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Hf2cancer: Exploring bidirectional interaction between cardiovascular diseases and cancer

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Hf2cancer: Exploring bidirectional interaction between cardiovascular diseases and cancer

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Table of contents

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Editorial: HF2Cancer: Exploring bidirectional interaction between cardiovascular diseases and cancer

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heart failure, cancer, anti-cancer drugs, population studies, risk factors, biomarkers, genetic variance

Editorial on the Research Topic
HF2Cancer: Exploring bidirectional interaction between cardiovascular diseases and cancer

Introduction

Cardiovascular diseases (CVDs) and cancer are responsible for 50% of all deaths in middle-aged people all over the word (1). Indeed, both diseases share common risk factors, including obesity, diabetes, lifestyle, and aging (2). They also exhibit common pathophysiological and genetic mechanisms such as inflammation, neuro-hormonal activation, oxidative stress, clonal hematopoiesis, and a dysfunctional immune system (3). These diseases are tightly linked (4), which is supported by recent epidemiological studies and case control studies, demonstrating that especially heart failure (HF) patients have a higher risk to develop cancer (5–9). Preclinical studies have shown that indeed, HF is an oncogenic stimulus (10–12), promoting tumor growth in distant organs such as the lung and colon. Furthermore, some of the cancer markers can estimate CV mortality and CVD (13).

A better understanding and early diagnosis of CVD and cancer development are critical to delivering timely and targeted prevention strategies and to reduce the world healthcare economic burden. Moreover, clinical awareness is essential to optimize treatment strategies of patients having developed cancer with a history of CVD.

In this Research Topic, we covered the expert reviews on epidemiological studies showing cancer itself leads to a higher risk of death from CVD, the novel nomogram model, some multimodal imaging guides and cardiac biomarkers to detect or predict the risk of CVDs in cancer patients, characterization of possible target molecules to prevent or treat cardiovascular damages induced by the cancer therapy.

Chianca et al. discussed and recapitulate the evidence on cardiovascular and oncological biomarkers in the field of cardio-oncology, focusing on their role in risk stratification, early detection of cardiotoxicity, follow-up, and prognostic assessment. Interestingly, these biomarkers may play key roles to better understand the common pathophysiology of both cancer and HF.

Posch et al. discussed how dynamic cardiotoxicity risk assessment can be achieved by monitoring left ventricular ejection fraction (LVEF), high-sensitive cardiac troponin T (hs-cTnT), and N-terminal pro B-type natriuretic peptide (NT-proBNP) in women with $HER2^{+}$ early breast cancer undergoing primarily trastuzumab-based therapy. They demonstrated that the pre-treatment and longitudinal LVEF trajectory but not hs-cTnT or NT-proBNP can be used for a dynamic assessment of cardiotoxicity risk in this group of patients.

Curtiaud et al. discussed the major etiologies and management of cardiogenic shock related to the acute cardiomyopathy and pulmonary embolism, myocarditis, Takotsubo syndrome, cardiac tamponade, cardiac herniation, neoplastic cardiac infiltration in cancer patient, suggesting an essential multidisciplinary collaboration between intensivists, cardiologists, cardiac surgeons, and oncologists in these critical situations.

In Turk and Kunej's screening of PubMed data base for genetic risk factors in patients with both cancer and CVD, and reviewing of the 181 articles and visualizing the gene-disease network by Cytoscape and the enrichment analysis, they demonstrated that genetic risk factors associated with the comorbidity of cancer and CVDs are significantly enriched in DNA damage repair (DDR) pathways. They suggested requirement of functional studies to elucidate contribution of DDR pathways in the pathophysiology to CVDs.

Guler et al. discussed the preclinical evidences that unravel the molecular pathways and targets in bilateral connection between cardiac injury (HF and early cardiac remodeling) and cancer. They specifically emphasized the cardiac- and cancer-secretoms as possible disease-specific biomarkers and therapeutic targets. They also highlighted the several studies speculating whether cardiovascular drugs are promoter or suppressor of cancer incidence.

Cao et al. showed that Hong Huang Decoction, a Chinese herbal medicine, restores the levels of interleukins such as IL-6, IL-10, superoxide dismutase (SOD), and nitric oxide (NO), as well as pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), thereby modulating oxidative stress to protect against antracyline-mediated cardio toxicity in breast cancer patients. They also mentioned that global longitudinal strain is a better detection method of myocardial damage in these patients as compare to LVEF.

Si et al. reported that in a patient with eosinophilic leukemia with progressive eosinophilic myocarditis, multimodality imaging can provide early diagnosis of fibroblast activation, and an anti-fibrotic therapy after heart failure can be a principle early treatment.

Zhang G. et al. summarized the mechanisms of ferroptosis, a new form of regulated cell death that was induced by doxorubicin, in respect to an iron accumulation and metabolism in cardiomyocytes. They also discussed the prevention and treatment of cardiotoxicity targeting ferroptosis.

Wang H. et al. developed and validated a nomogram based on Cox regression to predict the survival of around 20,000 patients with colorectal cancer recruited from 4 centers in China between 2011 and 2017, hoping to improve the prognostic evaluation ability.

Wang W. et al. showed that the signaling pathway that is involved in coiled-coil containing protein kinase (RhoA/ROCK), an inhibitory signal to block regeneration contributes to the inhibiteur de tyrosine kinase anti-VEGFR2, apatinib-mediated hypertension in the gastric cancer, and Y27632 as an inhibitor of RhoA/ROCK has a potential for the prevention and treatment of this type of hypertension.

Murtaza et al. evaluated 18 studies, and found that cancer patients with atrial fibrillation had higher mortality rate and recommended that extra care and specific measures must be taken in the management of cancer patients with new-onset atrial fibrillation.

Rushton et al. demonstrated that increased risk of cardiac event was associated with age (>60) and longer treatment with trastuzumab in $HER2^+$ breast cancer patients.

Wu et al. found that the serum adrenomedullin concentration is associated with the incidence of myocardial ischemic T wave change among lung cancer patients, suggesting adrenomedullin as a potentially valuable predictor for heart ischemia in lung cancer patients.

Li J. et al. demonstrated that coronary atherosclerotic heart disease (CAD) is an independent risk factor for cancer, and digestive, respiratory and urogenital cancers are independent risk factors for CAD. Indeed, they also showed that high alanine aminotransferase is correlated with low cancer incident.

Zhang S. et al. reported a 66-year-old woman with syncope caused by carotid sinus syndrome who was eventually diagnosed with advanced nasopharyngeal carcinoma and after diagnosis and treatment, the patient had no recurrence of syncope, suggesting that nasopharyngeal carcinoma is the potential intrinsic causes of syncope.

Conclusion and future challenges

We think that the Research Topic of *Heart failure (HF) to Cancer (HF2Cancer): Exploring bidirectional interaction between cardiovascular diseases and cancer* provides awareness of new challenges and future directions in the field of cardio-oncology from fundamental scientist to the clinicians. Thus, a better understanding and early diagnosis of HF and cancer development play key roles to delivering timely and targeted prevention strategies to reduce the world healthcare economic burden.

Author contributions

CN wrote the editorial information. Both CN and MC have approved the submitted version.

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Conflict of interest

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

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CASE REPORT
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Syncope as the Initial Manifestation of Advanced Nasopharyngeal Carcinoma: A Case Report

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Carotid sinus syndrome is a principal cause of syncope in the elderly. Syncope, associated with carotid sinus syndrome which is secondary to metastasis of advanced nasopharyngeal carcinoma, rarely occurs. The current study reported a 66-year-old woman, who presented with a history of frequent and recurrent syncope as the initial symptom, and was eventually diagnosed with advanced nasopharyngeal carcinoma. The positron emission tomography scan demonstrated a diagnosis of advanced nasopharyngeal carcinoma with involvement in carotid sheath space, and nasopharyngeal biopsy revealed non-keratinized nasopharyngeal carcinoma. After diagnosis and treatment, the patient had no recurrence of syncope. In summary, our case study suggests that great importance should be attached to potential intrinsic causes of syncope especially in the case of nasopharyngeal carcinoma, as it is an insidious malignancy which needs to be precisely identified.

Keywords: syncope, nasopharyngeal carcinoma, carotid sinus syndrome, diagnosis, case report

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INTRODUCTION

Carotid sinus syndrome is a principal cause of syncope among the elderly, accounting for 26 to 60% of elderly patients with inexplicable syncope (1, 2). Patients with advanced nasopharyngeal carcinoma usually present with blood-tinged sputum, nosebleed, hearing loss, headache and neck mass. Nevertheless, syncope, associated with the metastasis of advanced nasopharyngeal carcinoma, is very rare. The current study reported an elderly woman with nasopharyngeal carcinoma who had recurrent syncopal attacks as the initial presentation, suggesting that great importance should be attached to the rare causes of syncope especially among patients with nasopharyngeal carcinoma, since it is an insidious malignancy which needs to be precisely identified.

The informed consent was obtained from the patient. All the personal information has been anonymized before the analysis.

CASE REPORT

A 66-year-old woman presented with a history of frequent and recurrent syncope which had lasted for half a month, and the frequency was up to 2 to 4 times per week. She was suffering from palpitation, dizziness, and neck tightness before the attacks, then the loss of consciousness followed

by those symptoms. The syncopal episodes lasted about 30–60 Sec each time. Recovery would spontaneously occur with a complaint of weakness. Past medical history was only remarkable for hypertension and her parents both had coronary atherosclerotic heart disease.

On admission, she was in sinus rhythm with a heart rate of 74 beats/min and her blood pressure was 125/74 mmHg. Physical and neurological examination showed no obviously abnormal signs. The result of carotid sinus massage was negative. Electrocardiogram (ECG) indicated sinus bradycardia (58 beats/min). The color sonography cardiac ultrasound result was normal for the 66-year-old female. The 24-h ECG Holter monitor detected that the average heart rate was more than 60 beats/min, that the lowest heart rate was 38 beats/min, and that the longest RR interval was 1.73 Sec. There were no abnormalities in routine blood examination, electrolytes, glucose, liver and kidney function. The level of adrenocorticotropic hormone and catecholamine in plasma was normal. Similarly, electroencephalogram (EEG) was negative as computed tomography (CT) and angiography of the carotid and cerebral arteries were conducted.

