recurrence of hepatocellular carcinoma after liver transplantation. *PloS One*. (2013) 8:e72235. doi: 10.1371/journal.pone.0072235

35. Li H, Li XL, Cao S, Jia YN, Wang RL, Xu WL, et al. Decreased granzyme B(+)CD19(+)B cells are associated with tumor progression following liver transplantation. *Am J Cancer Res*. (2021) 11:4485–99.

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Research hot spots and trends in endocrine-related adverse events caused by immune checkpoint inhibitors: a bibliometric analysis and visualization research

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Background: In recent years, with the widespread use of immune checkpoint inhibitors (ICIs) in cancer treatment, the toxicity associated with immunotherapy of ICIs has attracted more attention from scholars. Endocrine toxicity is the most likely immune-related adverse events (irAEs) and is often irreversible, posing a significant clinical treatment challenge.

Methods: In this study, bibliometric methods were used to analyze relevant literature in screening endocrine-related adverse events caused by ICIs in the Web of Science core collection database (WoSCC) and to summarize the status, research hot spots, and future trends in this field.

Results: 321 countries, 297 institutions, 365 authors, and 305 journals had published 671 English documents on endocrine adverse reactions of ICIs as of 1 December, 2022. The United States, Japan, and China were the top three countries with the most publications. The University of Texas MD Anderson Cancer Center, Harvard Medical School, and Memorial Sloan Kettering Cancer Center were the top three research institutions in terms of publication output. F Stephen Hodi, from the Dana-Farber Cancer Institute in the United States, contributed the largest number of publications. Frontiers in Oncology, which was the most widely distributed publication in the field. The main keywords or clusters identified that current research hotspots include the management of endocrine-related adverse events, hypophysitis, thyroid dysfunction, type I diabetes mellitus, and the impact of endocrine adverse events on survival of patients in this field.

Conclusion: The basic knowledge structure of the field of endocrine-related adverse events of ICIs, including publication trends, authors, institutions,

countries, keywords, journals and publications, and cited documents, was visually analyzed in this bibliometric analysis. The research results comprehensively demonstrated the hot spots and future trends in the research field, as well as its broad prospects, thus providing a reference for the researchers.

KEYWORDS

immune checkpoint inhibitor, endocrine, adverse events, toxicity, bibliometrics, visual analysis

1 Introduction

Immune checkpoint inhibitors (ICIs) are monoclonal antibodies that achieve immune activation and tumor tissue damage by promoting signaling cascades of T cell function and can improve the immune system's efficiency in destroying tumor cells (1). The emergence and development of ICIs have brought new concepts and breakthroughs to tumor treatment, as well as unprecedented challenges. ICIs work by non-specific activation of the immune system; consequently, immune-related adverse events (irAEs) of ICIs are an inevitable treatment problem in the use of this class of drugs (2). irAE currently uses more mature cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors, programmed cell death receptor-1 (PD-1) inhibitors, and programmed cell death ligand-1 (PD-L1) inhibitors, among others, to varying degrees (3). These irAEs can affect any organ system, range in severity, and can be fatal (4). The endocrine toxicity of this class of drugs is one of the most prevalent irAEs associated with ICIs treatment. The toxicity related to immunotherapy with this class of drugs is a hot topic among scholars (5). The relevant published research (6) includes several summary studies in this field, but bibliometric analysis and visual analysis research have not yet been conducted. Therefore, this article used bibliometric analysis and visual processing to quantitatively and qualitatively evaluate the research trends in the field of endocrine toxicity caused by ICIs, to objectively reveal the field's research hot spots and development trends, and to provide literature data support for formulating research strategies and directions.

2 Method

2.1 Data source

The literature on endocrine-related adverse events caused by PD-1/PD-L1 monoclonal antibody drugs was screened in the Web of Science (WOS) core collection database using the following search strategy: keyword((TS=("PD-1"OR"PD-L1 "OR"CTLA-4"OR"Immune checkpoint inhibitors")) AND TS=("adversarial events"OR"side effects"OR"adverse events")) AND TS=("Endocrine"OR"diabetes"OR"thyroid" OR"adrenal gland"OR" hypophysis"). The time was set from the database's establishment to, 2022-12-01, and 716 articles were retrieved. Papers and review papers were retained, and the language was set to English; eventually, 671 articles were included. The documents were selected as fully recorded with cited references and then exported in.txt format.

2.2 Data processing

The research used CiteSpace 6.1.R6 with the following settings. Time slicing: January, 2004 to 1 December, 2022 (the first related research was published in, 2004), with one-year time slices. Node types: author, institution, keywords. If the node was the author: threshold (top N per slice) = 25, pruning = none; if the node was the institution: threshold (top N per slice) = 25, pruning = none; if the node was the keyword: threshold (top N per slice) = 25, pruning = pathfinder + pruning the merged network. Visual analysis was performed based on the parameter settings of each node to generate a knowledge map of authors, institutions, countries, and collaborations in the research field of endocrine-related adverse events caused by ICIs; co-occurrence, emergence, and clustering of keywords; a knowledge graph; and a timeline chart for keywords.

The documents retrieved from the WOS database were exported in plain text format with complete records and cited references as the contents. The data were imported into the VOSviewer 1.6.18 software, the calculation method was set to full calculation, and thresholds were established following the various analysis projects. After creating a visual map, the cooperation network was analyzed. Figure 1 depicts the research process and its essential steps.

3 Results

3.1 Posting trends

The first study on ICIs associated endocrine irAEs was published in, 2004. F. Monzani, an Italian scientist, published an article on thyroid autoimmunity and dysfunction associated with type I interferon therapy in "Clinical and Experimental Medicine." According to the publication trend (Figure 2), the annual

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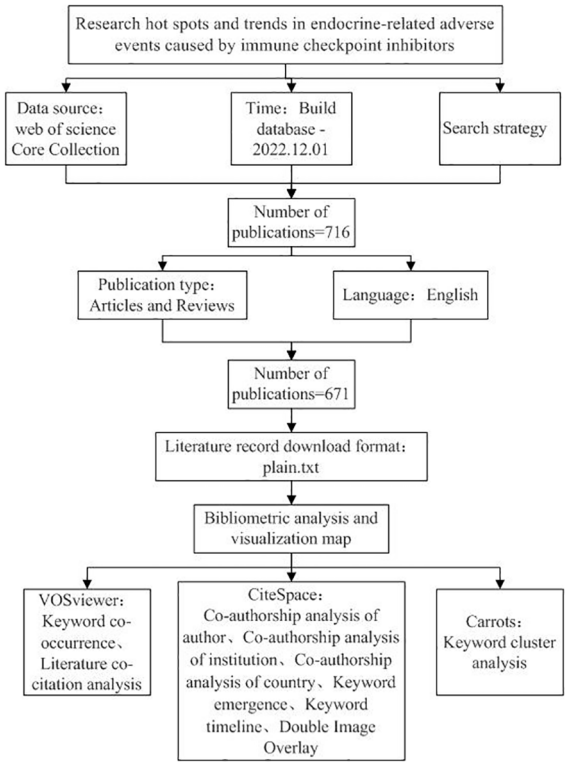

FIGURE 1
Frame flow diagram shows the detailed selection criteria and bibliometric analysis steps in this study.

publication volume had fluctuated, and then, in, 2018, it began to accelerate. After, 2019, the annual publication volume exceeded 100, reaching 143 in, 2021. In, 2022, no all data was included, and 115 articles were published. The trend was expected to be slightly lower than in, 2021.

3.2 Author collaboration network

Citespace was used to analyze an article's author, leading to Figure 3. The figure has 365 nodes and 801 connections, with a network density of 0.0121. Each node in the figure represents an individual author. The greater the node radius, the greater the number of articles published. The number of articles between each pair of nodes, represented by a connecting line, signifies the connection or collaboration between the authors. The cooperative relationship is closer when the connecting line is thicker. According to the findings, the authors in the field have established six collaborative networks. The research team led by F. Stephen Hodi is the largest. The team mainly studies the therapeutic effect and safety of nivolumab and ipilimumab in patients with advanced melanoma, as well as ipilimumab treatment and the effects and safety of metastatic melanoma. The second-largest team is led by Douglas B. Johnson, whose research focuses on the multi-system toxicity caused by ICIs, including endocrine toxicity, neurotoxicity, and cardiotoxicity, as well as the long-term effects of these toxicities on the treated population. Shintaro Iwama heads the third-largest team. This team mainly focuses on the clinical characteristics, treatment, and biomarker identification of endocrine dysfunction induced by ICIs, particularly in thyroid and pituitary dysfunction. Their research is relatively more comprehensive. Table 1 displays the top ten authors in the research field.

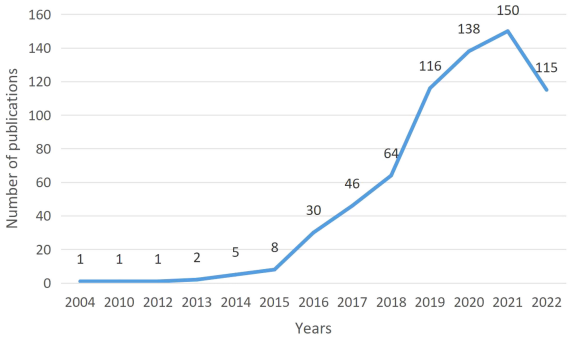

FIGURE 2
The number of publications per year from, 2004 to, 2022.

3.3 Institutional cooperation network

Figure 4 is obtained by analyzing the publishing institutions in the study's included literature using Citespace. The figure contains 297 nodes and 805 connections, with a network density of 0.0183. The study included 297 institutions, nine of which published more than ten articles (Table 2). The University of Texas MD Anderson Cancer Center was the institution with the most publications (25), followed by Harvard Medical School (22) and Memorial Sloan-Kettering Cancer Center (19). The issuing institutions comprise cancer research centers, medical schools, and comprehensive universities. Moreover, there are cooperative ties between institutions. It may be because most American research institutions make cooperation more convenient. Centrality is a

FIGURE 3
Map of author cooperation network in this field, connecting lines stand for author interaction.

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TABLE 1 The top ten authors in the research fields from, 2004-2022.

Rank	Author	Count	Year
1	F Stephen Hodi	10	2017
2	Douglas B Johnson	7	2015
3	Shintaro Iwama	7	2014
4	Alexander M Menzies	7	2017
5	Le Min	6	2018
6	Hiroshi Arima	6	2018
7	Agnese Barnabei	5	2013
8	Lisa Zimmer	5	2016
9	Anupam Kotwal	5	2020
10	Francesco Torino	5	2013

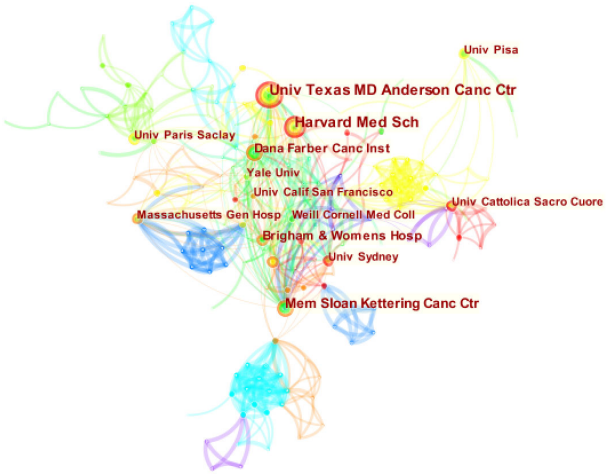

FIGURE 4
Map of institutional cooperation network in this field. Circle size means the number of institutions published articles; the connecting lines stand for mutual communication and interaction.

metric used to evaluate the significance of a network node's position. A value ≥ 0.1 indicates that it plays a significant role in the field's evolution, reflecting the current hot research direction (7). The centralities of the three research institutions, Memorial Sloan-Kettering Cancer Center, University of Sydney, and Weill Cornell Medical College are all greater than 0.1, indicating that their research is vital.

3.4 Analysis of national or regional cooperation networks

As shown in Figure 5, Citespace was used to determine the number of publications by country or region in the study's documents. The figure shows that 321 countries have published relevant documents in the field. The figure depicts 608 connections, with a network density of 0.0118. The United States has published the most research in the field (223), followed by Japan (80), China (75), Italy (64), and France (47). Table 3 lists the ten countries or regions with the most published articles. The United States, Japan, and China play a significant leadership role in the field. The centrality indicates that research significance in the United States and Japan is relatively high, indicating that researchers from these countries have published many influential publications. Meanwhile, China began research in the field relatively late. However, its research activity has increased in recent years, as its research quality still has room for improvement compared to the top two countries. -

3.5 Keywords

3.5.1 Keyword co-occurrence

Keywords are a high-level summary of the paper's topic. Co-occurrence analysis uses keywords as nodes and tailors them to form a map of nodes and connections. Vosviewer was used to generate keyword co-occurrence network diagrams and overlay visualization diagrams for visual analysis. With a minimum of

TABLE 2 The top ten institutions in the research fields from, 2004-2022.

Rank	Institution	Country	Count	Centrality	Year
1	University of Texas MD Anderson Cancer Center	USA	25	0.08	2017
2	Harvard Medical School	USA	22	0.02	2019
3	Memorial Sloan-Kettering Cancer Center	USA	19	0.19	2014
4	Dana-Farber Cancer Institute	USA	16	0.07	2017
5	Brigham and Women's Hospital	USA	14	0.01	2017
6	University of Pisa	Italy	11	0.03	2004
7	The University of Sydney	Australia	11	0.14	2017
8	University of California, San Francisco	USA	10	0.08	2017
9	Massachusetts General Hospital	USA	10	0.02	2019
10	Weill Cornell Medical College	USA	9	0.19	2014

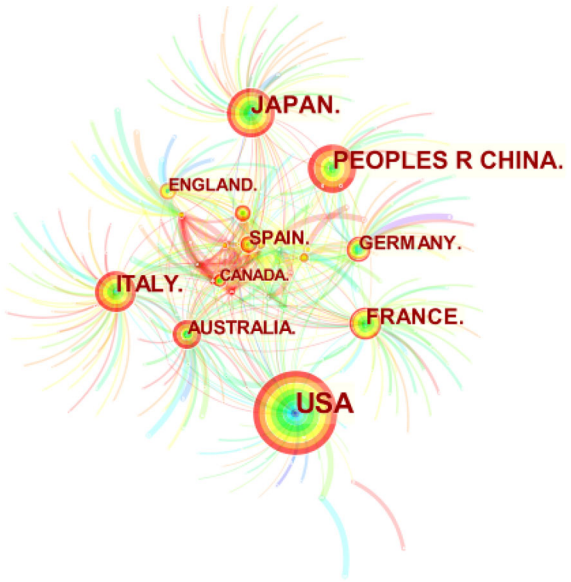

FIGURE 5
Map of national or regional cooperation network in this field. Circle size means the number of published articles.

five occurrences as the screening criterion, Figure 6A's co-occurrence network reveals that of the 1,753 keywords, 211 were screened out and divided into seven clusters. Cluster 1 (44 items, red) is an immune checkpoint inhibitor related to research on the pathogenesis and biomarkers of endocrine toxicity. Cluster 2 (38 items, green) is a clinical trial related to ICIs. Cluster 3 (34 items, blue) describes the clinical characteristics of immune checkpoint inhibitor-induced endocrine toxicity, including research on treatment and prognosis. Cluster 4 (32 items, yellow) is related to adrenal and pituitary dysfunction caused by ICIs. Cluster 5 (31 items, purple) is related to renal toxicity, liver toxicity, and pulmonary toxicity caused by ICIs, including related studies. Cluster 6 (22 items, light blue) studies diabetes-related adverse events caused by anti-PD-1 and PD-L1 monoclonal antibodies. Cluster 7 (10 items, orange) is a study on anti-PD-1 monoclonal antibody-induced thyroid dysfunction, including related research. The keyword overlay visualization diagram (Figure 6B) incorporates time factors. Various colors correspond to the year in which the keyword first appeared. The earlier the keyword appears, the greener the color; the later the keyword appears, the redder the redder it is. Figure 6C depicts the keyword co-occurrence map densities. Each point in the density visualization is colored according to the item's density at that point. The greater the density, the closer the color is to red, while the lower the density, the closer to blue. This map can determine the knowledge and research density in the field.