During hospitalization, her bedside ECG indicated sinus arrest and ventricular escape rhythm when she experienced syncope. These symptoms were followed by the loss of consciousness with blood pressure of 63/34 mmHg and pulse of 39 beats/min. Whereafter, the patient received treatment of temporary pacemaker during hospital stay. Simultaneously, the coronary angiography demonstrated coronary atherosclerosis without significant stenosis. However, she still had syncopal attacks after pacemaker implantation while ECG suggested that pacing rhythm alternated with sinus rhythm of 60 beats/min and blood pressure of 59/39 mmHg. The abdominal ultrasonography showed no evident adrenal mass but surprisingly detected multiple low echo-level masses in her liver. The contrast-enhanced abdominal CT revealed multiple low-density shadows of varied sizes in the liver, which were diagnosed as secondary malignant tumors. Systemic metabolic imaging were performed to identify the primary tumor. The positron emission tomography (PET) scan demonstrated an increased accumulation of FDG (2-deoxy-2-[18F] fluoro-D-glucose) in the thickening left naso-pharyngeal mucous membranes and the left carotid sheath space (**Figure 1**). Nasopharyngeal biopsy revealed non-keratinized nasopharyngeal carcinoma. Hence, palliative chemoradiotherapy was administered after the diagnosis of advanced nasopharyngeal carcinoma.

Three months after diagnosis and treatment, the left nasopharyngeal and carotid sheath space masses were significantly reduced in size, and the patient had no recurrence of syncope in 2 years. Currently, the patient has been still receiving palliative treatment.

DISCUSSION

Syncope is defined as a self-limited transient loss of consciousness due to a global reduction in cerebral perfusion (1, 3). Generally speaking, several causes may contribute to syncope. Except for some unexplained pathogenesis, syncope can be categorized as cardiac syncope, reflex syncope, orthostatic hypotension and cerebral syncope. For the cardiac syncope, the primary mechanism is a remarkable reduction in cardiac output due to cardiopulmonary disease, such as arrhythmia, structural heart disease, or pulmonary embolism that contribute to global cerebral hypoperfusion (4). Thus, cardiac syncope may be preceded by palpitations, shortness of breath, or chest pain. For patients with arrhythmic syncope, a pacemaker therapy may be of benefits. In reflex syncope, peripheral vasodilation and bradycardia induced by abnormal sympathetic or vagal reflexes can lead to sudden drop in blood pressure and decrease in cardiac output (5). According to the current pathophysiological mechanisms, reflex syncope can be summarized as vasovagal syncope, carotid sinus syndrome, and urinary syncope. Orthostatic hypotension (OH) is an excessive fall in blood pressure after standing which may result from inadequate intravascular volume, autonomic nervous system dysfunction, decreased venous return, or an inability to increase cardiac output in response to postural changes. Transient loss of consciousness with spontaneous recovery, which has a direct impact on cerebral parenchyma or cerebral vessels, can also be ascribed to cerebral syncope like epilepsy, transient ischemic attack (TIA) or blood supply disorders of vertebrobasilar arteries.

The current study reported a patient with carotid sinus syndrome accompanied by arrhythmia. In this case, the patient was an elderly female with presentation of cardiovascular disease like palpitation, pallor and syncope. The ECG indicated sinus arrest and ventricular escape rhythm during a syncopal attack. In addition, she had a past medical history of hypertension and a family history of cardiovascular disease, suggesting possibility of cardiac syncope. From another perspective, The PET scan demonstrated a lesion of carotid sheath space, secondary to advanced nasopharyngeal carcinoma, anatomically indicative of carotid sinus syndrome. Notably, our patient still had syncopal attacks and hypotension after the pacemaker therapy, which implied that cardiac syncope might be precluded. Therefore, it could be inferred that her syncope was related to carotid sinus syndrome.

Carotid sinus syndrome (CSS) is a clinical syndrome which presents as a group of symptoms including spontaneously sudden onset of dizziness, weakness, tinnitus or even syncope. The associated symptoms directly reflect carotid sinus hypersensitivity (CSH) due to compression or invasion of the carotid sinus. Four pathophysiological mechanisms of carotid sinus syndrome have been described (6): the cardio-inhibitory type, the vasodepressor type, the mixed type and the rare cerebral type. Under normal circumstances, a pacemaker therapy might alleviate syncope originated from the cardio-inhibitory type of CSS. However, the pacing might be ineffective if pure vasodepressor syncope develops. In this case, since the patient still had recurrences of syncope and hypotension after pacemaker implantation, the vasodepressor or mixed type of carotid sinus syndrome was probably the main pathogenesis.

In the setting of head and neck cancer, the causative mechanisms of syncope include irritation of carotid sinus or glossopharyngeal nerve (7). In this case, our patient was an

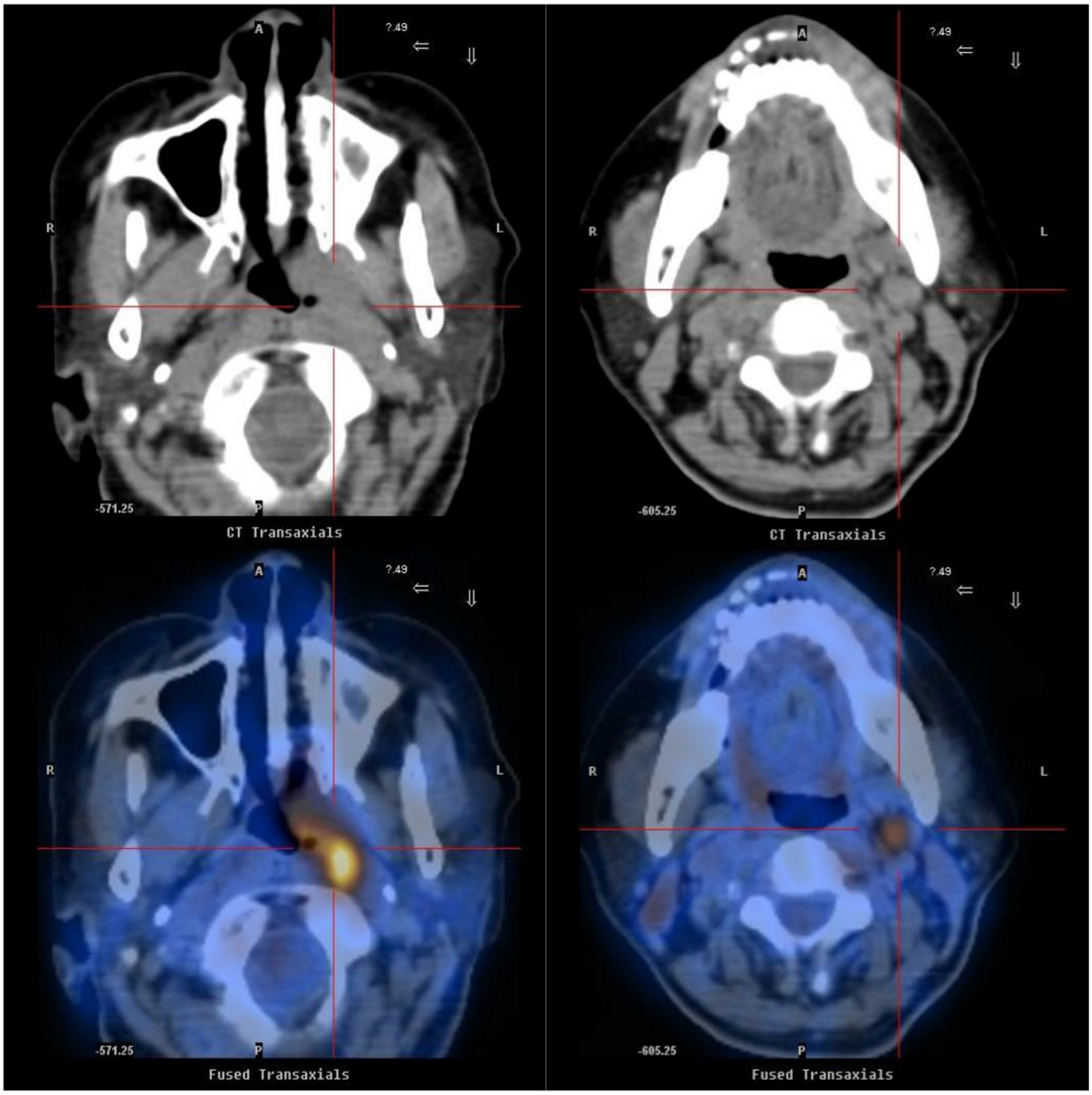

FIGURE 1 | Positron emission tomography (PET) scan shows left nasopharyngeal carcinoma extending to carotid sinus. PET scan demonstrated an increased accumulation of FDG (2-deoxy-2-[18F] fluoro-D-glucose) in the thickening left nasopharynx (left) and an increased accumulation of FDG in the left carotid sheath space (right).

elderly female ever diagnosed with nasopharyngeal carcinoma, who had syncopal episodes as the initial symptom. The PET scan indicated a diagnosis of advanced nasopharyngeal carcinoma with involvement in carotid sheath space, anatomically supporting the possibility of compression on the carotid sinus. It can be deduced that, recurrent syncopal attacks were associated with carotid sinus syndrome, secondary to mechanical compression and stimulation of the carotid sinus, resulting from the metastasis of advanced nasopharyngeal carcinoma. Accordingly, the exceptional causes of syncope should be taken into account, especially in the case of head and neck cancer like nasopharyngeal carcinoma.

The cases of syncope associated with nasopharyngeal carcinoma reported between 1990 and 2020 have been summarized (**Table 1**). The majority of nasopharyngeal carcinoma patients (86.36%) with syncope were males, indicating that male patients with nasopharyngeal carcinoma may be prone to developing syncope. Moreover, patients with syncopal attacks mostly have a late or advanced state of illness. According to the previous studies, most patients were diagnosed as clinical III stages, whose biopsy identifying low differentiated /undifferentiated squamous cell carcinoma. It is indicated that syncope may be associated with the metastasis of advanced nasopharyngeal carcinoma. Meanwhile, approximately one fifth of patients with nasopharyngeal carcinoma died consequently. Thus, syncope appears to be a poor prognostic sign of tumor invasion and metastasis. In addition, carotid sinus syndrome is found to be responsible for most syncope caused by

TABLE 1 | Summary of the cases of syncope associated with nasopharyngeal carcinoma.

Article	Age/gender	Time	Stages/typing	ECG	Treatment	Outcome	Cause	Possible etiology
Degirmenci (8)	56/M	NA	III/Low differentiated squamous cell carcinoma	Sinus bradycardia	Chemoradiotherapy, lymphadenectomy	No recurrence	Metastatic mass lesions compressing	CSS
Wang (9)	75/M	2 y	III/Low differentiated squamous cell carcinoma	Third degree A-V block	Chemoradiotherapy	No recurrence	Metastatic mass lesions compressing	CSS
Zhang (10)	75/M	2 m	III/NA	sinus arrest, sick sinus syndrome	Chemoradiotherapy	NA	Downregulation of NE secretion	CSS/CSH
Lin (11)	72/M	5 m	IV/Non-keratinizing squamous cell carcinoma	Normal	Radiotherapy	Death	Involvement of glossopharyngeal and vagus nerves	PSL
	66/M	5 m	IV/Undifferentiated squamous cell carcinoma	Normal	Chemoradiotherapy	NA	Involvement of glossopharyngeal and vagus nerves	PSL
Kala (12)	69/M	5 y	III/Low differentiated squamous cell carcinoma	Normal	Chemoradiotherapy	No recurrence	Irritation of vagus nerve	Irritation of vagus nerve
Atsuumi (13)	53/M	9 y	III/Low differentiated squamous cell carcinoma	NA	Chemotherapy	No recurrence	Irritation of vagus nerve	PSL
Tang (14)	54/M	NA	IV/Undifferentiated squamous cell carcinoma	Normal	Chemoradiotherapy	No recurrence	NA	CSS
	65/M	NA	IV/Undifferentiated squamous cell carcinoma	Sinus bradycardia	Chemoradiotherapy	No recurrence	NA	Glossopharyngeal neuralgia
	57/M	NA	IV/Undifferentiated squamous cell carcinoma	Normal	Chemoradiotherapy	No recurrence	NA	PSL
Chen-Scarabelli (7)	62/M	6 m	IV/Keratinizing squamous cell carcinoma	Bradycardia, long sinus pauses	Chemoradiotherapy	No recurrence	Metastatic mass lesions compressing	CSS/CSH
Wang (15)	50/M	6 m	III/Undifferentiated squamous cell carcinoma	Sinus arrest	Chemoradiotherapy	No recurrence	Metastatic mass lesions compressing	CSS, PSL, GNI
Tulchinsky (16); Wang (17); Li (18)	68/M 55/F 65/M	3 m NA 10 d	IV/Low differentiated squamous cell carcinoma III/ Undifferentiated non-keratinizing carcinoma III/ Undifferentiated non-keratinizing carcinoma	Junctional rhythm, ventricular escape NA Normal	Chemoradiotherapy Chemoradiotherapy Chemotherapy	Death No recurrence No recurrence	Metastatic mass lesions compressing Metastatic mass lesions compressing Metastatic mass lesions compressing	CSS CSS CSS
Zhang (19)	53/M 64/M	2 d 2 y	III/NA III/ Non-keratinizing squamous cell carcinoma	Atrial fibrillation bradycardia	Chemoradiotherapy Radiotherapy	No recurrence Recurrence	Irritation of vagus nerve Metastatic mass lesions compressing	PSL CSS
Yang (20)	56/M	7 y	III/Low differentiated squamous cell carcinoma	Third degree A-V block	Radiotherapy	Death	NA	CSS
	36/F	1 y	III/Low differentiated squamous cell carcinoma	Third degree A-V block	Radiotherapy	Death	NA	CSS
Zhou (21)	48/M	5 y	III/Low differentiated squamous cell carcinoma	Sinus bradycardia	Radiotherapy	NA	NA	CSS, GNI

(Continued)

TABLE 1 | Continued

Article	Age/gender	Time	Stages/typing	ECG	Treatment	Outcome	Cause	Possible etiology
Shen (22)	63/M	1 y	III/Low differentiated squamous cell carcinoma	Sinus bradycardia	Radiotherapy	No recurrence	NA	CSS
Our case	66/F	2 y	IV/Non-keratinizing squamous cell carcinoma	Third degree A-V block	Chemoradiotherapy	No recurrence	Metastatic mass lesions compressing	CSS

M, male; F, female; y, years; m,months; d, days; NA, not available; ECG, electrocardiogram; CSS, carotid sinus syndrome; PSL, parapharyngeal space lesions; GNI, glossopharyngeal nerve injury. Time: Period from time of syncope occurrence to time of diagnosis.

nasopharyngeal carcinoma, which is primarily attributed to the compression of metastatic mass lesions on carotid sinus.

The patient in this case was eventually diagnosed with advanced nasopharyngeal carcinoma. However, she did not suffer from typical symptoms like blood tinged sputum, nosebleed, hearing loss or headache. In contrast, her initial presentation was syncope associated with carotid sinus syndrome. Most physicians may neglect nasopharyngeal malignancies characterized by syncope and hypotension. It is recommended that doctors should evaluate the origin of syncope before the pacemaker treatment. Although a pacemaker can maintain a normal heart rate, the efficacy is limited when mentioning the vasodepressor or mixed type of carotid sinus syndrome.

To summarize, this was the first case of advanced nasopharyngeal carcinoma with recurrent syncope as an initial symptom. To our knowledge, syncope rarely occurred as the initial presentation of advanced nasopharyngeal carcinoma, and carotid sinus syndrome was considered as the potential intrinsic cause of syncope in nasopharyngeal carcinoma (7–10, 14–22). Hence, cranial imaging examinations and nasopharyngoscopy may be needed for these suspected patients. Chemoradiotherapy could be a better choice for controlling syncope (7–9, 12, 14, 15, 17, 19, 23). It is also advised that physicians should find out the origin of recurrent syncope through clinical condition and relevant tests, in the purpose of early diagnosis, precise treatment and improved prognosis.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Y-SG, S-CZ, and M-QL: case collection. Y-SG, S-CZ, M-QL, L-WZ, and K-YL: analysis of the case. S-CZ and M-QL: draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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Relationship Between Serum Concentration of Adrenomedullin and Myocardial Ischemic T Wave Changes in Patients With Lung Cancer

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Background: Patients with lung cancer are at increased risk for the development of cardiovascular diseases. Molecular markers for early diagnosis of cardiac ischemia are of great significance for the early prevention of cardiovascular events in patients with lung cancer. By evaluating the relationship between adrenomedullin (ADM) and myocardial ischemic T wave changes, the clinical value of circulating ADM as a predictor of myocardial ischemia in patients with lung cancer is confirmed.

Methods: We enrolled patients with lung cancer and healthy people from 2019 to 2021 and extracted a detailed ECG parameter. After adjustment for potential confounders, logistic regression was used to assess the association of clinical data. We performed analyses on differences in T wave between patients with lung cancer and healthy people, and the relationship between T wave and ADM among patients with lung cancer. Receiver operator characteristic (ROC) curves were drawn to confirm the diagnostic value of biomarkers.

Results: After adjusting for potential confounders, the incidence of T wave inversion or flattening in patients with lung cancer was higher than in healthy people (OR: 3.3228, $P = 0.02$). Also, further analysis of the data of lung cancer patients revealed that the ADM in lung cancer patients with T wave inversion or flat was higher than those with normal T wave (189.8 ± 51.9 vs. 131.9 ± 38.4, $p < 0.001$). The area under the ROC curve was 0.8137.

Conclusion: Among the patients with lung cancer, serum ADM concentration is associated with the incidence of the abnormal T wave. ADM might be a potentially valuable predictor for heart ischemia in patients with lung cancer.

Keywords: adrenomedullin (ADM), T wave inversion or flat, myocardial ischemia, cardiovascular diseases (CVDs), lung cancer

INTRODUCTION

Cancer and cardiovascular diseases (CVDs) are two major killers among humans across the world. Cancer incidence and mortality have been rising steadily over the past several decades (1). Recent data have indicated that CVDs have become a leading cause of morbidity and mortality among cancer survivors (2). This is largely because of tumor invasiveness (like direct compression or infiltration and several inflammatory factors) and cancer treatment-related cardiotoxicity (3). Prevention of CVDs has important clinical implications for cancer survivors.

Among all cancers worldwide, lung cancer is the most commonly diagnosed with the highest mortality, and the burden of this disease is considerable. In China, it was estimated that 2.09 million new cases of lung cancer occurred globally in 2018, ranking first among all cancer types (4, 5). Anatomically, the lung is adjacent to the heart, and pulmonary circulation is a closed circuit between the right side of the heart (right ventricle, RV) and the lungs. A lung tumor releases pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IFN-γ), chemokines, and soluble factors into the blood. These substances are then returned to the heart *via* pulmonary circulation and directly elicit catabolic responses in the heart (6, 7). However, the sensitivity and specificity of these indicators were not high enough to be specific biomarkers of CVDs. In addition to the properties of the tumor itself, clinical evidence has also shown that long term anti-cancer therapy can lead to a persistently increased CVDs risk (8). Platinum-based chemotherapy, microtubular agents, ALK inhibitors, endothelial growth factor receptor (EGFR) inhibitors, and immunotherapy represent principal clinical options for lung cancer therapy (9–11). All of these drugs have been shown to result in cardiotoxicity. Radiotherapy also plays a critical role in the management of early- or locally advanced-stage lung cancer. During radiotherapy for lung cancer, radiation often involves surrounding tissues and organs, especially the heart. Prolonged radiation exposure leads to capillary network and myocardium damage, eventually contributing to the development of myocardial, heart valve, and cusp fibrosis (12). Given that lung cancer has been known to have a higher risk of cardiovascular events, potential biomarkers to indicate susceptibility to heart ischemia need to be identified (13).

Adrenomedullin (ADM) is a 52-amino acid peptide hormone that was originally identified in extracts of human pheochromocytoma (14). The expression of ADM is widely distributed throughout the cardiovascular system and has been identified in blood vessels and the heart. There is increasing evidence that elevated circulating and tissue levels of ADM are associated with cardiovascular events. In a rat myocardial infarction (MI) model, the use of ADM has shown beneficial effects on survival and ameliorated the progression of left ventricular remodeling and heart failure (15, 16). A recent study revealed that ADM induces beneficial hemodynamic, hormonal, and myocardial changes, which can improve outcomes of patients with HF (17). In addition, various studies have indicated that imbalance in the expression of ADM is closely related to the occurrence and development of CVDs (18). ADM can be considered as biomarkers of various CVDs diagnoses, prognoses, and surveillance. The serum level of ADM has a significant positive correlation with the severity of CVDs. Because of the stability of ADM in whole blood, serum, or plasma, numerous studies have speculated that serum ADM levels can be used as an early warning indicator of cardiac ischemia.