3.5.2 Keyword cluster analysis

Figure 7 depicts a keyword clustering diagram created with Carrot2. As shown in the figure, current research hotspots include the management of endocrine-related adverse events, hypophysitis, thyroid dysfunction, type I diabetes mellitus, and the impact of endocrine adverse events on survival of patients are also a research focus in this field.

3.5.3 Keyword emergence

Keyword emergence refers to a significant increase in keyword frequency in a short period. Understanding the research that has received significant attention during this period can be used to determine the research hot spots and frontiers in the field (8). Figure 8 depicts the emergence analysis of keywords in research literature regarding endocrine-related adverse events caused by ICIs. Setting the parameters $\gamma[0,1] = 1.0$ and minimum duration = 1 and obtained 13 emergent words. The results show that research began in, 2004, and additional related research did not emerge until, 2010. Disease-related research focuses primarily on melanoma, drug-related research on anti-CTLA-4 monoclonal antibodies and anti-PD-1 monoclonal antibodies, and adverse events research on autoimmune hypophysitis and diabetes. In the past five years, research has primarily focused on diabetes-related adverse events caused by ICIs, endocrine-related adverse events caused by combined nivolumab and ipilimumab, and the impact of such adverse events on patient survival.

TABLE 3 The top ten countries or regional in the research fields from, 2004-2022.

Rank	Country	Count	Centrality	Year
1	USA	223	0.43	2010
2	Japan	80	0.43	2014
3	Peoples R China	75	0.13	2017
4	Italy	64	0.45	2013
5	France	47	0.33	2016
6	Australia	32	0.18	2015
7	Germany	31	0.19	2013
8	Spain	28	0.01	2016
9	England	27	0.12	2015
10	Canada	18	0.08	2017

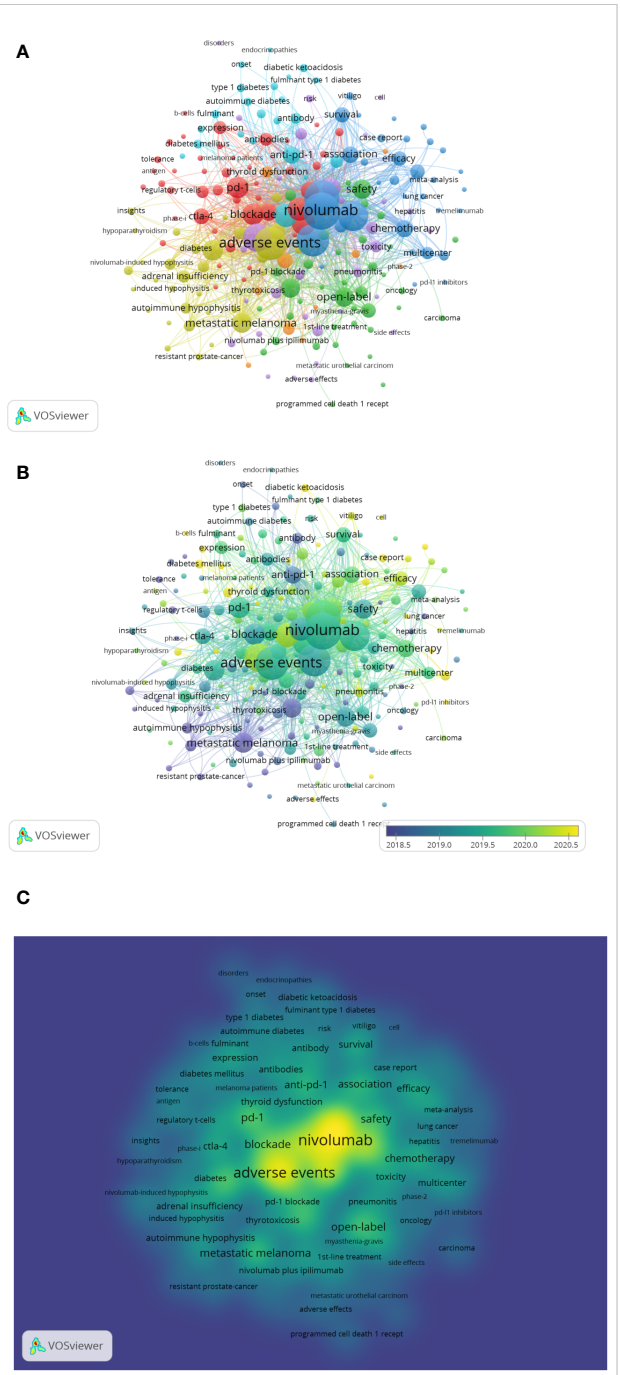

FIGURE 6
(A) Keyword co-occurrence analysis of network knowledge graph. The keywords fell into seven clusters based on colors: Cluster 1, 2, 3, 4, 5,6 and 7 are, respectively red, green, blue, yellow, purple, light blue and orange. The node size denotes the occurrence frequency. **(B)** Distribution of keywords according to the mean frequency of appearance, the different colours indicate the relevant year of publication. Yellow keywords came later than green keywords. **(C)** Keyword co-occurrence density map based on VOSviewer, indicates the occurrence frequency of keyword.

3.5.4 Keyword timeline chart

By selecting "timeline", a timeline map of document clustering was drawn, and the period and correlation of clustering were visually analyzed, as shown in Figure 9. Clusters #5, #7, and #8 have ceased to evolve. In contrast, clusters #0 (open-label), #1 (autoimmunity), #2 (antigen), #3 (the research fields represented by autoimmune hypophysitis), #4 (diabetes mellitus), and #6 (interferon-alpha) have a relatively long period and continue to the present day; hence, they are enduring research hot spots.

3.6 Top ten cited documents and co-cited documents

3.6.1 Top ten cited documents

The top 10 cited documents are listed (Table 4). The most cited document (1241 times) is the article "Immune-related adverse events with Immune checkpoint blockade: a comprehensive review" (9) by Michot, J.M., published in "European Journal of Cancer" (IF =10.002) in, 2016. The article systematically reviewed the mechanisms of irAEs of specific immune checkpoint molecules cytotoxic T lymphocyte-associated antigen-4(CTLA-4), programmed cell death protein (PD-1)and its ligand PD-L1, and the putative relationships between immunodystoxicity and antitumor efficacy. The relevant content of this article has become the basis for management guidelines.

3.6.2 Literature co-citation network

Vosviewer was used to analyze co-cited documents. A total of 12,738 co-cited documents were extracted. At least 20-times cited documents were used for data extraction, and 205 of these documents were obtained. A co-cited document network was generated (Figure 10). The documents were divided into four clusters according to the color blocks. Table 5 displays the ten documents with the most citations. Most cited documents were published in high-level journals; six of them were published in the magazine "New English J Med", with an impact factor of 176.079. All cited documents were likewise separated into four clusters. Cluster 1 (67 items, red) included comprehensive high-level medical journal research; Cluster 2 (58 items, green) included research on journals related to endocrinology; Cluster 3 (51 items, blue) included research on journals related to oncology; and Cluster 4 (29 items, yellow) included research on journals related to diabetes.

3.7 Leading journals

A total of 305 journals have published research-related articles. Table 6 displays the ten journals with the highest number of research publications. One hundred forty-six articles have been published, accounting for 21.40% of all literature. Most of JCR journals are Q1 or Q2. The journal with the most published articles is "Frontiers in Oncology" (20 articles, 2.93%), focusing primarily on cutting-edge tumor treatment research.

3.8 Double figure overlay

A double-figure overlay aims to reveal overall scientific contributions and interactions between journals (10). Publications and citations in the field can be described at the subject level. A

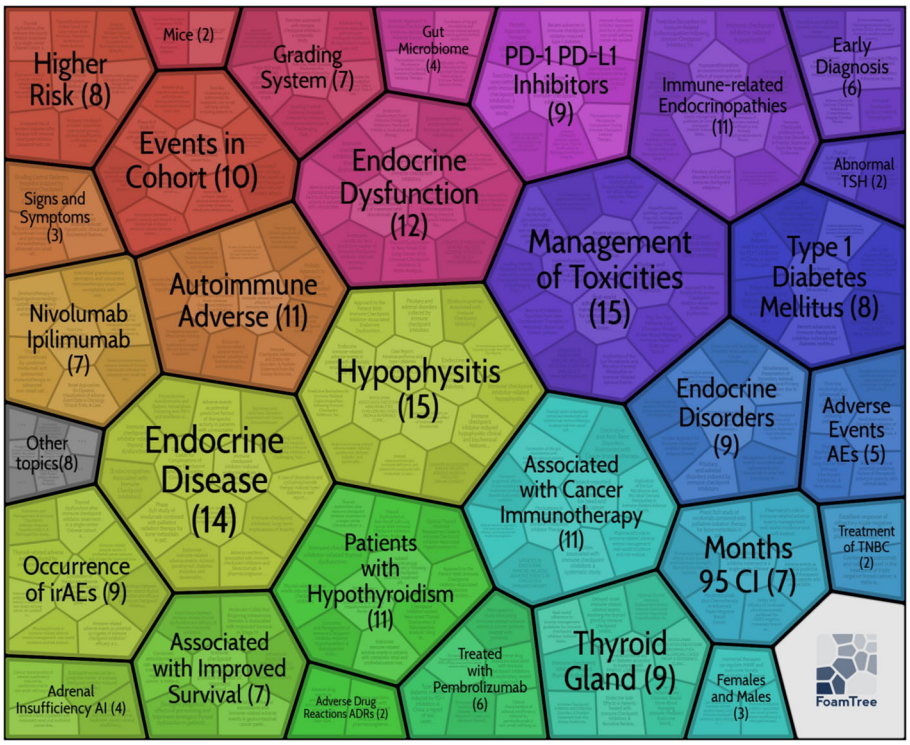

FIGURE 7
Keywords Cluster analysis atlas based on the carrot system, the numbers represent the frequency of keyword clustering.

citation bimap was constructed using Citespace's bimap overlay function. The left half represents the citing document, the right half denotes the cited document, and the curve represents the citation correlation line. The citing document is primarily affected by the cited document. This connection illustrates the flow and interconnectedness of knowledge in different research fields (Figure 11). By Z-score, medicine, medical, and clinical are the most frequently covered record fields, and research is affected by these fields.

Top 13 Keywords with the Strongest Citation Bursts

Keywords	Year	Strength	Begin	End	2004 - 2022
metastatic melanoma	2004	8.1	2010	2016	
anti ctla 4 antibody	2004	6.9	2013	2016	
autoimmune hypophysiti	2004	4.48	2013	2016	
safety	2004	3.68	2015	2016	
clinical activity	2004	3.29	2015	2018	
advanced melanoma	2004	6.25	2016	2018	
untreated melanoma	2004	3.42	2016	2018	
long term safety	2004	3.05	2016	2018	
mellitus	2004	3.99	2017	2018	
anti pd 1	2004	3.36	2017	2018	
nivolumab plus ipilimumab	2004	3.35	2017	2019	
immune checkpoint inhibitor	2004	3.07	2017	2018	
survival	2004	3.45	2021	2022	

FIGURE 8
The top 13 keywords with the most bursts. The year represented by the red line indicates the time period when the keyword was mainly influential.

4 Discussion

4.1 Research status and main results

ICIs are monoclonal antibodies that bind and inhibit CTLA-4 or PD-1 and its ligand PD-L1 to target two key signaling pathways related to T cell activation and exhaustion. ICIs, such as nivolumab, pembrolizumab, and ipilimumab, have been approved for treating numerous types of cancer in various combination regimens and are currently the cornerstone of cancer treatment. ICI-induced toxicity is autoimmune and known as irAEs, which may unpredictably affect any organ system (11). irAEs may manifest as endocrine disorders, including thyroid, pituitary, adrenal, and pancreatic disorders (12). In this study, we used data mining and visualization technology to draw a knowledge map of research on endocrine-related adverse events caused by ICIs. We comprehensively searched for literature on this topic published in the WOS core collection database before 1 December, 2022. We included a total of 671 publications for bibliometric analysis.

The number of publications in different years reflects, to some extent, the degree of researchers' attention to the field. In, 2004, Italian scientist F. Monzani published the first report on thyroid autoimmunity and dysfunction associated with type I interferon therapy in Clinical and Experimental Medicine. The study revealed that the side effects of type I interferons lead to multiple changes in thyroid function, some of which are related to autoimmunity, with hypothyroidism occurring more frequently than hyperthyroidism. It has been confirmed that CTLA-4 gene polymorphisms are

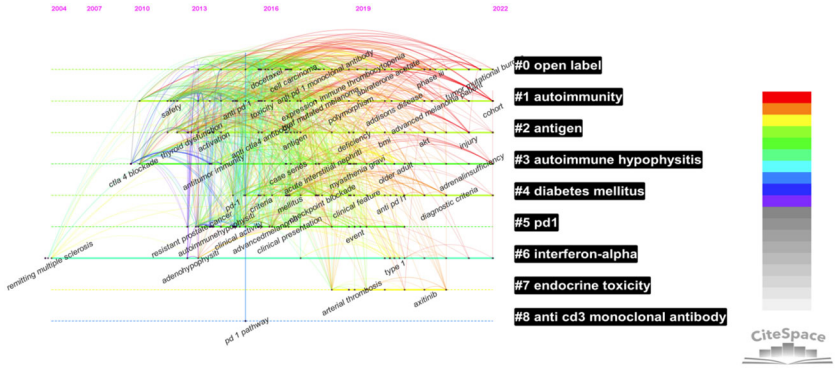

FIGURE 9
Keyword timeline visualization chart based on Citespace.

associated with thyroid dysfunction. From that point forward until, 2014, only 10 papers were published in eleven years. No relevant research results were published for many consecutive years, especially from, 2005 to, 2009. Since the first PD-1 inhibitor pembrolizumab was approved for market by the US Food and Drug Administration (FDA) in, 2014, Many types of ICIs have been approved and applied to the treatment of various tumors worldwide. An annual rise in the frequency of drug-related adverse reactions has coincided with the addition of new indications for ICIs in cancer therapy and the growing patient population. The negative consequences of irAEs on patients are now receiving more attention (13). Consequently, since, 2015, there has been a significant increase in the field of irAE research. The, 2018 Nobel Prize in Medicine or Physiology was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation" (14). Since then, it has further boosted the research heat in this field, leading to a more significant increase in the number of studies since, 2018. As of, 2021, the annual publication volume has reached 150 articles. Our research has found that as of December 1, 2022, 115 articles have been published in this field. Although the number of publications has slightly decreased compared to last year, it is still at a relatively high level. We believe that with the increasing clinical application of ICIs, research in this field will continue to receive widespread attention and remain a hot topic for future research.

4.2 Research hot spots and cutting-edge trends

Keyword clustering illustrates the direction of research hotspots in this field, whereas the timeline view depicts the evolution of related hotspots over time. Combining keyword co-occurrence with highly cited papers makes it possible to identify and detect research

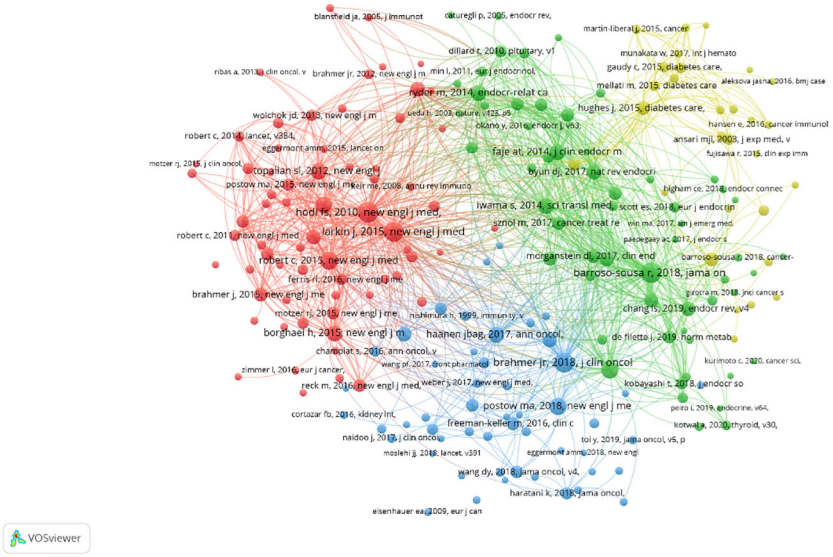

FIGURE 10
Document co-citation network diagram. The different colours of the nodes represent different types of documents, with larger nodes meaning articles that are collectively cited more frequently.