MATERIALS AND METHODS

Study Subjects

We performed a cross-sectional study that included patients with lung cancer and healthy populations who visited the Chongming branch of Xin Hua Hospital from 2019 to 2021 and extracted detailed information on ECG. The data were extracted from electronic medical records in the Chongming branch of Xin Hua Hospital. In this way, we identified 67 patients with lung cancer and 81 healthy people. Exclusion criteria were as follows: (1) abnormal thyroid function, (2) congenital heart disease, (3) a history of heart surgery, (4) severe valvular heart disease, (5) myocardial diseases, (6) coronary heart disease, (7) arrhythmia, and (8) diabetes. The local ethical committee approved the protocol of the study and waived the need for informed consent because of the observational characteristic of the study, and the study followed the principles outlined in the Declaration of Helsinki.

Electrocardiogram Recordings

Standard 10-s 12-lead ECGs of all the subjects were recorded. Inverted T waves with an amplitude $\geq$0.1 mV were considered to be abnormal, whereas those with peak amplitude between +1 and −1 mm were considered to be flat. All procedures and operations were performed by experienced cardiologists (**Figure 1**).

Serum Collection

Patients with lung cancer routinely underwent routine blood tests over the course of their treatment and follow-up stages. Residual blood from clinical tests was collected during these stages. Whole blood samples were obtained and kept at room temperature for 2 h or placed at 4°C overnight. Serum samples were collected by centrifugation (20 min at 1,000 × *g*), preserved, and stored at −80°C before analysis.

Enzyme-Linked Immunosorbent Assay

The frozen serum samples were used to measure the serum levels of ADM. ELISA was performed with commercial ELISA kits (Shanghai Tongwei Industrial Co. Ltd., TW10166) according to the manufacturer's instructions. Each independent experiment was performed three times.

Covariates

For each study subject, we extracted data on demographic information, physical examination, history and comorbidity, concomitants, laboratory tests, and prescribed related medications from electronic medical records, and conducted a retrospective review. All the data were checked and validated; if there were any outliers, we manually checked the original dataset.

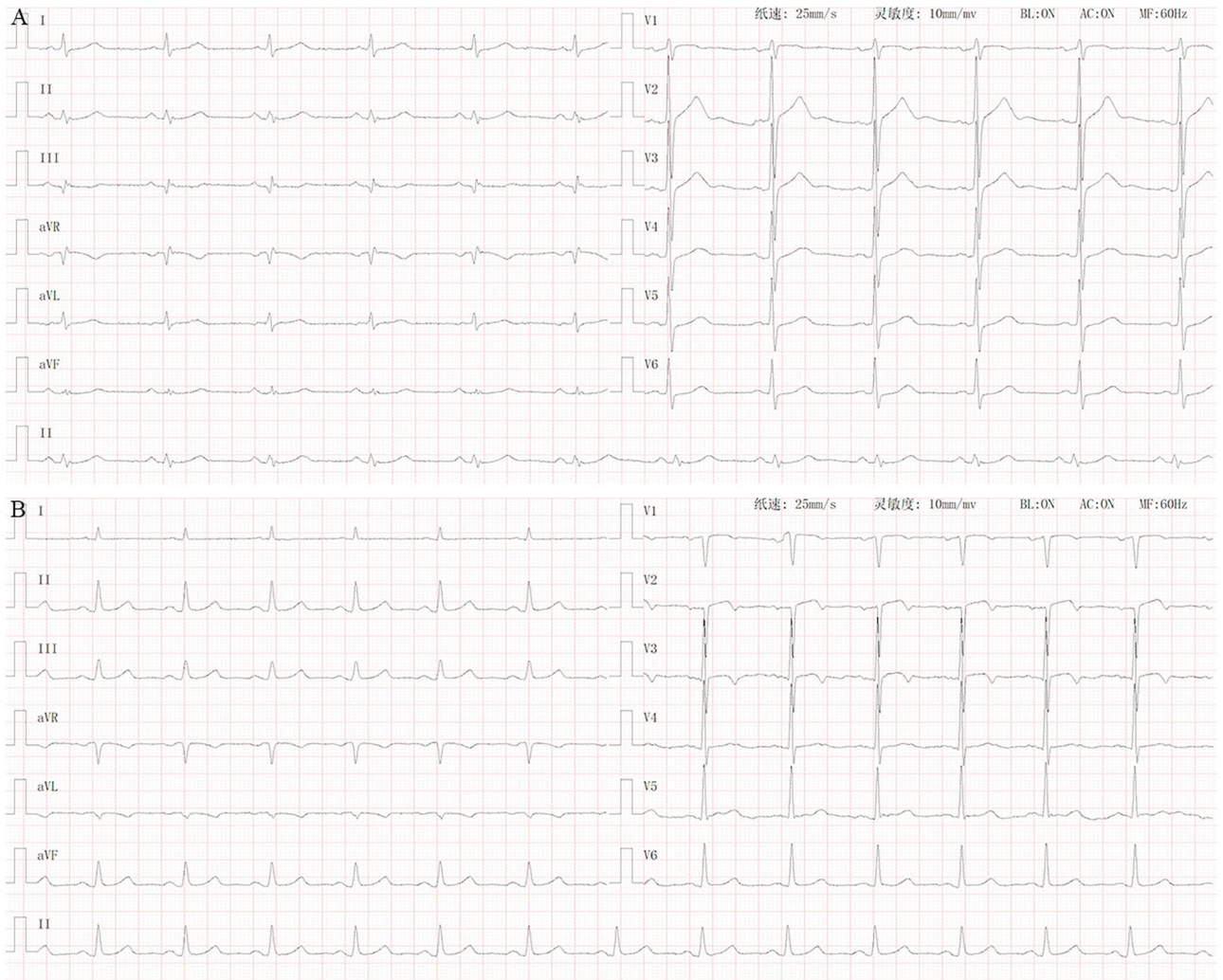

FIGURE 1 | Normal ECG vs. ECG of patients with lung cancer with T wave changes. **(A)** Normal ECG. **(B)** ECG of lung cancer patients with T wave inversion or flattening (I, aVL, V3, V4, and V5).

Statistical Analysis

Continuous variables were expressed as mean values ± SD, whereas categorical variables were expressed as frequencies between subjects with and without T wave inversion or flattening. Kolmogorov-Smirnov test was performed to examine whether continuous variables were normally distributed, and analyses were performed by Student's *t*-test. Associations between categorical variables were tested by Pearson's χ2 test. Logistics regression analysis was performed to evaluate risk factors for T wave inversion or flattening, and ROC analysis was performed to assess the efficiency of distinguishing patients with lung cancer with myocardial ischemic T wave change (T wave inversion or flattening) from patients without.

All the statistical analyses were performed using the STATA 15.1 software. A $P < 0.05$ was considered statistically significant.

RESULTS

Clinical Characteristics of the Subjects

The general characteristics of patients with lung cancer ($n = 67$) and health check-up people ($n = 82$), including gender, age, being older, history of drug use, and related risk factors are summarized in **Table 1**. Among all the enrolled individuals, 9 (11%) healthy people had T wave inversion or flattening, while 16 (23.9%) patients with lung cancer had T wave inversion or flattening. The rate of occurrence of T wave inversion or flattening in patients with lung cancer was higher than that in the healthy control population (23.9% vs. 11%, $p = 0.036$), and smokers among them were markedly more than healthy subjects (44.8% vs. 8.5%, $p < 0.001$). Baseline characteristics of the patients with lung cancer stratified by presence/absence of T wave inversion or flattening are summarized in **Table 2**. T wave inversion or flattening was more likely to be detected in female patients with

TABLE 1 | Characteristics of checkup people and patients with lung cancer.

Characteristics	Health people $n = 82$	Lung cancer patients $n = 67$	*P*-value
Male, n	63 (76.8%)	53 (79.1%)	0.74
Female	19 (23.2%)	14 (20.9%)	
Age, years	71.3 ± 7.0	69.8 ± 8.1	0.23
Older, n (%)	24 (29.2%)	15 (22.4%)	0.34
Current smoking, n	7 (8.5%)	30 (44.8%)	<0.001
Alcohol consumption, n	8 (9.8%)	13 (19.4%)	0.092
Hypertension, n	28 (34.1%)	33 (49.3%)	0.062
CAC, n	30 (36.7%)	8 (11.9%)	0.11
Pulmonary nodules, n	19 (23.2%)	18 (26.9%)	0.70
β-blocker, n	9 (11.0%)	4 (6.0%)	0.28
CCB, n	9 (11.0%)	4 (6.0%)	0.28
ACEI, n	7 (8.5%)	2 (3.0%)	0.16
ARB, n	4 (4.9%)	7 (10.4%)	0.20
T wave inversion or flat, n	9 (11.0%)	16 (23.9%)	0.036

Older, age ≥ 75 years; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; CAC, coronary artery calcification. Value was given as mean ± SD, or frequency (percentage).

TABLE 2 | Characteristics of lung cancer patients with T wave inversion or flattening.

Characteristics	T wave inversion or flat $n = 16$	Normal T wave $n = 51$	*P*-value
Male, n	9 (53%)	44 (86%)	0.010
Female	7 (47%)	7 (14%)	
Age, years	69.7 ± 9.8	69.9 ± 7.6	0.93
Older, n	3 (19.0%)	12 (24.0%)	0.69
Current smoking, n	3 (19.0%)	27 (53.0%)	0.016
Alcohol consumption, n	1 (6.0%)	12 (24.0%)	0.13
Hypertension, n	9 (56.0%)	24 (47.0%)	0.52
CAC	5 (31.3%)	3 (5.9%)	0.73
Pulmonary nodules, n	9 (56.3%)	9 (17.6%)	0.002
β-blocker, n	1 (6.0%)	3 (6.0%)	0.96
CCB, n	0 (0%)	4 (7.8%)	0.25
ACEI, n	0 (0%)	2 (3.9%)	0.42
ARB, n	2 (12%)	0 (0%)	
Chemotherapy, n	8 (50.0%)	30 (59.0%)	0.53
Lung cancer operation, n	2 (12.0%)	3 (6.0%)	0.38
Pleural effusion, n	4 (25.0%)	5 (10.0%)	0.15

Older, age ≥ 75 years; CAC, coronary artery calcification; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers. Value was given as mean ± SD, or frequency (percentage).

lung cancer (47 vs. 14%, $p = 0.01$). Compared to patients with normal T wave, patients with T wave change exhibited a higher proportion of pulmonary nodules (56.3 vs. 17.6%, $p = 0.002$) and a lower proportion of smokers (19 vs. 53%, $p = 0.016$).

T Wave Inversion or Flat in Healthy People and Patients With Lung Cancer

By using Logistics regression analysis, lung cancer patients were more likely to observe T wave inversion or flat compared with healthy controls…and medication use [angiotensin-converting enzyme inhibitor (ACEI), angiotensin receptor blockers (ARB), β-blocker, and calcium channel blocker (CCB)].