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TABLE 4 Statistical table of the top ten cited literatures in the field.

Rank	Title	First author	Publication Year	Total Citations	Average per Year
1	Immune-related adverse events with immune checkpoint blockade: a comprehensive review	Michot, J. M	2016	1241	177.29
2	Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group	Puzanov, I	2017	997	166.17
3	Safety Profile of Nivolumab Monotherapy: A Pooled Analysis of Patients With Advanced Melanoma	Almazor, M. E	2017	692	115.33
4	Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study	Marabelle, Aurelien	2020	657	219
5	Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination	Kao, Steven	2016	606	86.57
6	Management of toxicities of immune checkpoint inhibitors	Spain, Lavinia	2016	525	75
7	Incidence of Endocrine Dysfunction Following the Use of Different Immune Checkpoint Inhibitor Regimens A Systematic Review and Meta-analysis	Barroso-Sousa	2018	471	94.2
8	Myocarditis in Patients Treated With Immune Checkpoint Inhibitors	Mahmood, Syed S	2018	425	85
9	Pituitary Expression of CTLA-4 Mediates Hypophysitis Secondary to Administration of CTLA-4 Blocking Antibody	Awadalla, Magid	2014	399	44.33
10	Cutaneous, gastrointestinal, hepatic, endocrine, and renal side-effects of anti-PD-1 therapy	Hofmann, Lars	2016	393	56.14

TABLE 5 Statistical table of top ten co-cited literature in this field.

Rank	Title	First Author	Year	Journal	IF (2022)	Citations
1	Improved survival with ipilimumab in patients with metastatic melanoma	Hodi FS	2010	New Engl J Med	176.079	175
2	Incidence of Endocrine Dysfunction Following the Use of Different Immune Checkpoint Inhibitor Regimens: A Systematic Review and Meta-analysis	Barroso-sousa R	2018	Jama Oncology	33.006	167
3	Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline	Brahmer Jr	2018	J Clin Oncol	50.717	161
4	Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma	Larkin J	2015	New Engl J Med	176.079	157
5	Pembrolizumab versus Ipilimumab in Advanced Melanoma	Robert C	2015	New Engl J Med	176.079	128
6	Immune-Related Adverse Events Associated with Immune Checkpoint Blockade	Postow Ma	2018	New Engl J Med	176.079	125
7	Antibody-mediated thyroid dysfunction during T-cell checkpoint blockade in patients with non-small-cell lung cancer	Osorio JC	2017	Ann Oncol	51.769	124
8	Nivolumab in previously untreated melanoma without BRAF mutation	Robert C	2015	New Engl J Med	176.079	111
9	Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer	Borghaei H	2015	New Engl J Med	176.079	105
10	Ipilimumab-induced hypophysitis: a detailed longitudinal analysis in a large cohort of patients with metastatic melanoma	Faje At	2014	J Clin Endocr Metab	6.134	104

TABLE 6 The top 10 leading journals with the most published papers in the field.

Journal of Publication	Count	Percentage	IF	JCR(2022)
Frontiers in Oncology	20	2.93%	5.738	Q2
Cancers	18	2.64%	6.575	Q1
Oncologist	17	2.49%	5.837	Q2
Journal of Clinical Endocrinology & Metabolism	15	2.20%	6.134	Q1
Cancer Immunology, Immunotherapy	14	2.05%	6.63	Q1
European Journal of Cancer	14	2.05%	10.002	Q1
Journal for ImmunoTherapy of Cancer	14	2.05%	12.469	Q1
Journal of Oncology Pharmacy Practice	14	2.05%	1.416	Q4
Frontiers in Immunology	10	1.47%	8.786	Q1
Thyroid	10	1.47%	6.506	Q1

hotspots and frontiers with greater precision. In, 2004, research in this field began. Since, 2010, more research on metastatic melanoma has been published. Anti-CTLA-4 monoclonal antibodies and anti-PD-1 monoclonal antibodies have been the subject of many safety studies in this field. Nivolumab, ipilimumab, and pembrolizumab have become popular research topics in this field. After many safety studies on the use of single drugs emerged and grew increasingly comprehensive, studies on the safety of the combination or sequential use of anti-PD-1 and anti-CTLA-4 drugs appeared immediately (15, 16). In a phase 3 study, researchers compared the effectiveness of the combination of ipilimumab and nivolumab to that of either antibody alone. This combination elicited a more robust response. However, immune-related toxicities occur more frequently and at a higher grade than with either agent alone (17). In a separate exploratory study, the combination of ipilimumab and nivolumab was more effective than nivolumab alone regarding progression-free survival and overall survival landmarks (18). In, 2015, regulatory approval was granted for combination immunotherapy (19). However, due to the higher risk of toxicity associated with combination therapy, researchers are very interested in identifying the subgroups of patients most likely to benefit from combination therapy. So, they weigh the therapeutic efficacy of combination therapy with ICIs against the incidence of irAEs. Since, 2021, the patient population that may benefit from combination therapy has become a focal point of research in this field.

Comprehensive studies of adverse effects at different endocrine sites have started to appear and have reached their current degree of maturity as a result of the continual development of safety studies. According to reports, thyroid and pituitary toxicity are the most common endocrine toxicities of this class of drugs (20) and are also the most studied by researchers. Diabetes and adrenocortical insufficiency are uncommon endocrine toxicities associated with ICI treatment (21), but diabetic ketoacidosis (DKA) and adrenal crisis are frequently life-threatening. This study found that most of the field's research focuses on autoimmune hypophysitis resulting from ICIs, diabetes resulting from ICIs, and thyroid dysfunction resulting from ICIs.

The treatments most commonly associated with immune-related hypophysitis are anti-CTLA-4 monotherapy, anti-CTLA-4 and anti-PD-1 combination therapy, and anti-PD-1 or anti-PD-L1 monotherapy (22). Hypophysitis secondary to tumor immunotherapy is an emerging area of clinical research. It may be attributable to its non-specific symptoms and signs, which are frequently overlooked and can have severe consequences. Currently, cases of delayed hypophysitis occurring several months after the cessation of ICIs have garnered much scholarly interest (23, 24).

This study found that the study of diabetes-related adverse events caused by ICIs has become a hot topic. Diabetes caused by ICIs has been reported to be more prevalent following treatment with PD-1/PD-L1 inhibitors (25, 26). The disease progresses rapidly to islet failure, and the damage to islet B cell function is frequently irreversible (27). Most cases of this type of diabetes require daily insulin treatment over the long term (28). If diabetes caused by ICIs is not diagnosed and identified promptly, the risk of diabetic ketoacidosis (DKA) is elevated (21). In most ICIs-related diabetes cases (70%) described to date, DKA is the underlying cause. Consequently, this type of adverse reaction has also become a popular topic of study among academics.

There is a reported 6%–20% incidence of thyroid-related injuries with ICIs, including hyperthyroidism, hypothyroidism, thyroiditis, thyrotoxicosis, and thyroid storm, especially with the use of anti-PD-1 or anti-PD-L antibodies (29). It has been reported that PD-1/PD-L1 inhibitors cause a more significant proportion of hyperthyroid patients than CTLA-4 inhibitors. The combined use of ICIs will increase the proportion of hyperthyroid patients by at least threefold (30, 31). Consider the positive aspects of thyrotoxicity, thyroid irAEs are associated with better cancer outcomes, improved survival, and a better prognosis for patients who experience them (32, 33). It may be due to the effective activation of the immune system (34), and the specific mechanism has become the focus of research by scholars. Historically, most endocrine adverse reactions were caused by the involvement of a single gland. However, with the gradual increase in clinical use of ICIs, reports of involvement of two or more glands

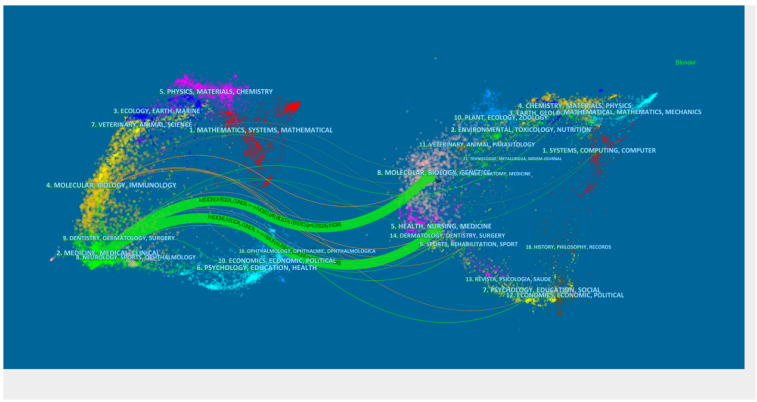

FIGURE 11
Double graph overlay of relevant literature on endocrine-related adverse events caused by ICIs. On the left were the citing journals, on the right were the cited journals, and the colored path represented the citation relationship.

are no longer uncommon. According to studies, the combination of thyroid and pancreatic injuries is the most prevalent multi-gland injury (35). Patients receiving ICIs must continue to monitor these endocrine-related events.

ICI-related endocrine adverse reactions are severe but quite manageable. Although they rarely lead to treatment discontinuation, many irAEs are irreversible (36). If endocrine irAEs can be effectively managed, the patient's prognosis will be drastically improved, and the patient's survival time will be extended. However, irreversible irAEs continue to have an impact on the quality of life of patients. This study found that the management of endocrine irAEs and the study of the survival of patients with irreversible endocrine adverse events may be future research hotspots in the field. Clinicians should focus on monitoring endocrine adverse reactions, the balance between endocrine adverse reactions and patient prognosis, and the accurate assessment of the balance between patient prognosis and the harm of adverse reactions to those who will benefit. In addition to strong interdisciplinary collaboration, as the number of patients receiving ICIs rises, it is critical that researchers in this field aggressively pursue efficient ways to predict biomarkers of the risk of endocrine adverse effects (37).

4.3 Limitations

Certainly, this paper has some limitations. The data was only retrieved from the WOSCC database, and the research literature of some countries may have been omitted. Furthermore, our study only included relevant literature in English, and studies published in other languages were excluded, which could cause a certain degree of bias in the analysis. Nonetheless, WOS remains the most commonly used database for scientometric analysis, and English is today's international lingua franca. In addition, recently published high-quality studies may not have received the attention they deserved due to citation delays and need to be updated in subsequent studies. Ultimately, we believe that these limitations may slightly impact the results, but will not have a significantly impact on the main trend in the field.

5 Conclusion

This study analyzed trends and hot spots in ICIs associated endocrine irAEs research. Based on the results, we believe that future research hotspots will mainly focus on the following aspects: Firstly, it is critical to accurately assess the balance between efficacy and adverse effects in patients with ICIs to facilitate the identification of these endocrine irAEs events and prognostic or survival analysis studies. Secondly, the continuous deepening and expansion of research on new irAEs, with the application of new ICIs drugs and combination therapy. Thirdly, conducting cohort studies to understand risk factors and patterns of onset. These future hotspots are crucial to promoting greater advancing on irAEs, which is where the potential of this study lies.

Author contributions

JZ: Writing – original draft. GL: Writing – review & editing, Data curation. XY: Writing – review & editing. CZ: Writing – review & editing, Data curation. BH: Writing – review & editing. MJ: Writing – review & editing, Methodology.

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Conflict of interest

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

Publisher's note

References

1. Naimi A, Mohammed RN, Raji A, Chupradit S, Yumashev AV, Suksatan W, et al. Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. *Cell Commun Signal*. (2022) 20:44. doi: 10.1186/s12964-022-00854-y

2. Wang DY, Salem JE, Cohen JV, Chandra S, Menzer C, Ye F, et al. Fatal toxic effects associated with immune checkpoint inhibitors: A systematic review and meta-analysis. *JAMA Oncol*. (2018) 4(12):1721–8. doi: 10.1001/jamaoncol.2018.3923

3. Chennamadhavuni A, Abushahin L, Jin N, Presley CJ, Manne A. Risk factors and biomarkers for immune-related adverse events: A practical guide to identifying high-risk patients and rechallenging immune checkpoint inhibitors. *Front Immunol*. (2022) 13:779691. doi: 10.3389/fimmu.2022.779691

4. Khoja L, Day D, Wei-Wu Chen T, Siu LL, Hansen AR. Tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors: a systematic review. *Ann Oncol*. (2017) 28:2377–85. doi: 10.1093/annonc/mdx286

5. Cardona Z, Sosman JA, Chandra S, Huang W. Endocrine side effects of immune checkpoint inhibitors. *Front Endocrinol (Lausanne)*. (2023) 14:1157805. doi: 10.3389/fendo.2023.1157805

6. Zhai Y, Ye X, Hu F, Xu J, Guo X, Zhuang Y, et al. Endocrine toxicity of immune checkpoint inhibitors: a real-world study leveraging US Food and Drug Administration adverse events reporting system. *J Immunother Cancer*. (2019) 7:286. doi: 10.1186/s40425-019-0754-2

7. Shen J, Shen H, Ke L, Chen J, Dang X, Liu B, et al. Knowledge mapping of immunotherapy for hepatocellular carcinoma: A bibliometric study. *Front Immunol*. (2022) 13:815575. doi: 10.3389/fimmu.2022.815575

8. Liu G, Zhao J, Tian G, Li S, Lu Y. Visualizing knowledge evolution trends and research hotspots of artificial intelligence in colorectal cancer: A bibliometric analysis. *Front Oncol*. (2022) 12:925924. doi: 10.3389/fonc.2022.925924

9. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer*. (2016) 54:139–48. doi: 10.1016/j.ejca.2015.11.016

10. Liu K, Zhao S, Li J, Zheng Y, Wu H, Kong J, et al. Knowledge mapping and research hotspots of immunotherapy in renal cell carcinoma: A text-mining study from 2002 to 2021. *Front Immunol*. (2022) 13:969217. doi: 10.3389/fimmu.2022.969217

11. Ramos-Casals M, Brahmer JR, Callahan MK, Flores-Chávez A, Keegan N, Khamashta MA, et al. Immune-related adverse events of checkpoint inhibitors. *Nat Rev Dis Primers*. (2020) 6:38. doi: 10.1038/s41572-020-0160-6

12. Wright JJ, Powers AC, Johnson DB. Endocrine toxicities of immune checkpoint inhibitors. *Nat Rev Endocrinol*. (2021) 17:389–99. doi: 10.1038/s41574-021-00484-3

13. Darnell EP, Mooradian MJ, Baruch EN, Yilmaz M, Reynolds KL. Immune-related adverse events (irAEs): Diagnosis, management, and clinical pearls. *Curr Oncol Rep*. (2020) 22:39. doi: 10.1007/s11912-020-0897-9

14. Bernadic M Jr, Duchon R, Aziri R, Mladosievicova B. New principles of cancer therapy give new hope for oncological patients. *Bratisl Lek Listy*. (2019) 120:15–8. doi: 10.4149/BLL_2019_002

15. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med*. (2013) 369:122–33. doi: 10.1056/NEJMoa1302369

16. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037) : a randomised, controlled open-label,phase 3 trial. *Lancet Oncol*. (2015) 16:375–84. doi: 10.1016/S1470-2045(15)70076-8