Serum ADM Levels in Patients With Lung Cancer With or Without T Wave Inversion or Flattening

Enzyme-linked immunosorbent assay (ELISA) was performed to assess serum levels of ADM. The level of ADM in serum was significantly higher in patients with T wave inversion or flattening than in those without, as shown in **Figure 2**. Average serum levels of ADM were 189.8 ± 51.9 in patients with abnormal T wave and 131.9 ± 38.4 in patients without. A logistic regression model indicated that ADM was associated with the incidence of T wave inversion or flattening [odds ratio (OR): 1.4102, $p = 0.036$, 95% confidence interval (CI): 1.0232–1.9437] after being adjusted for all the accepted confounders: gender, age, being older, hypertension, lung cancer operation, pleural effusion, pulmonary nodules, current smoker, alcohol consumption, and history of drug use (chemotherapy drug, ACEIs, ARBs, β-blockers, and CCBs).

To investigate the relationship between ADM and myocardial ischemic-related T wave alteration in patients with lung cancer, ROC analyses were performed to evaluate the diagnostic ability of ADM. As shown in **Figure 3**, a ROC curve for ADM is plotted to judge the reliability of the model and reflect the distinction between patients with or without T wave inversion or flattening, with an area under the curve (AUC) of.8137 (95% CI:0.69426–0.93324). ADM displayed acceptable sensitivity and specificity for the prediction of myocardial ischemia in patients with lung cancer.

DISCUSSION

We first demonstrated the reciprocal relationship between myocardial ischemia and lung cancer. We performed logistic regression analyses and found that the incidence of the ischemic T wave in patients with lung cancer was two times that in healthy subjects.

Myocardial ischemia is a chronic pathological condition, in which the heart receives oxygen slightly, and it is also an early pathological process during the occurrence and development

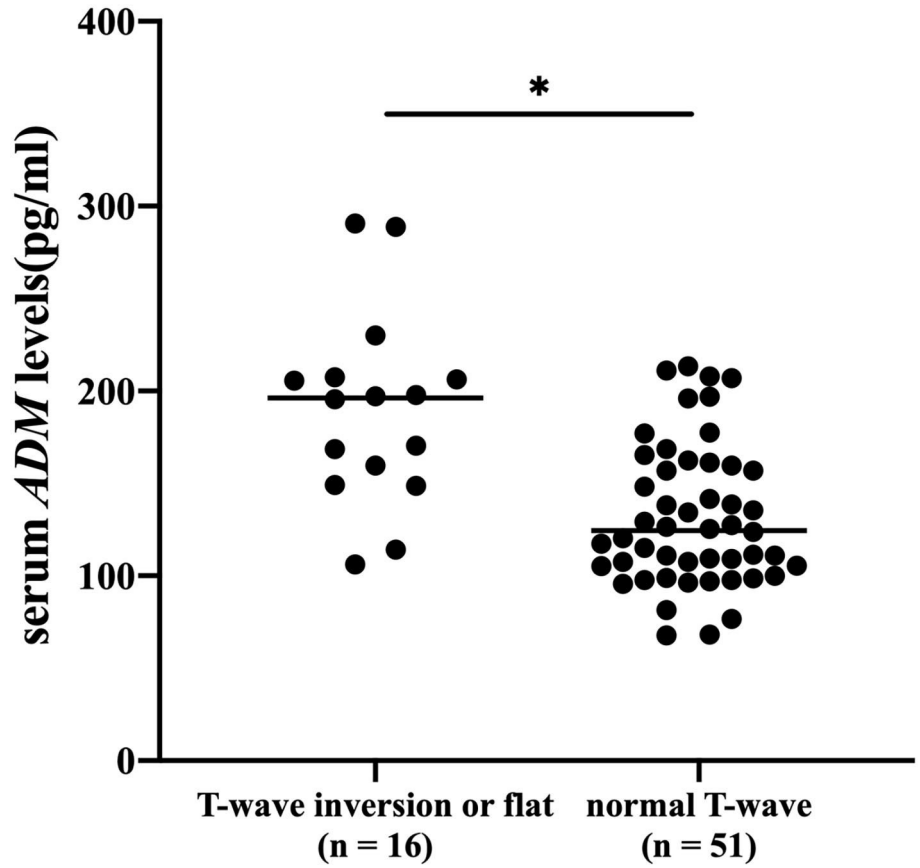

FIGURE 2 | Serum levels of adrenomedullin (ADM) (pg/ml) were measured in patients with lung cancer with or without T wave inversion or flattening. *$p < 0.05$.

of cancer-induced and anticancer-therapy induced CVDs (19). Prolonged ischemia leads to loss of heart contractility because of poor proliferation capability of myocardial cells, which consequently contributes to CVDs, including heart failure, arrhythmia, MI, etc. (20). Clinically, ECG is the most used method for monitoring myocardial ischemia. The leading ECG abnormality in myocardial ischemia is characterized by abnormal ventricular repolarization. Therefore, the QRS complex, which represents ventricular depolarization, is not the best index to diagnose ischemia. The T wave of the electrocardiogram is generated by transmural differences in action potential waveform. Under normal conditions, the T wave in human beings is usually positive except in aVR and often in V1. Causes of T wave inversion and flattening are related to abnormal depolarization. In previous studies, negative T wave in ischemic heart disease represents ischemia and is located in the subepicardial area (19). In contrast to the ST segment, acute ongoing ischemia does not cause a negative T wave. A negative or flat T wave appears when acute ongoing ischemia is vanishing or has already transitioned into chronic cardiac ischemia (21). Likewise, cancer-related or anticancer therapy-induced cardiac ischemia is a chronic process, which is consistent with pathological processes of the negative or flat T wave.

ECG signals collected from the surface of the human body inevitably suffer from EMG interference, power frequency interference, and some drugs. Therefore, the serodiagnostic method would be a better choice. ADM is a vasoactive peptide whose main functions are vasodilatation and maintaining vascular integrity and decreasing vascular leakage. A recent study has suggested that circulating ADM is a useful biomarker for the diagnosis of CVDs. It has been demonstrated that serum ADM levels were elevated in heart failure (HF) patients, and its change in response to the pathophysiologic changes of HF. After adjusting for other biomarkers, including natriuretic peptide (BNP) and atrial natriuretic peptide (ANP), etc., serum ADM remained a strong predictor of HF. A subsequent clinical study has shown a strong association between high levels of serum ADM and the prognosis of HF (22). Higher levels of ADM reflect residual tissue congestion and are independently associated with a higher risk of hospital readmissions (14, 23). Moreover, one study found elevated serum levels of ADM in patients with acute myocardial infarction (AMI). In the acute stage of AMI, circulating

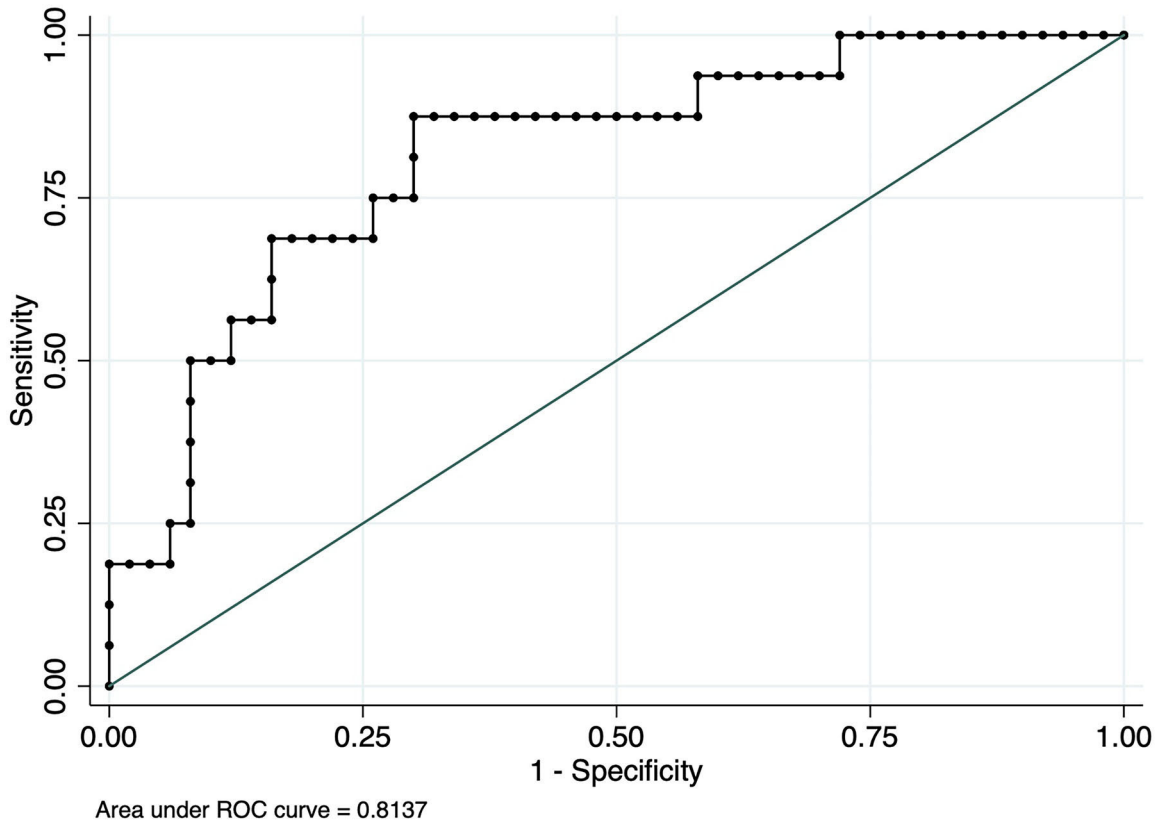

FIGURE 3 | Receiver operator characteristic (ROC) curve analysis using serum ADM to distinguish myocardial ischemia-related T wave in patients with lung cancer.

levels of ADM elevate and peak within 24 h and gradually decrease over a 3-week period (24). However, few studies have focused on ADM related to cardiac ischemia, especially among patients with cancer who face a high lifetime risk of CVDs.

In this study, we sought to find a useful biomarker for the early detection of myocardial ischemia in patients with lung cancer. By using logistics regression analysis, we adjusted for influencing factors including gender, age, history of cardiovascular and cerebrovascular diseases, lifestyle risk factors, and history of related drug therapy; the results indicated an independent positive association between serum ADM level and myocardial ischemia-related ECG change (T wave inversion or flattening) among the patients with lung cancer. Overall, our results demonstrated that patients with lung cancer with higher ADM levels have a tendency to increase the risk of myocardial ischemia compared to lung cancer patients with lower ADM levels. The current clinical guidelines do not offer specific recommendations for heart monitoring or the use of cardioprotective drugs in patients with lung cancer. Therefore, ADM may be used as a potential indicator to guide heart monitor and prevention strategies for patients with lung cancer.

CONCLUSION

Elevated serum ADM levels in patients with lung cancer are correlated with increased incidence of the negative or flat T wave, which has been implicated in chronic myocardial ischemia. ADM may be an early biomarker of lung cancer-related myocardial ischemia.

LIMITATIONS

There are few limitations to this study. First, the study, with a retrospective study design, suffered from a relatively small sample size due to single-center data and relatively strict exclusion criteria. Further large-scale studies are needed to validate the clinical utility of ADM as a practical biomarker for myocardial ischemia patients with lung cancer. In addition, this study is a cross-sectional case-control one, and prospective follow-up studies should be conducted to better assess the predictive value of biomarkers for patients with lung cancer. Third, factors leading to the occurrence of T wave inversion or flattening are very complex. Although we have taken the confounding factors as comprehensively as possible, there

would still be some other unmeasured confounders causing bias in the propensity-score-match cohort. Moreover, the type of lung cancer may also affect the incidence rate of myocardial ischemia in patients with lung cancer. We did not perform data analysis for different types of lung cancer because of insufficient subgroup data within each type of lung cancer 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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Xin Hua Hospital, Affiliated to Shanghai Jiao Tong University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-sW, Z-xW, CW, and D-wL: conception and design. FJ and Y-sW: administrative support. CW, D-wL, and Y-wJ: provision of study materials or patients. FJ, CW, D-wL, and Y-wJ: collection and assembly of data. Z-xW, CW, D-wL, and Y-wJ: data analysis and interpretation. All authors wrote the manuscript, approved the final manuscript, and have read and agreed to the published version of the manuscript.

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Coronary Atherosclerotic Disease and Cancer: Risk Factors and Interrelation

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Background: In our clinical work, we found that cancer patients were susceptible to coronary atherosclerotic heart disease (CAD). However, less is known about the relationship between CAD and cancer. The present study aimed to identify the risk factors for CAD and cancer, as well as the relationship between CAD and cancer.

Methods: In this retrospective study, 1600 patients between January 2012 and June 2019 were enrolled and divided into groups according to whether they had CAD or cancer. Single-factor and multivariate analysis methods were applied to examine the risk factors for CAD and cancer.

Results: (1) Cancer prevalence was significantly higher in patients with CAD than in patients without CAD (47.2 vs. 20.9%). The prevalence of CAD in cancer and non-cancer patients was 78.9 and 52.4%, respectively. (2) Multivariable logistic regression showed that patients with cancer had a higher risk of developing CAD than non-cancer patients (OR: 2.024, 95% CI: 1.475 to 2.778, $p < 0.001$). Respiratory (OR: 1.981, 95% CI: 1.236–3.175, $p = 0.005$), digestive (OR: 1.899, 95% CI: 1.177–3.064, $p = 0.009$) and urogenital (OR: 3.595, 95% CI: 1.696–7.620, $p = 0.001$) cancers were significantly associated with a higher risk of CAD compared with no cancer. (3) Patients with CAD also had a higher risk of developing cancer than non-CAD patients (OR = 2.157, 95% CI: 1.603 to 2.902, $p < 0.001$). Patients in the Alanine aminotransferase (ALT) level $\geq$ 40 U/L group had a lower risk of cancer than patients in the ALT level $<$ 20 U/L group (OR: 0.490, 95% CI: 0.333–0.722, $p < 0.001$). (4) An integrated variable ($Y = 0.205 \times 10^{-1}$ age $- 0.595 \times 10^{-2}$ HGB $- 0.116 \times 10^{-1}$ ALT $+ 0.135$ FIB) was identified for monitoring the occurrence of cancer among CAD patients, with an AUC of 0.720 and clinical sensitivity/specificity of 0.617/0.711.

Conclusion: (1) We discovered that CAD was an independent risk factor for cancer and vice versa. (2) Digestive, respiratory and urogenital cancers were independent risk factors for CAD. (3) We created a formula for the prediction of cancer among CAD patients. (4) ALT, usually considered a risk factor, was proven to be a protective factor for cancer in this study.

Keywords: coronary atherosclerosis, cancer, risk factors, prediction model, ALT

INTRODUCTION

With the improvement of living standards, coronary atherosclerotic heart disease (CAD) and cancer have ranked as leading causes of death worldwide (1, 2). In 2017, a total of 126.5 million people were diagnosed with ischemic heart disease (IHD), and 10.6 million new cases of IHD were registered, resulting in 8.9 million deaths (1). Likewise, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths were reported in 2020 (2). Considering the similar high morbidity and mortality of CAD and cancer, the relationship of their occurrence should be studied.

Coronary atherosclerotic heart disease and cancer were originally thought of as two separate diseases. Currently, accumulating studies have focused on the relevance of these two diseases. Cholesterol was reported to be positively associated with CAD and negatively associated with cancer, indicating a negative correlation between CAD and cancer (3), while some reports showed that colorectal cancer was positively related to CAD but with no significant difference (4). In our clinical work, we found that patients with cancer were more susceptible to CAD, and vice versa. From another point, CAD and cancer appear to share common risk factors. Male sex (5, 6), smoking (7, 8), diabetes (7, 9) and high body mass index (10, 11) have all been reported to be associated with the two diseases. All this evidence suggests a probable relationship between CAD and cancer. However, there is still a lack of large-scale studies on the relevance of CAD and cancer. Whether there are other factors involved in CAD and cancer, and whether each of these two diseases is a risk factor for the other needs further exploration.

In this retrospective study, applying single-factor and multivariate analysis methods, we analyzed the risk factors for CAD and cancer, trying to explore the answer to these questions.

MATERIALS AND METHODS

Study Sample

In this retrospective study, we used data from 1,600 inpatients at TangDu Hospital from January 2012 to June 2019. The following patients were excluded from the present study: (1) patients who had not undergone coronary angiography (CAG); (2) patients with uncertain diagnoses of CAD and cancers; and (3) patients with incomplete medical data. The definition of CAD was stenosis of the coronary artery greater than or equal to 50% by CAG. The diagnosis of cancer was based on histopathological examination. This study was approved by the TangDu Hospital Medical Ethics Committee, which waived the requirement for informed consent (approved ID: K202106-07).

Information Collection

We collected the patients' basic information, medical histories and laboratory examination data, which were obtained from the Electronic Medical Records (EMRs) and Laboratory Information Management System (LIS) of TangDu Hospital. Hypertension was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg or use of antihypertensive drugs. Diabetes was defined as fasting glucose level $\geq$ 7.0 mmol/L (126 mg/dl) or 2-h post load glucose level $\geq$ 11.0 mmol/L (200 mg/dl), or on the medications for diabetes currently. The laboratory examination included a routine blood examination, biochemical tests, coagulation tests and tumor biomarkers.

Statistics

The statistical analysis was performed by SPSS version 26.0. A "*T* test" was applied for measurement data that conformed to a normal distribution, and for this test, the data are shown as the mean $\pm$ SD. Other measurement data that did not comply with a normal distribution were subjected to the Mann–Whitney test, for which the data are described as the median (interquartile range). The Pearson $\chi 2$ test was used for enumeration data, and the Mann–Whitney test was used for ranked data. These data are expressed as n (%). Multivariable analysis was conducted through binary logistic regression. Receiver operating characteristic (ROC) analysis and Youden's index were performed to identify possible cutoff points and clinical sensitivities/specificities. The optimal coefficient of indicators was obtained by calculating the maximum value of the area under the curve (AUC) using MATLAB (version 7.0). A p-value of < 0.05 indicated statistical significance.

RESULTS

Baseline Characteristics

Baseline Characteristics of Patients With and Without Coronary Atherosclerotic Heart Disease

A total of 1,600 patients, 996 with CAD and 604 without CAD, were enrolled in this study. **Table 1** exhibits the comparisons of the baseline characteristics between patients with and without CAD. Through single-factor analysis, we concluded that the risk factors, including sex, age, smoking, drinking, and medical history of ischemic stroke, diabetes, and hypertension, were significantly correlated with CAD (all $p < 0.01$). Patients with CAD presented a higher index in glycaemia ($p < 0.001$), glycosylated hemoglobin (HbA1c) ($p < 0.001$), low-density lipoprotein-cholesterol (LDL-C) ($p = 0.007$), aspartate aminotransferase (AST) ($p < 0.001$), globulin ($p = 0.043$), direct bilirubin ($p = 0.024$), blood urea nitrogen (BUN) ($p < 0.001$), creatine (Cr) ($p < 0.001$), fibrinogen (FIB) ($p < 0.001$), and D-dimer ($p < 0.001$) than non-CAD patients. Compared with non-CAD patients, CAD patients had a lower level of red blood cell count (RBC) ($p = 0.007$), hemoglobin (HGB) ($p = 0.018$), high-density lipoprotein-cholesterol (HDL-C) ($p < 0.001$), apolipoprotein A (APOA) ($p = 0.005$), and albumin ($p < 0.001$) (**Table 1**).

As shown in **Table 1**, a challenging finding was that of all the patients we monitored in this study, 47.2% of the CAD patients had a history of cancer, and for non-CAD patients, this figure was 20.9% ($p < 0.001$). Furthermore, there was a significant difference in the distribution of cancer types between the non-CAD and CAD groups ($p < 0.001$). CAD patients had the highest prevalence of respiratory cancer (18.5%), followed

TABLE 1 | Baseline characteristics of patients with/without CAD and with/without cancer.