17. Paz-Ares LG, Ramalingam SS, Ciuleanu TE, Lee JS, Urban L, Caro RB, et al. First-line nivolumab plus ipilimumab in advanced NSCLC: 4-year outcomes from the randomized, open-label, phase 3 checkMate 227 part 1 trial. *J Thorac Oncol*. (2022) 17(2):289–308. doi: 10.1016/j.jtho.2021.09.010

18. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, et al. Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma. *J Clin Oncol*. (2022) 40:127–37. doi: 10.1200/JCO.21.02229

19. Sullivan RJ. Adjuvant nivolumab plus ipilimumab for resected stage IV melanoma. *Lancet*. (2020) 395:1524–5. doi: 10.1016/S0140-6736(20)31044-8

20. Chera A, Stancu AL, Bucur O. Thyroid-related adverse events induced by immune checkpoint inhibitors. *Front Endocrinol (Lausanne)*. (2022) 13:1010279. doi: 10.3389/fendo.2022.1010279

21. Chang LS, Barroso-Sousa R, Tolaney SM, Hodi FS, Kaiser UB, Min L. Endocrine toxicity of cancer immunotherapy targeting immune checkpoints. *Endocr Rev*. (2019) 40:17–65. doi: 10.1210/er.2018-00006

22. Ferrari SM, Fallahi P, Galetta F, Citi E, Benvenga S, Antonelli A. Thyroid disorders induced by checkpoint inhibitors. *Rev Endocrine Metab Disord*. (2018) 19:325–33. doi: 10.1007/s11154-018-9463-2

23. Antoniou S, Bazazo G, Röckl L, Papadakis M, Berg C. Late-onset hypophysitis after discontinuation of nivolumab treatment for advanced skin melanoma: A case report. *BMC Endocr Disord*. (2021) 21:191. doi: 10.1186/s12902-021-00854-y

24. Otsubo K, Nakatomi K, Furukawa R, Ashida K, Yoneshima Y, Nakanishi Y, et al. Two cases of late-onset secondary adrenal insufficiency after discontinuation of nivolumab. *Ann Oncol*. (2017) 28:3106–7. doi: 10.1093/annonc/mdx497

25. Hickmott L, de la Peña H, Turner H, Ahmed F, Protheroe A, Grossman A, et al. Anti-PD-L1 atezolizumab-induced autoimmune diabetes: a case report and review of the literature. *Target Oncol*. (2017) 12:235–41. doi: 10.1007/s11523-017-0480-y

26. Paschou SA, Stefanaki K, Psaltopoulou T, Liontos M, Koutsoukos K, Zagouri F, et al. How we treat endocrine complications of immune checkpoint inhibitors. *ESMO Open*. (2021) 6:100011. doi: 10.1016/j.esmoop.2020.100011

27. Zheng Z, Liu Y, Yang J, Tan C, Zhou L, Wang X, et al. Diabetes mellitus induced by immune checkpoint inhibitors. *Diabetes Metab Res Rev*. (2021) 37:e3366. doi: 10.1002/dmrr.3366

28. Okura N, Asano M, Uchino J, Morimoto Y, Iwasaku M, Kaneko Y, et al. Endocrinopathies associated with immune checkpoint inhibitor cancer treatment: a review. *J Clin Med*. (2020) 9:2033. doi: 10.3390/jcm9072033

29. El Sabbagh R, Azar NS, Eid AA, Azar ST. Thyroid dysfunctions due to immune checkpoint inhibitors: A review. *Int J Gen Med*. (2020) 13:1003–9. doi: 10.2147/IJGM.S261433

30. De Filette J, Andreescu CE, Cools F, Bravenboer B, Velkeniers B. A systematic review and meta-analysis of endocrine-related adverse events associated with immune checkpoint inhibitors. *Hormone Metab Res*. (2019) 51:145–56. doi: 10.1055/a-0843-3366

31. Stelmachowska-Banasí M, Czajka-Oraniec I. Management of endocrine immune-related adverse events of immune checkpoint inhibitors: An updated review. *Endoc Connect*. (2020) 9:R207–28. doi: 10.1530/EC-20-0342

32. Basak EA, van der Meer JWM, Hurkmans DP, Schreurs MWJ, Oomen-de Hoop E, van der Veldt AAM, et al. Overt thyroid dysfunction and anti-thyroid antibodies predict response to anti-PD-1 immunotherapy in cancer patients. *Thyroid*. (2020) 30:966–73. doi: 10.1089/thy.2019.0726

33. Chieng JHL, Htet ZW, Zhao JJ, Tai ES, Tay SH, Huang Y, et al. Clinical presentation of immune-related endocrine adverse events during immune checkpoint inhibitor treatment. *Cancers*. (2022) 14:2687. doi: 10.3390/cancers14112687

34. Muir CA, Tsang VHM, Menzies AM, Clifton-Bligh RJ. Immune related adverse events of the thyroid - A narrative review. *Front Endocrinol (Lausanne)*. (2022) 13:886930. doi: 10.3389/fendo.2022.886930

35. Zhao Z, Wang X, Bao XQ, Ning J, Shang M, Zhang D. Autoimmune polyendocrine syndrome induced by immune checkpoint inhibitors: a systematic review. *Cancer Immunol Immunother*. (2021) 70:1527–40. doi: 10.1007/s00262-020-02699-1

36. O'Reilly A, Hughes P, Mann J, Lai Z, Teh JJ, Mclean E, et al. An immunotherapy survivor population: health-related quality of life and toxicity in patients with metastatic melanoma treated with immune checkpoint inhibitors. *Support Care Cancer*. (2020) 28:561–70. doi: 10.1007/s00520-019-04818-w

37. Nakamura Y. Biomarkers for immune checkpoint inhibitor-mediated tumor response and adverse events. *Front Med (Lausanne)*. (2019) 6:119. doi: 10.3389/fmed.2019.00119

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Assessment of bidirectional relationships between hypothyroidism and endometrial cancer: a two-sample Mendelian randomization study

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Objective: Hypothyroidism, characterized by reduced thyroid hormone levels, and endometrial cancer, a prevalent gynecological malignancy, have been suggested to have a potential association in previous observational studies. However, the causal relationship between them remains uncertain. This study aimed to investigate the causal relationship between hypothyroidism and endometrial cancer using a bilateral Mendelian randomization approach.

Methods: A bidirectional two-sample Mendelian randomization study was conducted using summary statistics from genome-wide association studies to identify genetic variants associated with hypothyroidism and endometrial cancer. The inverse variance weighting method was used as the main analysis, and sensitivity analyses were conducted to validate the MR results.

Results: The results of our analysis did not support a causal effect of hypothyroidism (OR: 0.93, p=0.08) or autoimmune hypothyroidism (OR: 0.98, p=0.39) on endometrial cancer risk. In the reverse MR analysis, we did not find a significant causal effect of endometrial cancer on hypothyroidism (OR: 0.96, p=0.75) or autoimmune hypothyroidism (OR: 0.92, p=0.50). Based on subgroup analysis by pathological subtypes of endometrial cancer, the above findings were further substantiated (all p-value >0.05).

Conclusions: Our Mendelian randomization analysis suggests a lack of causal association between hypothyroidism and endometrial cancer. To gain a deeper understanding of this association, it is essential to conduct large-scale randomized controlled trials in the future to validate our findings.

KEYWORDS

hypothyroidism, autoimmune hypothyroidism, endometrial cancer, Mendelian randomization, causality

1 Introduction

Endometrial cancer (EC) is a malignant epithelial tumor originating in the endometrium, ranking as the most common cancer type in developed countries and the second most common in developing countries (1, 2). In 2020, a significant number of newly diagnosed cases were reported worldwide, with 417,367 cases and 97,370 deaths attributed to this disease (2). Notably, China has observed a high incidence of EC, with approximately 84,520 new cases reported in 2022 (3). Unlike other types of cancer that have experienced declining incidence rates over the past two decades, the global incidence of endometrial cancer has continued to rise steadily (1). Furthermore, there is a significant trend towards an earlier age of onset, especially observed in South Africa and specific Asian countries with a considerably high incidence rate (4). Of particular concern is the fact that around 10% to 15% of EC patients are diagnosed at an advanced stage, resulting in a relatively low 5-year survival rate of only 10% to 20% (5). Given these challenges, it is imperative to prioritize the identification of innovative risk factors in order to mitigate the forthcoming healthcare burden.

Hypothyroidism, a prevalent endocrine disorder characterized by reduced thyroid hormone production, affects a significant number of individuals with varying rates of prevalence among women ranging from 0.6% to 12% (6). The relationship between EC and hypothyroidism has been investigated extensively (7). Some studies have found that hypothyroidism is a common comorbidity among patients with endometrial cancer (6, 8). Additionally, it has been observed that 15.3% of EC patients had a prior diagnosis of hypothyroidism, and an additional 8.5% exhibited biochemical evidence of subclinical hypothyroidism based on baseline blood tests (9). Although epidemiological studies suggest a potential link between hypothyroidism and EC, these observations are based on observational data, which are susceptible to confounding factors and bias. Gaining a comprehensive understanding of the causal association between hypothyroidism and endometrial cancer holds significant clinical implications for effective patient management. Regrettably, those previous reports did not provide any information on exact causal relationships between hypothyroidism on endometrial cancer.

Mendelian randomization (MR) is a powerful analytical approach that addresses the limitations inherent in observational methods by employing genetic variants, predominantly single nucleotide polymorphisms (SNPs), as instrumental variables (IVs) to evaluate potential causal associations between exposures and outcomes (10, 11). The random assignment of genetic variants at conception mimics the process of randomization in controlled trials, thereby minimizing the influence of confounding factors (11). Furthermore, as genetic variants precede the onset of disease, they mitigate reverse causation. Importantly, the selected IVs is associated with the exposure but not with any confounders in the exposure-outcome relationship, nor does it exert any other effects on the outcome apart from through the exposure (12). Understanding the causality and biology underlying this association between EC and hypothyroidism is important for deciphering the etiology and can provide therapeutic insights. Therefore, this study aims to contribute to the current knowledge of the causal relationship between endometrial cancer and hypothyroidism using an MR approach to potentially provide a unique perspective.

2 Methods

2.1 Study design

We present a concise overview of the bidirectional MR design, as depicted in Figure 1. MR analysis is based on three key assumptions (1): The selected instrumental variable, a genetic variant, exhibits a robust association with the exposure; (2) The genetic variant is not associated with any confounding factors; (3) The genetic variants solely influence the outcome through the exposure and not via alternative pathways. To conduct our study, we utilized summary-level data obtained from published genome-wide association studies (GWAS) investigating hypothyroidism and EC (13). Bidirectional MR was performed to evaluate the causal effects of hypothyroidism on EC (forward MR), as well as the effects of EC on hypothyroidism (reverse MR). In the forward MR analysis, we first identified genetic variants associated with hypothyroidism to infer the causal relationship between hypothyroidism and EC. Subsequently, in the reverse MR analysis, we utilized genetic variants associated with EC to infer the causal relationship between EC and hypothyroidism.

2.2 Data sources

The summary-level data for the two-sample MR study was obtained from the GWAS database (https://gwas.mrcieu.ac.uk/). Specifically, we utilized data on hypothyroidism (GWAS ID: finn-b-HYPOTHYROIDISM) and autoimmune hypothyroidism (GWAS ID: finn-b-E4_HYTHY_AI_STRICT). Additionally, the data on EC (GWAS ID: ebi-a-GCST006464), endometrioid EC (GWAS ID: ebi-a-GCST006465), and non-endometrioid EC (GWAS ID: ebi-a-GCST006466) was also extracted from the GWAS database. The initial GWAS studies were conducted with approval from the relevant ethics committee, and all participants provided informed consent. Details of the included cohorts were listed in Supplementary Table S1.

2.3 Selection of genetic instrumental variables

Two thresholds were used to select the IVs. The first threshold selected SNPs less than the genome-wide statistical significance threshold ($p < 5 \times 10^{-8}$) to serve as IVs. Unfortunately, after we selected SNPs, only a small number of non-endometrioid EC were selected as IVs, and to explore more relations between hypothyroidism and non-endometrioid EC to obtain more comprehensive results, we used the second threshold that identified SNPs that were smaller than the locus-wide significance level and selected them as the second IVs set to find more potential causal associations ($p < 1 \times 10^{-7}$). The independence of SNPs was evaluated using stringent criteria ($r^2 \leq 0.001$; window size =10,000 kb).

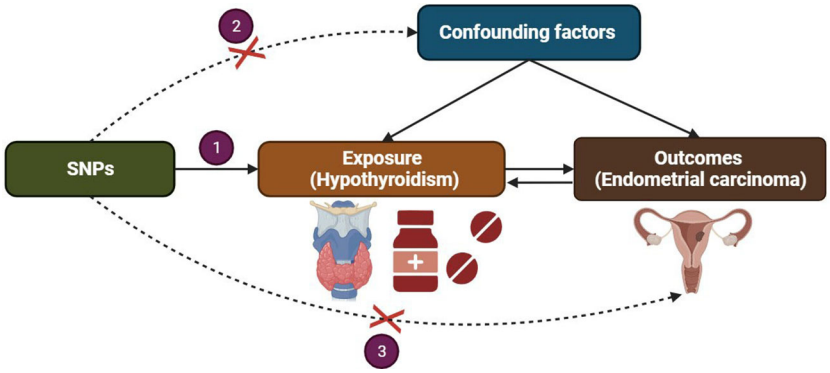

FIGURE 1
Three assumptions of Mendelian randomization.

2.4 Statistical analysis

The primary analysis employed the inverse variance weighted (IVW) method to obtain an unbiased estimate of the causal relationship (12). Additional methods, including the MR Egger and weighted mode, were applied to estimate causal effects under different conditions (14). The weighted median method combined data from multiple genetic variants into a single causal estimate, providing consistent results if at least half of the weight derived from valid instrumental variables (14). The MR-Egger method assessed directional pleiotropy and provided a consistent estimate of the causal effect (15). The intercept of MR-Egger regression was calculated to evaluate horizontal pleiotropy, with a p-value >0.05 indicating weak evidence of pleiotropic effects in the causal analysis (16). Cochran's Q test, derived from IVW estimation, detected heterogeneity among instrumental variables. Sensitivity analysis was conducted using a leave-one-out method, sequentially removing one SNPs and using the remaining SNPs as instrumental variables for two-sample MR analysis, which assessed the degree of influence of each SNPs on the causal association. Additionally, a reverse-direction MR analysis was performed to investigate the possibility of a reverse-direction causal relationship. The TwoSampleMR package for R software (version 4.2.0) was used for all analyses (17).

3 Results

3.1 Instrumental variables

All IVs utilized in our analysis exhibited F-statistics exceeding 10, indicating a robust predictive capability for exposure and minimal bias resulting from weak IVs in our investigation. Most of the exposure analyses showed moderate heterogeneity (all p-values for Cochrane's Q > 0.05), which prompted the adoption of the random-effects IVW MR method. Comprehensive details regarding the chosen SNPs are available in Supplementary Tables S2-S13.

3.2 Causal effect of hypothyroidism on the risk of EC

Our investigation revealed no significant causal effect of hypothyroidism on the risk of EC [odds ratio (OR) = 0.93, 95% confidence interval (CI): 0.87–1.01, p = 0.08]. Both MR-Egger analysis (OR = 0.86, 95% CI: 0.70–1.06, p = 0.17) and weighted median analysis (OR = 0.94, 95% CI: 0.85–1.03, p = 0.20) supported this finding (Figure 2A). Furthermore, we conducted additional analysis to explore the potential causal association between autoimmune hypothyroidism and the risk of EC. Similarly, no causality for genetically predicted autoimmune hypothyroidism on EC risk using the IVW method (OR = 0.98, 95% CI: 0.92–1.03, p = 0.39) (Figure 2A). The results obtained from weighted median and MR-Egger analyses were consistent with those from the IVW analysis (all p-values > 0.05) (Figure 2A).

3.3 Causal effect of EC on the risk of hypothyroidism

Figure 2B presents the findings of MR analysis examining the causal effect of EC on the risk of hypothyroidism. We observed genetic liability to EC was not causally associated with risk of hypothyroidism based on IVW analysis (OR = 0.96, 95% CI: 0.77–1.21, p= 0.75). The results of weighted median and MR-Egger are consistent with those of IVW (all p-value > 0.05) (Figure 2B). Furthermore, the results from three different MR methods did not reveal any statistically significant associations between EC and autoimmune hypothyroidism examined (all p-values >0.05) (Figure 2B).

3.4 Subgroup analysis based on pathological subtypes of EC

To strengthen our findings, we conducted a subgroup analysis based on the pathological subtypes of endometrial cancer. The results from this analysis were consistent with the above findings,

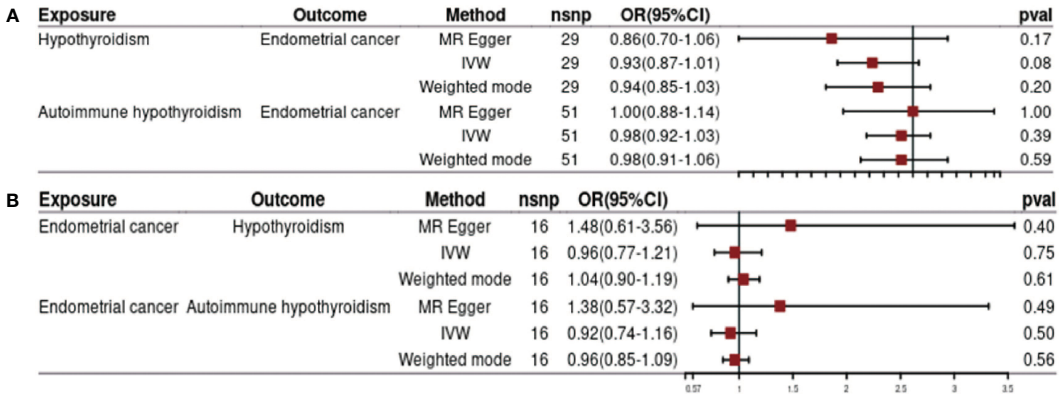

FIGURE 2
Impact of hypothyroidism on endometrial cancer **(A)** and effects of endometrial cancer on hypothyroidism **(B)**.

suggesting no significant causal effect of hypothyroidism (both general and autoimmune) and different pathological subtypes of endometrial cancer (all p-values >0.05) (Figure 3). In the reverse MR analysis, we did not find evidence of a potential causal effect of different pathological subtypes of EC on hypothyroidism or autoimmune hypothyroidism (all p-values >0.05) (Figure 4). These subgroup analyses enhance the robustness and reliability of our main findings.

3.5 Sensitivity analyses

MR-Egger intercept analysis estimates are presented for sensitivity analyses and indicated the absence of horizontal pleiotropy (all p-values >0.05). These findings affirm the validity and unbiased nature of the instrumental variables employed in our study, unaffected by other genetic or environmental factors. To further validate the aforementioned results, we performed a leave-one-out analysis. The leave-one-out sensitivity analysis demonstrated consistent outcomes across all variables (Supplementary Figures S1-S12).

4 Discussion

Hypothyroidism is a commonly occurring condition characterized by insufficient levels of thyroid hormone (18). Hypothyroidism may contribute to carcinogenesis, as thyroid hormones and TSH possess the capacity to directly stimulate tumorigenesis through various mechanisms encompassing cell surface receptors, estrogen pathways, augmented angiogenesis, and gene expression modification (19). Furthermore, hypothyroidism exhibits associations with diabetes mellitus and cardiovascular disorders, both of which showcase associations with escalated cancer susceptibility (20). Previous studies have investigated the relationship between hypothyroidism and different types of cancer. These studies have found associations between hypothyroidism and decreased risks of breast cancer, thyroid cancer, and hepatocellular carcinoma (6, 21). Unfortunately, they did not report a causal relationship between hypothyroidism and EC risk.

To fill this gap. we conducted first MR analyses to investigate the causal relationship between hypothyroidism and endometrial cancer. Our findings indicate that there is no causal relationship

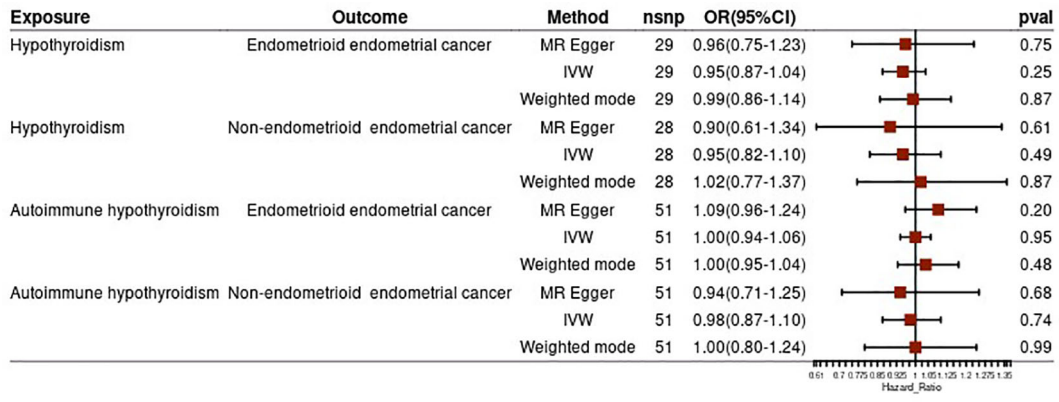

FIGURE 3
Subgroup analysis of effect of hypothyroidism on endometrial cancer risk.

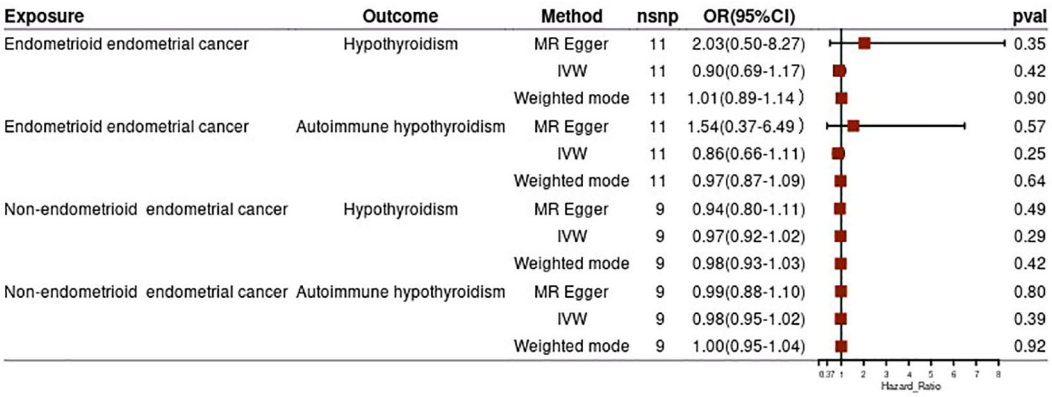

FIGURE 4
Subgroup analysis of effect of endometrial cancer on hypothyroidism.

between hypothyroidism or autoimmune hypothyroidism and endometrial cancer. The endometrioid subtype, which accounts for approximately 80% of EC cases, exhibits estrogen responsiveness and typically presents with a favorable prognosis (22, 23). Conversely, the less frequent non-endometrioid subtypes, such as serous and clear cells, are not as responsive to estrogen and are often associated with an unfavorable prognosis (22, 23). To further investigate the potential causal relationships between hypothyroidism and EC susceptibility, we performed subgroup analyses based on above two pathological subtypes. In accordance with the above findings, we also did not find any causal effect of hypothyroidism on the risk of endometrioid or non-endometrioid EC. Furthermore, we also observed any causal effect of endometrioid or non-endometrioid EC on hypothyroidism, which confirmed the robustness of the above findings. In a previous large cohort study involving 1314 EC patients, the relationship between hypothyroidism and the risk of EC was investigated. The results showed that there was no significant association between a history of hypothyroidism and the risk of EC, which in agreement with our finding (24). These findings suggest that factors other than hypothyroidism may play a more prominent role in the pathogenesis of endometrial cancer.

The lack of a causal relationship between hypothyroidism and endometrial cancer observed in our MR analysis contradicts the findings of previous observational studies (7, 9). However, there are several possible reasons for the disparity between our findings and those of previous studies. Firstly, residual confounding may have influenced the results of previous observational studies. These studies rely on observational data, which are susceptible to confounding factors that may distort the association between hypothyroidism and endometrial cancer. By contrast, Mendelian randomization analysis leverages genetic variants as instrumental variables to minimize confounding biases and provide more reliable estimates of causal effects. Secondly, reverse causation is another potential explanation for the discrepancy. Observational studies are vulnerable to reverse causation, as the temporal ordering of events is often challenging to establish definitively. Lastly, measurement errors in the exposure and outcome variables could have contributed to the inconsistent findings. In observational studies, misclassification or inaccuracies in diagnosing hypothyroidism or endometrial cancer can introduce bias into the results, leading to inconsistent associations.

One strength of our study lies in the utilization of the MR method, which offers a valuable framework for evaluating causal relationships and addresses the limitations associated with observational studies. Through the implementation of genetic variants as IVs, we successfully estimated the causal link between EC risk and hypothyroidism while mitigating confounding and reverse causality. To ensure the credibility of our IVs, we exclusively selected SNPs with robust associations and high instrument strength, as indicated by F-statistics exceeding 10. This rigorous criterion bolstered comparability between the exposure and outcome samples, thereby enhancing the reliability of our conclusions. Furthermore, to minimize the impact of sample overlap, we obtained exposure and outcome datasets from different databases, reducing potential interference (25). Additionally, we conducted a comprehensive sensitivity analysis and subgroup analysis. This meticulous examination allowed us to evaluate the robustness and reliability of our findings from various perspectives.

While our study provides robust evidence to refute a direct causal association between hypothyroidism and endometrial cancer, it is essential to acknowledge some limitations. Firstly, our study assumes that the genetic variants used as instrumental variables are independent of potential confounding factors. Although this assumption is reasonable, unmeasured confounders could still impact the estimated results. Additionally, our study focused on European populations, necessitating further research in other ethnic groups to validate our findings. Furthermore, our study did not explore the potential mechanistic pathways that may mediate the observed associations. Future investigations should consider examining how hypothyroidism may indirectly influence

endometrial cancer risk through mechanisms such as altered estrogen metabolism or insulin resistance.

While a causal relationship has not been observed between hypothyroidism and endometrial cancer, both may play a role in the progression of each other's diseases. Dysregulation of immune modulation in the body may potentially explain the intricate relationship between the two. In recent years, Human Leukocyte Antigen-G (HLA-G) has become a research hotspot in studying the relationship between cancer risk and certain autoimmune diseases (26–28). The role of HLA-G has been extensively studied in various inflammatory conditions, especially in autoimmune diseases such as hypothyroidism (28). It is believed that the weakening of the immune suppressive state is one of the factors leading to the development of autoimmune diseases, and HLA-G may serve as a mechanism to counteract the damage in these diseases (28). The expression of HLA-G has been observed to be associated with tumor staging, prognosis, and circulating levels in various types of cancer (29). In endometrial cancer, researchers have also found upregulation of HLA-G expression, and the increased soluble HLA-G levels are associated with advanced pathological staging, metastasis, and poor prognosis in endometrial cancer patients (30, 31). However, there is currently no research definitively stating whether HLA-G is involved in the pathogenesis and progression mechanisms of hypothyroidism and endometrial cancer. Future studies need to further explore this area.

Given the clinical implications of understanding the relationship between hypothyroidism and endometrial cancer, our study contributes valuable insights for individual health management and prevention strategies. While hypothyroidism does not appear to increase the risk of endometrial cancer directly, it is crucial to account for other critical factors when assessing overall risk. Considering the multifactorial nature of endometrial cancer, comprehensive approaches that consider various risk factors such as obesity, diabetes mellitus, postmenopausal estrogen replacement, ovarian dysfunction, infertility, nulliparity, and tamoxifen use are necessary for tailored prevention and early detection strategies (32). Given these limitations, large-scale randomized controlled trials are warranted to validate our findings and provide a deeper understanding of the relationship between hypothyroidism and endometrial cancer. Such trials would help elucidate the causal nature of the association and potentially inform clinical management and interventions for patients with hypothyroidism and endometrial cancer.

5 Conclusion

In conclusion, our bidirectional MR study suggests a lack of causal association between hypothyroidism and endometrial cancer. These findings highlight the importance of conducting rigorous randomized controlled trials to elucidate the true nature of this association. Further research is needed to explore other potential risk factors and pathways involved in the development of endometrial cancer, contributing to improved prevention and treatment strategies for this prevalent gynecological malignancy.

Data availability statement

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

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

BW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. YL: Data curation, Investigation, Methodology, Software, Writing – original draft. TL: Data curation, Formal analysis, Methodology, Software, Writing – original draft. SX: Formal analysis, Methodology, Writing – original draft. JP: Data curation, Formal analysis, Methodology, Writing – original draft. JL: Conceptualization, Formal analysis, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JY: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1308208/full#supplementary-material

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. (2023) 73:17–48. doi: 10.3322/caac.21763

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. (2020) 70:7–30. doi: 10.3322/caac.21590

3. Xia C, Dong X, Li H, Cao M, Sun D, He S, et al. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. *Chin Med J (Engl)*. (2022) 135:584–90. doi: 10.1097/CM9.0000000000002108

4. Renaud MC, Le T, Sogc-Goc-Scc P, Practice Guidelines C, Special C. Epidemiology and investigations for suspected endometrial cancer. *J Obstet Gynaecol Can*. (2013) 35:380–1. doi: 10.1016/S1701–2163(15)30970–1

5. Oaknin A, Bosse TJ, Creutzberg CL, Giornelli G, Harter P, Joly F, et al. Endometrial cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. (2022) 33:860–77. doi: 10.1016/j.annonc.2022.05.009

6. Lu L, Wan B, Li L, Sun M. Hypothyroidism has a protective causal association with hepatocellular carcinoma: A two-sample Mendelian randomization study. *Front Endocrinol (Lausanne)*. (2022) 13:987401. doi: 10.3389/fendo.2022.987401

7. Wang Y, Zhou R, Wang J. Relationship between hypothyroidism and endometrial cancer. *Aging Dis*. (2019) 10:190–6. doi: 10.14336/AD.2018.0224

8. Vilos GA, Oraif A, Vilos AG, Ettler H, Edris F, Abu-Rafea B. Long-term clinical outcomes after resectoscopic endometrial ablation of nonatypical endometrial hyperplasia in women with abnormal uterine bleeding. *J Minim Invasive Gynecol*. (2015) 22:704. doi: 10.1016/j.jmig.2015.02.017

9. Barr CE, Barr CE, Njoku K, Njoku K, Hotchkies L, Hotchkies L, et al. Does clinical and biochemical thyroid dysfunction impact on endometrial cancer survival outcomes? A prospective database study. *Cancers*. (2021) 13:5444–. doi: 10.3390/cancers13215444

10. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Smith GD. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. *Stat Med*. (2008) 27:1133–63. doi: 10.1002/sim.3034

11. George DS, Shah E. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. (2003) 1:1. doi: 10.1093/ije/dyg070

12. Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing Mendelian randomization investigations: update for summer 2023. *Wellcome Open Res*. (2019) 4:186. doi: 10.12688/wellcomeopenres.15555.3

13. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res*. (2017) 26:2333–55. doi: 10.1177/0962280215597579

14. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. (2016) 40:304–14. doi: 10.1002/gepi.21965

15. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. (2015) 44:512–25. doi: 10.1093/ije/dyv080

16. Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat Med*. (2015) 34:2926–40. doi: 10.1002/sim.6522

17. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife*. (2018) 7:e34408. doi: 10.7554/eLife.34408

18. Wilson SA, Stem LA, Bruehlman RD. Hypothyroidism: diagnosis and treatment. *Am Fam Physician*. (2021) 103:605–13.

19. Moeller LC, Fuhrer D. Thyroid hormone, thyroid hormone receptors, and cancer: a clinical perspective. *Endocr Relat Cancer*. (2013) 20:R19–29. doi: 10.1530/ERC-12–0219