	Total	Non-CAD	CAD	*p*	Non-cancer	Cancer	*p*
Number	1,600	604	996		1,004	596	
Basic information							
Male gender (%)	1,062 (66.4)	318 (52.6)	744 (74.7)	< 0.001	638 (63.5)	424 (71.1)	0.002
Age (years)	60.7 ± 10.77	56.3 ± 10.47	63.4 ± 10.04	< 0.001	57.8 ± 10.88	65.7 ± 8.49	< 0.001
SBP (mmHg)	128.7 ± 16.56	126.9 ± 15.18	129.8 ± 17.26	0.001	128.2 ± 16.47	129.5 ± 16.69	0.141
DBP (mmHg)	78.0 ± 10.47	78.0 ± 10.00	78.1 ± 10.74	0.910	78.2 ± 10.68	77.8 ± 10.10	0.433
Smokers	714 (44.8)	207 (34.3)	507 (50.9)	< 0.001	413 (41.1)	301 (50.5)	< 0.001
Drinkers	290 (18.4)	52 (8.6)	238 (23.9)	< 0.001	162 (16.1)	128 (21.5)	0.008
Medical histories							
Hypertension	817 (51.1)	264 (43.7%)	553 (55.5%)	< 0.001	498 (49.6)	319 (53.7)	0.113
Diabetes	261 (16.3)	31 (5.1)	230 (23.1)	< 0.001	140 (13.9)	121 (20.3)	0.001
Ischemic stroke	119 (7.4)	29 (4.8)	90 (9.0)	0.002	69 (6.9)	50 (8.4)	0.264
CAD	996 (62.3)	–	–	–	526 (52.4)	470 (78.9)	< 0.001
Routine blood examination							
RBC (10^{12}/L)	4.43 ± 1.15	4.53 ± 1.72	4.37 ± 0.56	0.007	4.50 ± 1.38	4.30 ± 0.55	0.001
HGB (g/L)	135.24 ± 17.58	136.55 ± 16.55	134.45 ± 18.14	0.018	137.75 ± 16.69	131.02 ± 18.24	< 0.001
PLT (10^9/L)	200.01 ± 66.94	196.96 ± 63.13	201.84 ± 69.10	0.158	202.37 ± 66.77	196.04 ± 67.11	0.067
Blood biochemical tests							
Glycemia (mmol/L)	5.96 ± 1.84	5.55 ± 1.17	6.26 ± 2.15	< 0.001	5.95 ± 1.96	5.97 ± 1.59	0.881
HbA1c (%)	6.51 ± 1.57	5.83 ± 0.91	6.71 ± 1.67	< 0.001	6.36 ± 1.44	6.78 ± 1.74	0.020
TC (mmol/L)	3.90 ± 0.99	3.87 ± 0.91	3.92 ± 1.03	0.390	3.93 ± 0.92	3.83 ± 1.10	0.059
TG (mmol/L)	1.56 ± 1.04	1.54 ± 0.93	1.58 ± 1.10	0.489	1.61 ± 1.04	1.47 ± 1.02	0.018
HDL-C (mmol/L)	1.00 ± 0.25	1.04 ± 0.27	0.98 ± 0.24	< 0.001	1.01 ± 0.26	0.99 ± 0.24	0.093
LDL-C (mmol/L)	2.16 ± 0.76	2.09 ± 0.73	2.21 ± 0.78	0.007	2.19 ± 0.75	2.11 ± 0.78	0.061
APOA (mmol/L)	1.15 ± 0.25	1.20 ± 0.25	1.13 ± 0.25	0.005	1.12 ± 0.26	1.12 ± 0.23	0.062
APOB (mmol/L)	0.81 ± 0.26	0.81 ± 0.28	0.81 ± 0.26	0.985	0.82 ± 0.26	0.78 ± 0.26	0.107
AST (U/L)	25.00 (15.00)	24.00 (11.00)	26.00 (20.00)	< 0.001	26.00 (17.00)	24.00 (13.00)	0.001
ALT (U/L)	27.00 (20.00)	27.00 (19.00)	27.00 (21.00)	0.433	28.00 (21.00)	25.00 (19.00)	< 0.001
Albumin (g/L)	40.97 ± 4.07	41.86 ± 3.80	40.44 ± 4.14	< 0.001	41.43 ± 3.83	40.21 ± 4.35	< 0.001
Globulin (g/L)	26.35 ± 10.43	25.65 ± 6.21	26.76 ± 12.25	0.043	25.79 ± 6.56	27.27 ± 14.72	0.007
Total bilirubin (umol/L)	14.89 ± 9.32	15.48 ± 11.82	14.54 ± 7.42	0.055	14.97 ± 7.63	14.75 ± 11.59	0.651
Direct bilirubin (umol/L)	5.20 (3.20)	5.00 (3.00)	5.35 (3.24)	0.024	5.10 (3.17)	5.30 (3.31)	0.089
BUN (mmol/L)	5.33 ± 1.63	5.12 ± 1.51	5.46 ± 1.69	< 0.001	5.28 ± 1.60	5.42 ± 1.69	0.099
Cr (umol/L)	68.60 ± 27.49	64.83 ± 16.26	70.86 ± 32.22	< 0.001	67.86 ± 31.70	69.82 ± 18.41	0.169
Coagulation tests							
FIB (g/L)	2.83 ± 0.89	2.60 ± 0.70	2.97 ± 0.97	< 0.001	2.68 ± 0.80	3.08 ± 0.99	< 0.001
D-Dimer (ug/mL)	0.46 (0.51)	0.41 (0.46)	0.50 (0.56)	< 0.001	0.43 (0.47)	0.53 (0.61)	< 0.001
Tumor markers							
CA724 (U/mL)	1.70 (2.72)	1.49 (4.42)	1.77 (2.49)	0.552	1.21 (1.74)	1.80 (2.92)	0.063
CEA (ng/mL)	2.67 (2.84)	2.28 (1.79)	2.86 (3.60)	< 0.001	2.03 (1.14)	2.79 (3.25)	< 0.001
AFP (ng/mL)	2.46 (1.63)	2.63 (2.03)	2.34 (1.67)	0.014	2.48 (1.70)	2.45 (1.64)	0.970
Ferritin (ug/L)	158.00 (181.27)	187.00 (171.90)	149.50 (187.98)	0.072	190.80 (221.60)	154.60 (180.01)	0.179
NSE (ng/mL)	13.37 (7.07)	13.10 (7.64)	13.43 (6.94)	0.924	11.57 (3.77)	13.80 (7.37)	0.013
CA19-9 (U/mL)	11.88 (12.65)	9.72 (9.46)	12.90 (12.92)	0.030	8.54 (7.62)	12.73 (12.98)	0.010
CA125 (U/mL)	13.44 (12.71)	12.17 (10.93)	13.75 (12.91)	0.236	9.91 (7.13)	13.92 (13.31)	< 0.001
CA153 (U/mL)	11.99 (8.62)	11.24 (4.88)	12.43 (9.91)	0.227	11.53 (10.81)	12.02 (7.77)	0.837
CYFRA21-1 (ng/mL)	3.05 (2.99)	2.55 (2.43)	3.14 (3.70)	0.008	2.42 (1.89)	3.14 (3.59)	0.007
CA50 (U/mL)	8.03 (6.44)	8.03 (6.76)	8.02 (6.58)	0.733	7.39 (3.16)	8.04 (6.72)	0.439
SCC (ng/mL)	0.48 (0.24)	0.46 (0.24)	0.50 (0.24)	0.858	0.51 (0.10)	0.48 (0.24)	0.491
Cancer	596 (37.3)	126 (20.9)	470 (47.2)	< 0.001	–	–	–
Cancer types				< 0.001			
Respiratory system[a]	230 (14.4)	46 (7.6)	184 (18.5)				
Digestive system[b]	220 (13.8)	46 (7.6)	174 (17.5)				
Urogenital system[c]	84 (5.3)	12 (2.0)	72 (7.2)				
Superficial organ[d]	62 (3.9)	22 (3.6)	40 (4.0)				

[a] *Respiratory system cancer includes nasopharyngeal and pulmonary cancers.*

[b] *Digestive system cancer includes esophageal, gastric, colon, liver, gallbladder and pancreatic cancers.*

[c] *Urogenital system cancer includes uterine, ovarian, renal, ureteral, bladder, prostatic and penile cancers.*

[d] *Superficial organ cancer includes thyroid, breast and other superficial organ cancers.*

ALT, alanine aminotransferase; AFP, alpha fetoprotein; APOA/APOB, apolipoprotein A/B; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CAD, coronary atherosclerotic disease; CEA, carcinoembryonic antigen; Cr, creatine; CYFRA21-1, cytokeratin 19 fragment; DBP/SBP, diastolic/systolic blood pressure; FIB, fibrinogen; HbA1c, glycosylated hemoglobin; HDL/LDL-C, high/low-density lipoprotein-cholesterol; HGB, hemoglobin; NSE, neuron specific enolase; PLT, platelet; SCC, squamous cell carcinoma antigen; TC, total cholesterol and TG, triglyceride.

Measurement values that conform to normal distribution are described as mean ± SD, others as median (interquartile range). Enumeration data was expressed as n (%).

by digestive (17.5%), urogenital (7.2%), and superficial organ (4.0%) cancers. Patients without CAD had the highest prevalence of respiratory and digestive cancer (both 7.6%), followed by superficial organ (3.6%), and urogenital (2.0%) cancer. Our analysis also proved that patients with CAD had a higher level of carcinoembryonic antigen (CEA) ($p < 0.001$), CA19-9 ($p = 0.030$) and cytokeratin 19 fragment (CYFRA21-1) ($p = 0.008$) than non-CAD patients (**Table 1**).

Baseline Characteristics of Patients With and Without Cancer

The study subjects included 596 cancer patients and 1,004 non-cancer patients. **Table 1** exhibits the comparisons of the baseline characteristics between patients with and without cancer. The single-factor analysis revealed that factors, including sex, age, smoking, drinking and medical history of diabetes, were significantly different ($p < 0.01$) between the two groups. Patients with cancer showed a higher index in HbA1c ($p = 0.020$), globulin ($p = 0.007$), FIB ($p < 0.001$), D-dimer ($p < 0.001$), CEA ($p < 0.001$), neuron specific enolase (NSE) ($p = 0.013$), CA19-9 ($p = 0.010$), CA125 ($p < 0.001$), CYFRA21-1 ($p = 0.007$), but a lower level of RBC ($p = 0.001$), HGB ($p < 0.001$), triglyceride (TG) ($p = 0.018$), AST ($p = 0.001$), alanine aminotransferase (ALT) ($p < 0.001$) and albumin ($p < 0.001$) than patients without cancer. Surprisingly, of all the samples we analyzed, 78.9% of the cancer patients had a history of CAD, and for non-cancer patients, this figure was 52.4% ($p < 0.001$) (**Table 1**).