20. Thvilum M, Brandt F, Almind D, Christensen K, Brix TH, Hegedüs L. Type and Extent of Somatic Morbidity before and after the Diagnosis of Hypothyroidism. A Nationwide Register Study. *PLoS One*. (2013) 8:e75789. doi: 10.1530/endoabs.32.P986

21. Yuan S, Kar S, Vithayathil M, Carter P, Mason AM, Burgess S, et al. Causal associations of thyroid function and dysfunction with overall, breast and thyroid cancer: A two-sample Mendelian randomization study. *Int J Cancer*. (2020) 147:1895–903. doi: 10.1002/ijc.32988

22. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. *Lancet*. (2005) 366:491–505. doi: 10.1016/S0140–6736(05)67063–8

23. Wang X, Glubb DM, O'Mara TA. 10 Years of GWAS discovery in endometrial cancer: Aetiology, function and translation. *EBioMedicine*. (2022) 77:103895. doi: 10.1016/j.ebiom.2022.103895

24. Kang JH, Kueck AS, Stevens R, Curhan G, De Vivo I, Rosner B, et al. A large cohort study of hypothyroidism and hyperthyroidism in relation to gynecologic cancers. *Obstet Gynecol Int*. (2013) 2013:743721. doi: 10.1155/2013/743721

25. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. *Genet Epidemiol*. (2016) 40:597–608. doi: 10.1002/gepi.21998

26. Negrini S, Contini P, Murdaca G, Puppo F. HLA-G in allergy: does it play an immunoregulatory role? *Front Immunol*. (2021) 12:789684. doi: 10.3389/fimmu.2021.789684

27. Murdaca G, Calamaro P, Lantieri F, Pigozzi S, Mastracci L, Grillo F, et al. HLA-G expression in gastric carcinoma: clinicopathological correlations and prognostic impact. *Virchows Arch*. (2018) 473:425–33. doi: 10.1007/s00428–018–2379–0

28. Gonzalez A, Rebmann V, LeMaoult J, Horn PA, Carosella ED, Alegre E. The immunosuppressive molecule HLA-G and its clinical implications. *Crit Rev Clin Lab Sci*. (2012) 49:63–84. doi: 10.3109/10408363.2012.677947

29. Guo X, Zhang J, Shang J, Cheng Y, Tian S, Yao Y. Human leukocyte antigen-G in gynaecological tumours. *Int J Immunogenet*. (2023) 50:163–76. doi: 10.1111/iji.12626

30. Bai Y, Liu W, Xie Y, Liang J, Wang F, Li C. Human leukocyte antigen-G (HLA-G) expression plays an important role in the diagnosis and grading of endometrial cancer. *J Obstet Gynaecol*. (2022) 42:641–7. doi: 10.1080/01443615.2021.1920007

31. Ben Yahia H, Babay W, Bortolotti D, Boujelbene N, Laaribi AB, Zidi N, et al. Increased plasmatic soluble HLA-G levels in endometrial cancer. *Mol Immunol*. (2018) 99:82–6. doi: 10.1016/j.molimm.2018.04.007

32. Ignatov A, Ortmann O. Endocrine risk factors of endometrial cancer: Polycystic ovary syndrome, oral contraceptives, infertility, tamoxifen. *Cancers (Basel)*. (2020) 12:1766. doi: 10.3390/cancers12071766

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Apolipoprotein A-I levels in the survival of patients with colorectal cancer: a retrospective study

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Background: Abnormal lipid levels have been associated with cancer incidence and progression. However, limited studies have investigated the relationship between apolipoprotein A-I (ApoA-I) and colorectal cancer (CRC). This study assessed the significance of ApoA-I levels in progression-free survival (PFS) and overall survival (OS) of patients with CRC.

Methods: Survival curves were compared using Kaplan–Meier analysis, while the predictive values of various lipid indicators in CRC prognosis were evaluated based on receiver operating characteristic curves. The factors influencing PFS and OS in patients with CRC were analyzed using Cox proportional hazards regression models. Finally, the relationship between ApoA-I level and disease recurrence was investigated through logistic regression analysis. The optimal Apo-I level was determined through maximally selected rank statistics.

Results: Using the optimal ApoA-I cutoff value (0.9 g/L), the 1,270 patients with CRC were categorized into low (< 0.9 g/L, 275 cases) and high (≥0.9 g/L, 995 cases) ApoA-I groups. Compared with other lipid indicators, ApoA-I demonstrated superior predictive accuracy. The high ApoA-I group exhibited significantly higher survival rates than the low ApoA-I group (PFS, 64.8% vs. 45.2%, $P < 0.001$; OS, 66.1% vs. 48.6%, $P < 0.001$). Each one-standard-deviation increase in ApoA-I level was related to a 12.0% decrease in PFS risk (hazard ratio [HR] 0.880; 95% confidence interval [CI], 0.801–0.968; $P = 0.009$) and an 11.2% decrease in OS risk (HR 0.888; 95%CI, 0.806–0.978; $P = 0.015$). Logistic regression analysis revealed that patients with low ApoA-I had a 32.5% increased risk of disease recurrence (odds ratio [OR] 0.675; 95%CI, 0.481–0.946; $P = 0.0225$) compared with those with high ApoA-I. PFS/OS nomograms based on ApoA-I demonstrated excellent prognostic prediction accuracy.

Conclusions: Serum ApoA-I level may be a valuable and non-invasive tool for predicting PFS and OS in patients with CRC.

KEYWORDS

colorectal cancer, prognostic, recurrence, ApoA-I, survival

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Background

Colorectal cancer (CRC) is among the most prevalent malignancies and poses a significant threat to human health. In Western countries, CRC ranks as the second leading cause of cancer-related fatalities (1). The situation in China is no less daunting, where CRC is the third leading cause of cancer-related death (2). Generally, the risk of CRC increases rapidly with age. In recent years, patients with CRC aged <65 years in the United States have accounted for 45% of cases. Patients with early-stage CRC who undergo surgical treatment can achieve a survival rate of >90%. However, 60% of CRC cases are diagnosed at an advanced stage, with a higher proportion of patients experiencing distant metastasis, leading to a survival rate of <20% for metastatic CRC (3). Therefore, widespread concern exists regarding the need to identify factors that can guide clinical treatment and predict the outcome of patients with CRC.

Numerous prognostic factors utilizing peripheral biochemical biomarkers have been validated (4–6). Dysregulated lipid levels have been consistently linked to the occurrence, risk, and progression of CRC. Dysregulation of lipid metabolism has a significant impact on the tumor microenvironment, suggesting a potential interaction between lipid metabolism and immune response (7). Increasing evidence suggests that lipid metabolism is a key determinant affecting immune therapy and clinical responses in cancer patients (8). Dysregulated lipid metabolism can enhance the occurrence, establishment, and metastatic potential of tumor cells (9). Controlling systemic lipid metabolism may contribute to improving responses to immunotherapy, with lipid metabolism interventions serving as regulators of anticancer immune responses and catalysts for anticancer immunotherapy, offering significant therapeutic potential (10). Therefore, lipid metabolism, especially that of apolipoprotein A-I (ApoA-I), and its relationship with cancer have recently attracted significant attention. ApoA-I is primarily synthesized in the liver and is a significant constituent of high-density lipoproteins (HDL). ApoA-I possesses multiple biological functions, including cholesterol transport, antioxidant properties, anti-inflammatory effects, and anticoagulant activities (11). Pulcrano et al. indicated that ApoA-I is a beneficial lipoprotein, particularly as a protective factor against cardiovascular diseases (12). ApoA-I is closely associated with the onset, progression, and prognosis of various cancer types, including renal cell carcinoma (13), esophageal squamous cell carcinoma (14), nasopharyngeal carcinoma (15), ovarian cancer (16), non-small cell lung cancer (17), and bladder cancer (18). Nevertheless, research investigating the relationship between ApoA-I and the prognosis of patients with CRC is scarce (19). The role of ApoA-I in cancer remains controversial, with some studies suggesting anti-cancer effects (20, 21) and other studies reporting a positive correlation between ApoA-I level and breast cancer risk (22).

In this context, the present study utilized clinical cohort analysis to explore the relationship between ApoA-I levels and the progression-free survival (PFS) and overall survival (OS) of patients with CRC who underwent surgical treatment. Additionally, a predictive nomogram based on ApoA-I was developed to offer novel insights into the prognostic evaluation of patients with CRC.

Methods

Participants

This study included 1,270 patients with CRC who received treatment at the First Affiliated Hospital of Guangxi Medical University between 2015 and 2017. The eligibility criteria for inclusion were: 1. no preoperative treatments including radiotherapy, chemotherapy, or immunotherapy; 2. postoperative pathological confirmation of CRC; 3. availability of complete clinical and pathological data; and 4. age ≥18 years. The exclusion criteria included: 1. history of dyslipidemia and 2. the presence of acute and chronic diseases such as hereditary hyperlipidemia, other malignant tumors, liver or kidney dysfunction, and cardiovascular diseases, which could potentially interfere with the study results.

Data collection

Various clinical data, including age, sex, body mass index (BMI), medical history, serum lipid parameters, postoperative pathological examination reports (tumor location, size, TNM stage, neural/vascular invasion, and differentiation degree), postoperative radiotherapy, and chemotherapy, were collected. The assessed lipid indicators included ApoA-I, ApoB, ApoA-I/ApoB, total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and lipoprotein (a) [Lp(a)]. The clinical and pathological data collected were obtained from the electronic medical records system of the research center.

Follow-up

All patients were followed up through telephone consultations and regular outpatient visits until January 2023, resulting in a total follow-up period ranging from 1 month to 106 months (median 64.9 months). OS and PFS were assessed in the prognostic follow-up cohort. OS was defined as the duration from the date of surgery until either death attributed to CRC or the last follow-up, whereas PFS was defined as the time elapsed from the date of surgery to the occurrence of local or distant disease recurrence.

Statistical analysis

Continuous data were reported as means ± standard deviation (SD) or medians (interquartile range [IQR]). T-tests were performed for comparisons between groups. Categorical data were presented as counts (%) and compared using the χ^2 test. The optimal threshold of ApoA-I was determined using maximally

selected rank statistics. Kaplan–Meier (K–M) analysis with log-rank test was employed to compare survival curves between the low and high ApoA-I groups according to the threshold value. The area under the receiver operating characteristic (ROC) curve (AUC) was used to analyze the predictive value of various lipid indicators for CRC prognosis. To investigate the continuous association between ApoA-I and survival, restricted cubic splines (RCS) were employed. The Cox proportional hazards regression models were applied to investigate the variables that influenced both PFS and OS in patients with CRC. Logistic regression analysis was performed to evaluate the correlation between ApoA-I levels and CRC recurrence. Finally, nomograms were constructed using statistically significant indicators, and discrimination and calibration were evaluated using the C-index, ROC curve, and calibration curve. The clinical utility of the nomogram was evaluated using decision curve analysis (DCA). $P < 0.05$ was considered statistically significant.

Results

Comparisons of general clinical data between patients with CRC according to ApoA-I level

Among the 1,270 patients with CRC, the mean age was 59.22 ± 12.65 years, and 63.3% were male. A total of 623 patients (49.1%) had colon cancer, and 647 patients (50.9%) had rectal cancer. Recurrence occurred in 349 patients (27.5%) and 517 patients (40.7%) died. Clinicopathological staging revealed 318 cases (25.0%) of stage I–II and 952 cases (75.0%) of stage III–IV CRC.

The 1,270 patients with CRC were divided into two groups based on the optimal ApoA-I cutoff: low (ApoA-I <0.9 g/L, 275 cases) and high (ApoA-I ≥0.9 g/L, 995 cases) (Figure S1). ApoA-I level was significantly associated with several factors, including sex, hypertension, diabetes, T stage, M stage, tumor location, tumor size, carcinoembryonic antigen (CEA) level, length of hospital stay, and hospitalization costs. Additionally, patients with a low ApoA-I level had a significantly higher overall mortality rate than those with a high ApoA-I level, with a difference of 16.7% (53.8% vs. 37.1%; $P <$ 0.001) (Table S1). Furthermore, the investigation of the distribution of median ApoA-I levels among various clinicopathological characteristics revealed notably lower ApoA-I levels in male patients with stage III–IV CRC, patients who experienced recurrence, and patients who died (Figure S2).

Comparison of the prognostic values of serum lipids

To compare the predictive value of lipid indicators for the outcome of patients with CRC, ROC curves were generated for the 3- and 5-year outcomes, and the AUCs were calculated. Among all lipid indicators, ApoA-I had the highest AUC for both 3- and 5-year PFS (Figures S3A, B). Similarly, compared with HDL, ApoA-I/ApoB, TG, TC, ApoB, LDL, and Lp(a), ApoA-I exhibited the highest predictive accuracy for 3- and 5-year OS (Figures S3C, D).

Kaplan–Meier survival analysis

The high ApoA-I group had significantly higher survival rates compared to the low ApoA-I group at the same time points, indicating a significant association between ApoA-I levels and PFS and OS in patients with CRC (PFS: 64.8% vs. 45.2%, $P < 0.001$; OS: 66.1% vs. 48.6%, $P < 0.001$) (Figure 1). Considering the prognostic implications of TNM stage, tumor location, and CEA level, a subgroup analysis was performed using the K–M method among patients diagnosed with CRC. In the TNM stage subgroup analyses, the low ApoA-I group displayed worse PFS and OS than the high ApoA-I group in both stage I–II and III–IV CRC (Figure 2). In the tumor location subgroup analyses, the low ApoA-I group also exhibited worse PFS and OS than the high ApoA-I group (Figures S4, S5). In the CEA subgroup analyses, ApoA-I effectively differentiated between PFS and OS in patients with CRC, with better discrimination in the high CEA group than in the normal CEA group (Figure S6).

Prognostic value of ApoA-I

Figure 3 illustrates the utilization of RCS to visually depict the flexible relationship between ApoA-I levels and both PFS and OS across various adjusted models. The results indicated a gradual decrease in the hazard ratio [HR] for survival with increasing ApoA-I levels. This trend remained consistent across different models. Tables 1 and 2 show the results of more detailed analyses of the relationships between ApoA-I levels and PFS and OS. As a continuous variable, every SD increase in ApoA-I was associated with a 12.0% decrease in PFS risk (HR 0.880; 95% confidence interval [CI], 0.801–0.968; $P = 0.009$) and an 11.2% decrease in OS risk (HR 0.888; 95%CI, 0.806–0.978; $P = 0.015$) in patients with CRC. High ApoA-I levels were associated with a 27.9% lower risk of adverse PFS (HR 0.721; 95%CI, 0.59–0.881; $P = 0.001$) and a 26.4% lower risk of adverse OS (HR 0.736; 95%CI, 0.599–0.905; $P = 0.004$) compared with low ApoA-I levels. Furthermore, quartile analysis indicated that compared with the reference level Q1 (~0.92), Q2 (0.92–1.06), Q3 (1.06–1.25), and Q4 (1.25+) were associated with decreased risks of adverse PFS and OS. In the multivariate forest plots of PFS and OS, ApoA-I was an independent protective factor in most subgroups (Figure S7). Additionally, examination of the relationship between ApoA-I and postoperative recurrence showed a higher risk of recurrence in the low ApoA-I group than in the high ApoA-I group (25.7% vs. 33.8%, $P =$ 0.010). Multivariate logistic regression analysis revealed that for every SD increase in ApoA-I, the risk of disease recurrence decreased by 54% (odds ratio [OR], 0.460; 95%CI, 0.250–0.830; $P = 0.010$). Compared with the high ApoA-I group (≥0.9 g/L), the low ApoA-I group (<0.9 g/L) had a 32.5% higher risk of disease recurrence (OR, 0.675; 95%CI, 0.481–0.946; $P = 0.0225$) (Table 3).

ApoA-I-based prediction nomograms

Preoperative ApoA-I, age, T stage, N stage, M stage, and CEA independently predicted both PFS and OS (Tables S2, S3). Consequently, nomograms utilizing these indicators were created

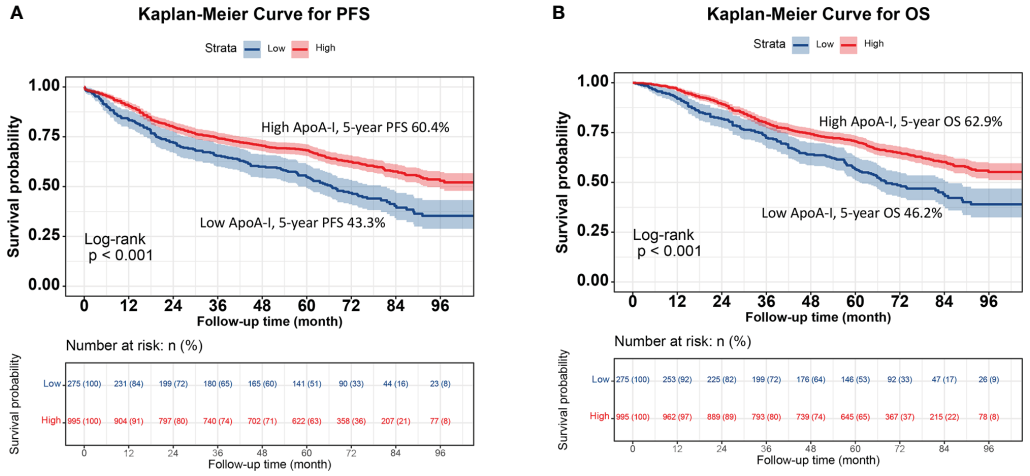

FIGURE 1
Kaplan-Meier curve of ApoA-I for PFS and OS in patients with colorectal cancer. **(A)**, Kaplan-Meier curve for PFS (High ApoA-I vs Low ApoA-I; 60.4% vs 43.3%); **(B)**, Kaplan-Meier curve for OS (High ApoA-I vs Low ApoA-I; 62.9% vs 46.2%).

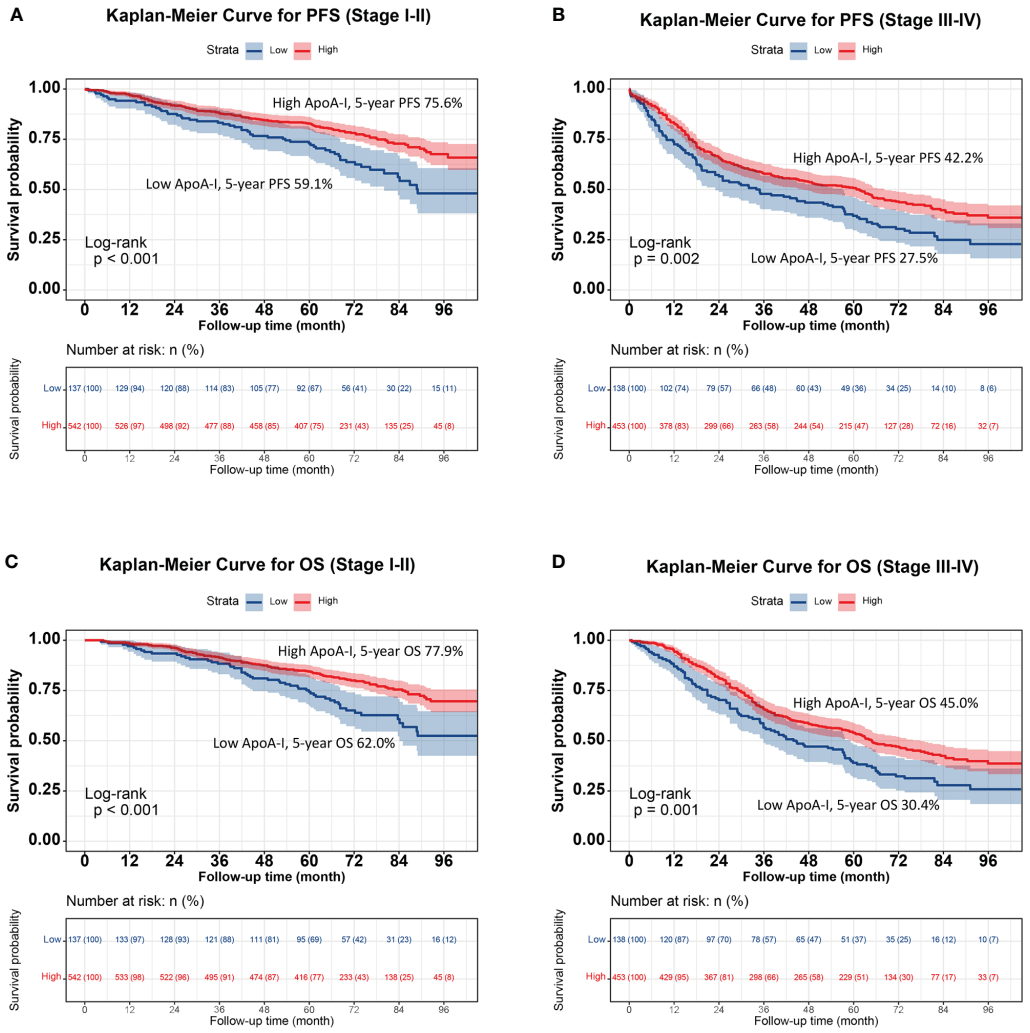

FIGURE 2
Stratified survival analysis of ApoA-I based on TNM stage subgroup. **(A)**, Kaplan-Meier curve for PFS (Stage I-II); **(B)**, Kaplan-Meier curve for OS (Stage I-II); **(C)**, Kaplan-Meier curve for OS (Stage III-IV); **(D)**, Kaplan-Meier curve for OS (Stage III-IV).

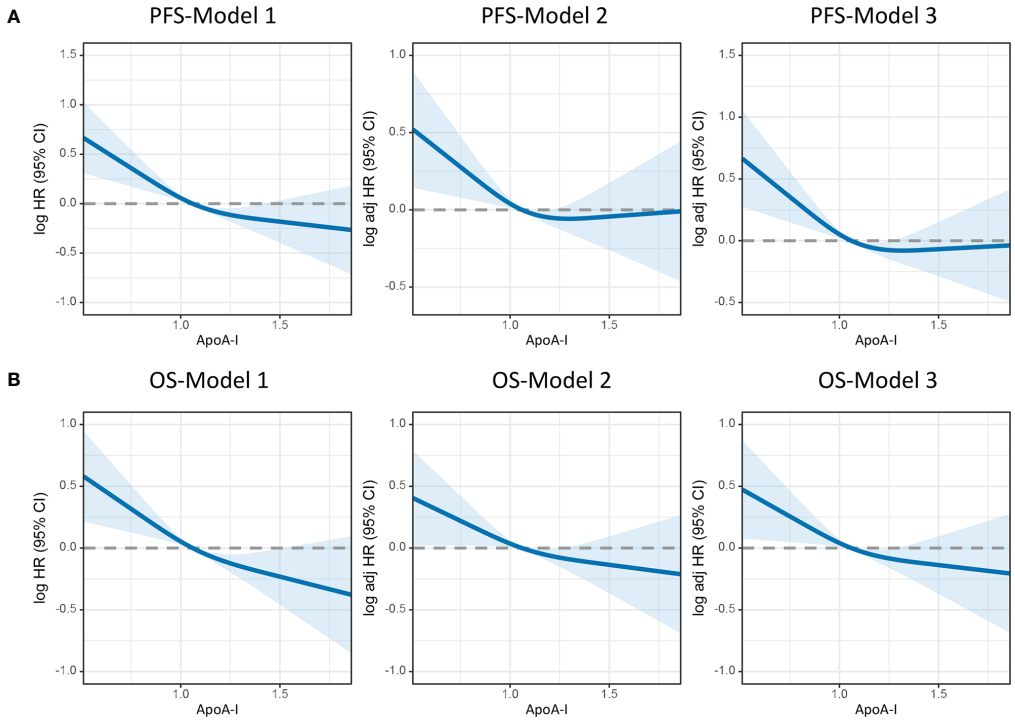

FIGURE 3
Restricted cubic spline model analysis was conducted to assess the correlation between ApoA-I levels and PFS/OS in patients with colorectal cancer. **(A)**, PFS; **(B)**, OS. Model 1: Not adjusted. Model 2: Adjusted for sex, age, and BMI. Model 3: Adjusted for sex, age, BMI, hypertension, diabetes, T stage, N stage, M stage, tumor size, perineural invasion, vascular invasion, differentiation, radiotherapy, chemotherapy.

to forecast 1–5-year PFS and OS for patients diagnosed with CRC (Figures 4A, B). The C-index of the nomograms indicated their good predictive performance for OS (0.726) and PFS (0.719) in patients with CRC. Further ROC analysis demonstrated the high predictive performance of these nomograms for PFS and OS, with 1-, 3-, and 5-year AUC values of (0.799 vs. 0.776 vs. 0.764) and (0.767 vs. 0.773 vs. 0.766), respectively (Figure S8). The calibration curves exhibited satisfactory concordance between the predicted values and actual clinical outcomes (Figure S9). Moreover, DCA to compare the clinical benefits of ApoA-I-based nomograms with those of traditional tumor staging showed superior clinical benefits of the ApoA-I-based nomograms for both PFS and OS, (Figure S10A, B). Subsequently, based on the median scores of the nomograms, the patients were divided into high-score and low-score groups. The high-scoring group showed significantly worse PFS and OS compared to the low-scoring group (Figure S11A, B).

Discussion

Mounting evidence suggests the critical role of systemic inflammation in both cancer onset and progression (23, 24).

TABLE 1 Association between ApoA-I and PFS of patients with colorectal cancer.

ApoA-I	Model 1	p value	Model 2	p value	Model 3	p value
Continuous (per SD)	0.837 (0.768,0.913)	<0.001	0.905 (0.826,0.992)	0.032	0.88 (0.801,0.968)	0.009
Cutoff value (High)	0.629 (0.522,0.757)	<0.001	0.75 (0.616,0.912)	0.004	0.721 (0.59,0.881)	0.001
Quartiles						
Q1 (~0.92)	ref		ref		ref	
Q2 (0.92~1.06)	0.612 (0.484,0.773)	<0.001	0.669 (0.527,0.848)	0.001	0.637 (0.5,0.812)	<0.001
Q3 (1.06~1.25)	0.757 (0.607,0.944)	0.013	0.84 (0.669,1.055)	0.133	0.783 (0.621,0.988)	0.04
Q4 (1.25~)	0.627 (0.495,0.795)	<0.001	0.785 (0.609,1.01)	0.06	0.736 (0.567,0.955)	0.021
p for trend		0.001		0.168		0.071

Model 1: Not adjusted.
Model 2: Adjusted for sex, age, and BMI.
Model 3: Adjusted for sex, age, BMI, hypertension, diabetes, T stage, N stage, M stage, tumor size, perineural invasion, vascular invasion, differentiation, radiotherapy, chemotherapy.

TABLE 2 Association between ApoA-I and OS of patients with colorectal cancer.

ApoA-I	Model 1	p value	Model 2	p value	Model 3	p value
Continuous (per SD)	0.836 (0.765,0.913)	<0.001	0.896 (0.818,0.983)	0.02	0.888 (0.806,0.978)	0.015
Cutoff value (High)	0.622 (0.514,0.752)	<0.001	0.74 (0.606,0.904)	0.003	0.736 (0.599,0.905)	0.004
Quartiles						
Q1 (~0.92)	ref		ref		ref	
Q2 (0.92~1.06)	0.616 (0.485,0.783)	<0.001	0.69 (0.541,0.88)	0.003	0.677 (0.529,0.868)	0.002
Q3 (1.06~1.25)	0.742 (0.591,0.932)	0.01	0.813 (0.643,1.027)	0.083	0.782 (0.615,0.993)	0.044
Q4 (1.25~)	0.611 (0.478,0.78)	<0.001	0.743 (0.574,0.962)	0.024	0.732 (0.559,0.958)	0.023
p for trend		0.001		0.059		0.051

Model 1: Not adjusted.
Model 2: Adjusted for sex, age, and BMI.
Model 3: Adjusted for sex, age, BMI, hypertension, diabetes, T stage, N stage, M stage, tumor size, perineural invasion, vascular invasion, differentiation, radiotherapy, chemotherapy.

Serum markers of systemic inflammation, such as serum C-reactive protein and procalcitonin, have been identified as adverse prognostic factors in various cancers (25, 26). Interestingly, serum ApoA-I levels have shown a significant association with systemic inflammatory markers. In their study involving 144 patients with CRC, Sirnio et al. (26) observed a robust negative relationship between serum ApoA-I levels and systemic inflammatory markers, including serum C-reactive protein level, interleukin-8 level, and blood neutrophil count. Furthermore, the authors reported a notable association between low serum ApoA-I levels and advanced T and TNM stages. Consequently, serum ApoA-I levels show promise as an indicator of both systemic inflammation and tumor progression. Similarly, the results of the present study revealed a significant association between ApoA-I levels and tumor progression. Low ApoA-I levels were significantly correlated with advanced pathological staging, larger tumor diameters, and higher CEA levels. Additionally, compared with patients with high ApoA-I levels, patients with low ApoA-I levels exhibited higher rates of recurrence, mortality, and hospitalization burden. Thus, decreased ApoA-I levels may serve as an indicator of aggressive tumor behavior and poor prognosis. The correlation between serum ApoA-I levels and tumor characteristics, as revealed in this study, can provide tailored guidance for treatment decisions and prognosis assessment.

ApoA-I itself possesses antitumor properties by reducing angiogenesis, altering immune cells, enhancing cholesterol efflux, and reversing sterol transport in cancer cells, which may inhibit tumor cell proliferation or growth (21). Although ApoA-I is linked to the development of various tumors and is a potential biochemical marker for diagnosing various cancers (27, 28), limited research has explored the correlation between ApoA-I level and the outcomes of patients with CRC. A study by Quan et al. (19) involving 508 participants, suggested that ApoA-I could serve as a prognostic factor in patients with metastatic CRC treated with bevacizumab. Zhang et al.'s meta-analysis found that serum ApoA-I could serve as a non-invasive marker for predicting the prognosis of various tumors, including CRC (29). Sirniö et al. also identified serum ApoA-I as a promising additional prognostic parameter for CRC (26). However, these studies were limited by small sample sizes or focused mainly on late-stage patients. Gu et al. evaluated four key blood lipid factors and established a novel lipoprotein cholesterol-apolipoprotein score for predicting the prognosis of patients undergoing CRC resection (30). In our study, we compared the prognostic values of eight common blood lipid factors and found that ApoA-I was the optimal blood lipid factor for predicting

TABLE 3 Association between ApoA-I and recurrence of patients with colorectal cancer.

ApoA-I	Model 1	p value	Model 2	p value	Model 3	p value
Continuous (per SD)	0.487 (0.297,0.798)	0.0043	0.619 (0.354,1.082)	0.0921	0.46 (0.25,0.83)	0.01
Cutoff value (High)	0.678 (0.509,0.904)	0.008	0.678 (0.509,0.904)	0.008	0.675 (0.481,0.946)	0.0225
Quartiles						
Q1 (~0.92)	ref		ref		ref	
Q2 (0.92~1.06)	0.714 (0.505,1.007)	0.055	0.725(0.494,1.065)	0.101	0.668(0.451,0.989)	0.044
Q3 (1.06~1.25)	0.813 (0.579,1.141)	0.231	0.865(0.592,1.264)	0.455	0.757(0.511,1.123)	0.166
Q4 (1.25~)	0.67 (0.473,0.948)	0.024	0.825(0.553,1.229)	0.344	0.685(0.452,1.037)	0.074
p for trend		0.541		0.658		0.365

Model 1: Not adjusted.
Model 2: Adjusted for sex, age, and BMI.
Model 3: Adjusted for sex, age, BMI, hypertension, diabetes, T stage, N stage, M stage, tumor size, perineural invasion, vascular invasion, differentiation, radiotherapy, chemotherapy.

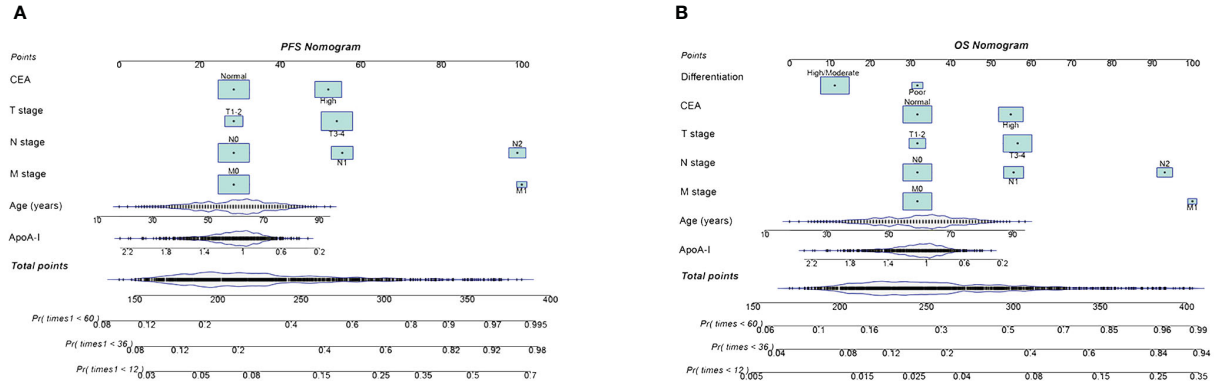

FIGURE 4
Construction the PFS and OS nomograms in CRC patients. **(A)**, PFS nomogram; **(B)**, OS nomogram.These nomograms comprise specific clinical features, with each feature corresponding to a specific point. A straight line can be drawn through these points on the axis to calculate the score for each feature. After summing the scores of these features, positioned on the total point axis, the probability of risk can be calculated by drawing downward to the prediction axis. Based on the results of Cox proportional hazards regression models, we developed these nomograms to predict the PFS/OS in CRC patients.

PFS and OS in CRC patients. This provides valuable insight for the clinical use of blood lipid factors in assessing the prognosis of CRC patients. Furthermore, unlike previous studies, we extensively explored the relationship between ApoA-I and PFS, OS, and recurrence in CRC patients. The results of the present study revealed higher PFS and OS rates in the low ApoA-I group compared to those in the high ApoA-I group, with improvements of 17.1% and 16.7%, respectively. Multivariate analysis indicated that ApoA-I was an independent predictor of PFS and OS in patients with CRC, with HR values of 0.778 and 0.776, respectively, irrespective of the TNM stage. Moreover, ApoA-I was an independent predictive factor for disease recurrence in patients with CRC. These findings suggest that ApoA-I may be a valuable prognostic indicator for individuals diagnosed with CRC and offer new insights into previous literature.

Despite the widespread utilization of TNM staging as the primary tool for treatment decisions and prognosis evaluation in patients with CRC, it is important to note that patient outcomes can vary significantly even within the same pathological stage. Therefore, identifying effective prognostic tools that complement TNM staging is crucial. Within the TNM subgroups, ApoA-I level effectively differentiated between patient outcomes in both stage I–II and III–IV CRC. This finding underscores the utility of ApoA-I as a valuable adjunct to TNM staging for prognostic assessments. Additionally, ApoA-I level was an effective prognostic predictor in both colon and rectal cancers. Within the CEA subgroups, ApoA-I effectively distinguished patient outcomes in each subgroup, with stronger prognostic discrimination in the high CEA subgroup than in the normal CEA subgroup. Collectively, these findings suggest that serum ApoA-I level may be a valuable indicator for predicting the outcome of patients with CRC.

While the current prognostic classification for CRC primarily relies on TNM stage, the predictive value of a single parameter is limited. Therefore, comprehensively integrating multiple indicators are essential for accurate prognostic prediction in patients with CRC. In the present study, besides ApoA-I, patient age and T stage, N stage, M stage, and CEA level were identified as independent influencing factors in patients with CRC. Incorporating various effective prognostic parameters is invaluable when evaluating the outcome of patients with CRC. Thus, these variables were integrated to construct ApoA-I-based prediction nomograms. The C-index and calibration curves indicated that the ApoA-I-based nomograms exhibited excellent predictive accuracy. Furthermore, compared with the traditional TNM staging system, the ApoA-I-based nomograms offered superior clinical benefits. These findings suggest that ApoA-I-based nomograms incorporating a range of prognostic parameters are effective tools for predicting the outcome of patients with CRC. These tools can provide personalized assistance for clinical decision-making in the care of patients with CRC.

Study strengths

Through its analysis of a large clinical cohort, the results of this support serum ApoA-I levels as a promising biomarker for predicting the prognosis of patients with CRC. Multiple study outcomes, including PFS, OS, and disease recurrence, were incorporated to explore the prognostic value of serum ApoA-I levels in patients with CRC. Among all serum lipid markers, ApoA-I exhibited the highest predictive accuracy for 3- and 5-year PFS/OS and OS. This finding highlights the significance of ApoA-I as the most representative indicator of lipid metabolism in predicting the outcome of patients with CRC. Furthermore, serum ApoA-I levels can serve as useful prognostic complements to TNM staging and CEA markers. By integrating prognostic variables, predictive nomograms based on ApoA-I were developed and their excellent prognostic prediction efficacy was confirmed. These findings demonstrated the strength of this study.

Limitations

This study has several limitations. Firstly, the retrospective single-center design made it difficult to completely avoid potential

selection bias. Secondly, the study population consisted solely of Chinese individuals. Further exploration is required to determine the applicability of these findings to other populations. Finally, the ApoA-I-based nomograms developed in this study require extensive external validation in diverse populations before they can be applied in clinical practice.

Conclusion

Decreased serum ApoA-I levels are associated with stronger tumor invasiveness, greater disease burden, and poorer prognosis. Serum ApoA-I levels are an independent factor affecting the prognosis of patients and are a useful supplement to TNM staging. The ApoA-I-based nomograms are effective tools for the comprehensive assessment of prognosis in patients with CRC. These findings have the potential to offer personalized guidance for treatment decisions and prognosis assessment.

Data availability statement

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

Ethics statement

The studies involving humans were approved by The First Affiliated Hospital, Guangxi Medical University (registration number: NO.2022-KY-(043)). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HX: Data curation, Investigation, Software, Writing – original draft, Writing – review & editing. LW: Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. QW: Formal analysis, Project administration, Validation, Writing – review & editing. ST: Funding acquisition, Resources, Visualization, Writing – review & editing. JG: Conceptualization, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1318416/full#supplementary-material

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics. *CA Cancer J Clin*. (2022) 72:7–33. doi: 10.3322/caac.21708

2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China. *CA Cancer J Clin*. (2016) 66:115–32. doi: 10.3322/caac.21338

3. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics. *2023 CA Cancer J Clin*. (2023) 73:233–54. doi: 10.3322/caac.21772

4. Xie H, Ruan G, Ge Y, Zhang Q, Zhang H, Lin S, et al. Inflammatory burden as a prognostic biomarker for cancer. *Clin Nutr*. (2022) 41:1236–43. doi: 10.1016/j.clnu.2022.04.019

5. Xie H, Wei L, Liu M, Liang Y, Yuan G, Gao S, et al. Prognostic significance of preoperative prognostic immune and nutritional index in patients with stage I-III colorectal cancer. *BMC Cancer*. (2022) 22:1316. doi: 10.1186/s12885-022-10405-w

6. Cao Y, Peng T, Li H, Yang M, Wu L, Zhou Z, et al. Development and validation of MMR prediction model based on simplified clinicopathological features and serum tumour markers. *EBioMedicine*. (2020) 61:103060. doi: 10.1016/j.ebiom.2020.103060

7. Wei ZQ, Du XX, Yu YS, Shen P, Chen YG, Li LJ. Plasmid-mediated KPC-2 in a Klebsiella pneumoniae isolate from China. *Antimicrob Agents Chemother*. (2007) 51:763–5. doi: 10.1128/AAC.01053-06

8. Bleve A, Durante B, Sica A, Consonni FM. Lipid metabolism and cancer immunotherapy: immunosuppressive myeloid cells at the crossroad. *J Mol Sci*. (2020) 16:5845. doi: 10.3390/ijms21165845

9. Martin-Perez M, Urdiroz-Urricelqui U, Bigas C, Benitah SA. The role of lipids in cancer progression and metastasis. *Cell Metab*. (2022) 34:1675–99. doi: 10.1016/j.cmet.2022.09.023

10. King RJ, Singh PK, Mehla K. The cholesterol pathway: impact on immunity and cancer. *Trends Immunol*. (2022) 43:78–92. doi: 10.1016/j.it.2021.11.007

11. Besler C, Lüscher TF, Landmesser U. Molecular mechanisms of vascular effects of High-density lipoprotein: alterations in cardiovascular disease. *EMBO Mol Med*. (2012) 4:251–68. doi: 10.1002/emmm.201200224

12. Pulcrano G, Iula DV, de Luca C, Roscetto E, Vollaro A, Rossano F, et al. Clonal dissemination of Klebsiella pneumoniae ST512 carrying blaKPC-3 in a hospital in southern Italy. *Apmis*. (2014) 122:42–6. doi: 10.1111/apm.12097

13. Guo S, He X, Chen Q, Yang G, Yao K, Dong P, et al. The effect of preoperative apolipoprotein A-I on the prognosis of surgical renal cell carcinoma: A retrospective large sample study. *Med (Baltimore)*. (2016) 95:e3147. doi: 10.1097/MD.0000000000003147

14. Wang XP, Li XH, Zhang L, Lin JH, Huang H, Kang T, et al. High level of serum apolipoprotein A-I is a favorable prognostic factor for overall survival in esophageal squamous cell carcinoma. *BMC Cancer*. (2016) 16:516. doi: 10.1186/s12885-016-2502-z

15. Chang H, Wei JW, Chen K, Zhang S, Han F, Lu LX, et al. Apolipoprotein A-I is a prognosticator of nasopharyngeal carcinoma in the era of intensity-modulated radiotherapy. *J Cancer*. (2018) 9:702–10. doi: 10.7150/jca.22836

16. Su F, Kozak KR, Imaizumi S, Gao F, Amneus MW, Grijalva V, et al. (apoA-I) and apoA-I mimetic peptides inhibit tumor development in a mouse model of ovarian cancer. *Proc Natl Acad Sci U.S.A*. (2010) 107:19997–20002. doi: 10.1073/pnas.1009010107

17. Shi H, Huang H, Pu J, Shi D, Ning Y, Dong Y, et al. Decreased pretherapy serum apolipoprotein A-I is associated with extent of metastasis and poor prognosis of non-small-cell lung cancer. *Onco Targets Ther*. (2018) 11:6995–7003. doi: 10.2147/OTT

18. Shang Z, Wang J, Wang X, Yan H, Cui B, Jia C, et al. Preoperative serum apolipoprotein A-I levels predict long-term survival in non-muscle-invasive bladder cancer patients. *Cancer Manag Res*. (2018) 10:1177–90. doi: 10.2147/CMAR

19. Quan Q, Huang Y, Chen Q, Qiu H, Hu Q, Rong Y, et al. Impact of serum apolipoprotein A-I on prognosis and bevacizumab efficacy in patients with metastatic colorectal cancer: a propensity score-matched analysis. *Transl Oncol*. (2017) 10:288–94. doi: 10.1016/j.tranon.2017.01.006

20. Georgila K, Vyrla D, Drakos E, Apolipoprotein A-I. (ApoA-I), immunity, inflammation and cancer. *Cancers (Basel)*. (2019) 8:1097. doi: 10.3390/cancers11081097

21. Zamanian-Daryoush M, Lindner D, Tallant TC, Wang Z, Buffa J, Klipfell E, et al. The cardioprotective protein apolipoprotein A1 promotes potent anti-tumorigenic effects. *J Biol Chem*. (2013) 288:21237–52. doi: 10.1074/jbc.M113.468967

22. Martin LJ, Melnichouk O, Huszti E, Connelly PW, Greenberg CV, Minkin S, et al. lipoproteins, and risk of breast cancer: a nested case-control study using multiple time points. *J Natl Cancer Inst*. (2015) 5:djv032. doi: 10.1093/jnci/djv032

23. Diakos CI, Charles KA, McMillan DC, Clarke SJ. Cancer-related inflammation and treatment effectiveness. *Lancet Oncol*. (2014) 15:e493–503. doi: 10.1016/S1470-2045(14)70263-3

24. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. (2008) 454:436–44. doi: 10.1038/nature07205

25. Xie H, Yuan K, Ruan G, Wei L, Zhang H, Ge Y, et al. Improving the assessment of malnutrition in cancer: Using systemic inflammation markers as a supplement to the inflammation items of the GLIM criteria. *Clin Nutr*. (2023) 42:2036–44. doi: 10.1016/j.clnu.2023.08.020

26. Sirniö P, Väyrynen JP, Klintrup K, Mäkelä J, Mäkinen MJ, Karttunen TJ, et al. Decreased serum apolipoprotein A1 levels are associated with poor survival and systemic inflammatory response in colorectal cancer. *Sci Rep*. (2017) 7:5374. doi: 10.1038/s41598-017-05415-9

27. Li C, Li H, Zhang T, Li J, Liu L, Chang J. Discovery of Apo-A1 as a potential bladder cancer biomarker by urine proteomics and analysis. *Biochem Biophys Res Commun*. (2014) 446:1047–52. doi: 10.1016/j.bbrc.2014.03.053

28. Yao X, Gordon EM, Figueroa DM, Barochia AV, Levine SJ. Emerging roles of apolipoprotein E and apolipoprotein A-I in the pathogenesis and treatment of lung disease. *Am J Respir Cell Mol Biol*. (2016) 55:159–69. doi: 10.1165/rcmb.2016-0060TR

29. Zhang Y, Yang X. Prognostic significance of pretreatment apolipoprotein A-I as a noninvasive biomarker in cancer survivors: A meta-analysis. *Dis Markers*. (2018) 2018:1034037. doi: 10.1155/2018/1034037

30. Gu JN, Yao S, Cao YH, Deng SH, Mao FW, Jiang HY, et al. Novel parameter based on lipid indicators ratio improves prognostic value of plasma lipid levels in resectable colorectal cancer patients. *World J Gastrointest Surg*. (2021) 13:689–701. doi: 10.4240/wjgs.v13.i7.689

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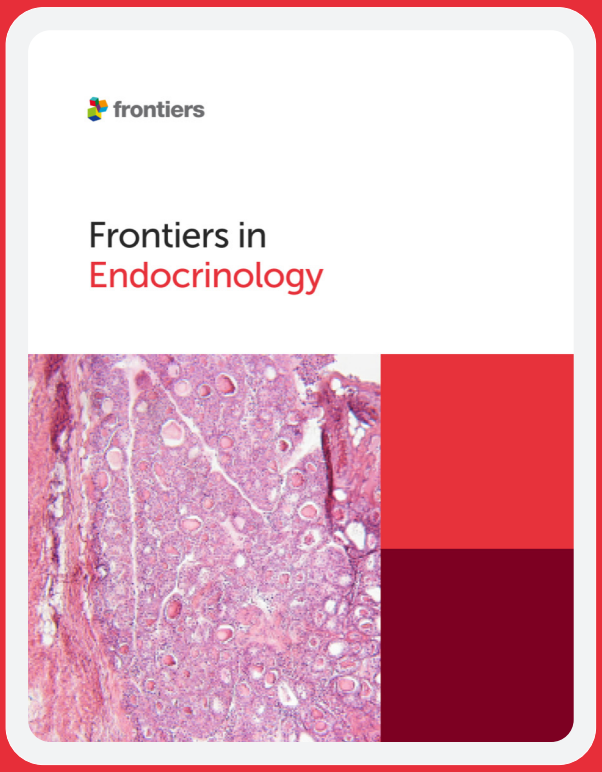

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