Baseline Characteristics of Patients With or Without Coronary Atherosclerotic Heart Disease/Cancer

Table 2 summarizes the comparisons of the baseline characteristics of patients in CAD-/cancer- ($n = 478$), CAD-/cancer + ($n = 126$), CAD + /cancer- ($n = 526$) and CAD + /cancer + groups ($n = 470$). The CAD + /cancer + group exhibited an older age than the CAD-/cancer- (66.6 ± 8.41 vs. 54.7 ± 10.47), CAD-/cancer + (66.6 ± 8.41 vs. 62.5 ± 8.02) and CAD + /cancer- (66.6 ± 8.41 vs. 60.6 ± 10.52) groups (all $p < 0.001$). Lower levels of albumin and higher levels of FIB and D-dimer were shown in the CAD + /cancer + group than the CAD-/ cancer-, CAD-/cancer + and CAD + /cancer- groups (all $p < 0.05$).

Multivariate Analysis for Risk Factors for Coronary Atherosclerotic Heart Disease

In this study, multivariate logistic regression analysis was performed to assess factors related to CAD, with CAD as the dependent variable and factors including age, sex, drinking, smoking, HGB, serum lipids (total cholesterol (TC), TG, HDL-C, LDL-C), liver function indexes (AST, ALT, direct bilirubin, albumin and globulin), Cr, BUN, D-dimer, fibrinogen, and history of hypertension, diabetes, stroke and cancers as the independent variables. The data obtained in the process of multivariate analyses proved a strong association between CAD and the following characteristics: male (OR: 2.558, 95% CI: 1.812–3.610, $p < 0.001$), age $\geq$ 45 years (45–60 years: OR: 2.010; 60–75 years: OR: 3.834; $\geq$ 75 years: OR: 9.791; all $p < 0.05$), hypertension (OR: 1.464, 95% CI: 1.114–1.924, $p = 0.006$), diabetes (OR: 4.879, 95% CI: 3.052–7.800, $p < 0.001$), drinking (OR: 3.333, 95% CI: 2.186–5.082, $p < 0.001$), an LDL-C level $\geq$ 1.8 mmol/L (1.8–2.6 mmol/L: OR: 1.382; $\geq$ 2.6 mmol/L: OR: 1.641; all $p < 0.05$), an AST level $\geq$ 40 U/L (OR: 3.053, 95% CI: 1.976–4.716, $p < 0.001$), an FIB level $\geq$ 3.5 g/L (OR: 2.599, 95% CI: 1.631–4.143, $p < 0.001$), and an HGB level $\geq$ 131 g/L (OR: 0.636, 95% CI: 0.455–0.889, $p = 0.008$) (**Table 3**).

Importantly, an interesting finding was that patients with cancer were particularly vulnerable to CAD (OR: 2.024, 95% CI: 1.475–2.778, $p < 0.001$). Respiratory, digestive and urogenital cancers were significantly associated with a higher risk of CAD compared with no cancer (OR: 1.981, 95% CI: 1.236–3.175, $p = 0.005$; OR: 1.899, 95% CI: 1.177–3.064, $p = 0.009$; OR: 3.595, 95% CI: 1.696–7.620, $p = 0.001$), after adjustment for age, sex, drinking, smoking, history of hypertension, diabetes and stroke and HGB, TC, TG, HDL-C, LDL-C, AST, ALT, direct bilirubin, albumin, globulin, Cr, BUN, D-dimer and FIB levels. The multivariate logistic regression results are visualized in **Table 3**.

Multivariate Analysis for Risk Factors for Cancer

Then, a multivariate logistic regression method was used to analyze the factors related to cancer, with cancer as the dependent variable and factors including age, sex, drinking, smoking, HGB, serum lipids (TC, TG, HDL-C, LDL-C), liver function indexes (AST, ALT, albumin, globulin), D-dimer, FIB, and history of diabetes and CAD as the independent variables. Through the multivariate analyses, we found that age (45–60 years: OR: 5.359; 60–75 years: OR: 15.193; $\geq$ 75 years: OR = 18.179; all $p \leq 0.001$), smoking (OR: 1.651, 95% CI: 1.247–2.184, $p < 0.001$), an FIB levels (2.5–3.5 g/L: OR: 1.354; $\geq$ 3.5 g/L: OR: 1.926; all $p < 0.05$) were positively correlated with cancer, while HGB levels (OR: 0.743, 95% CI: 0.560–0.986, $p = 0.040$) was negatively related to cancer. There was also a significant difference in the incidence of cancer between the ALT level $\geq$ 40 U/L group and the ALT level < 20 U/L group (OR: 0.490, 95% CI: 0.333–0.722, $p < 0.001$). And it is worth noticing that CAD patients were more prone to suffer from cancer compared to those without CAD (OR: 2.157, 95% CI: 1.603–2.902, $p < 0.001$) (**Table 4**).

Multivariate Analysis for Risk Factors for Combined Coronary Atherosclerotic Heart Disease and Cancer

The risk factors for combined CAD and cancer were determined by multivariate logistic regression, as shown in **Table 5**. The CAD + /cancer + group was compared with the CAD-/ cancer-, CAD-/cancer + and CAD + /cancer- groups, respectively. After adjustment for age, sex, history of hypertension, diabetes and ischemic stroke, drinking, smoking, serum lipids (TC, TG, HDL-C, LDL-C), liver function indexes (AST, ALT, albumin, globulin), Cr, BUN and D-dimer, an FIB level $\geq$ 2.5 g/L was positively

TABLE 2 | Baseline characteristics of patients in CAD–/ cancer–, CAD-/cancer + , CAD + /cancer– and CAD + /cancer + groups.

	CAD-/cancer-	CAD-/cancer +	CAD + /cancer-	CAD + /cancer +	p1*	p2#	p3†
Number	478	126	526	470			
Basic information							
Male gender (%)	251 (52.5)	67 (53.2)	387 (73.6)	357 (76.0)	< 0.001	<0.001	0.388
Age (years)	54.7 ± 10.47	62.5 ± 8.02	60.6 ± 10.52	66.6 ± 8.41	< 0.001	<0.001	< 0.001
SBP (mmHg)	127.0 ± 15.35	126.8 ± 14.58	129.4 ± 17.36	130.2 ± 17.15	0.002	0.039	0.445
DBP (mmHg)	78.1 ± 10.02	77.6 ± 9.93	78.3 ± 11.25	77.8 ± 10.15	0.661	0.864	0.483
Smokers	158 (33.1)	49 (38.9)	255 (48.5)	252 (53.6)	< 0.001	0.003	0.168
Drinkers	35 (7.3)	17 (13.5)	127 (24.1)	111 (23.6)	< 0.001	0.011	0.793
Medical histories							
Hypertension	211 (44.1)	53 (42.1)	287 (54.6)	266 (56.6)	< 0.001	0.003	0.471
Diabetes	28 (5.9)	3 (2.4)	112 (21.3)	118 (25.1)	< 0.001	<0.001	0.154
Ischemic stroke	14 (2.9)	15 (11.9)	55 (10.5)	35 (7.4)	0.002	0.109	0.098
Routine blood examination							
RBC (10^{12}/L)	4.57 ± 1.92	4.38 ± 0.47	4.44 ± 0.55	4.28 ± 0.57	0.002	0.050	< 0.001
HGB (g/L)	137.27 ± 16.33	133.83 ± 17.12	138.19 ± 17.00	130.27 ± 18.47	< 0.001	0.051	< 0.001
PLT (10^9/L)	200.22 ± 61.83	184.67 ± 66.65	204.31 ± 70.93	199.08 ± 66.97	0.786	0.032	0.233
Blood biochemical tests							
Glycemia (mmol/L)	5.57 ± 1.23	5.46 ± 0.93	6.37 ± 2.46	6.13 ± 1.72	< 0.001	<0.001	0.114
HbA1c (%)	5.83 ± 0.91	5.85 ± 0.96	6.56 ± 1.56	6.91 ± 1.79	< 0.001	0.001	0.085
TC (mmol/L)	3.91 ± 0.90	3.72 ± 0.94	3.96 ± 0.95	3.86 ± 1.14	0.518	0.263	0.149
TG (mmol/L)	1.59 ± 0.98	1.32 ± 0.60	1.63 ± 1.09	1.51 ± 1.11	0.300	0.109	0.118
HDL-C (mmol/L)	1.05 ± 0.28	1.02 ± 0.23	0.98 ± 0.25	0.98 ± 0.24	< 0.001	0.120	0.930
LDL-C (mmol/L)	2.11 ± 0.73	2.01 ± 0.74	2.26 ± 0.77	2.14 ± 0.79	0.674	0.151	0.024
APOA (mmol/L)	1.20 ± 0.26	1.19 ± 0.22	1.14 ± 0.26	1.11 ± 0.23	0.003	0.077	0.200
APOB (mmol/L)	0.82 ± 0.28	0.76 ± 0.25	0.82 ± 0.25	0.79 ± 0.27	0.339	0.576	0.185
AST (U/L)	24 (11)	25 (12)	27 (25)	23 (14)	0.947	0.365	< 0.001
ALT (U/L)	27 (21)	26 (21)	29 (23)	24 (18)	0.003	0.121	< 0.001
Albumin (g/L)	42.01 ± 3.70	41.30 ± 4.13	40.91 ± 3.86	39.92 ± 4.37	< 0.001	0.002	< 0.001
Globulin (g/L)	25.42 ± 6.22	26.51 ± 6.11	26.12 ± 6.84	27.47 ± 16.27	0.012	0.523	0.087
Total bilirubin (umol/L)	14.87 ± 7.32	14.06 (8.45)	13.39 (7.82)	12.60 (7.86)	0.050	0.029	0.037
Direct bilirubin (umol/L)	5.35 ± 3.23	5.40 (3.64)	5.40 (3.22)	5.30 (3.30)	0.126	0.617	0.982
BUN (mmol/L)	5.06 ± 1.42	5.33 ± 1.78	5.47 ± 1.72	5.44 ± 1.67	< 0.001	0.536	0.753
Cr (umol/L)	64.38 ± 16.43	66.54 ± 15.55	70.99 ± 40.59	70.71 ± 19.02	< 0.001	0.025	0.889
Coagulation tests							
FIB (g/L)	2.52 ± 0.65	2.89 ± 0.80	2.83 ± 0.89	3.13 ± 1.02	< 0.001	0.013	< 0.001
D-Dimer (ug/mL)	0.40 (0.44)	0.44 (0.48)	0.47 (0.51)	0.55 (0.64)	< 0.001	0.001	< 0.001

**p1 represents comparison between the CAD-/cancer- and CAD + /cancer + groups.*
#p2 represents comparison between the CAD-/cancer + and CAD + /cancer + groups.
†p3 represents comparison between the CAD + /cancer- and CAD + /cancer + groups.
ALT, alanine aminotransferase; AFP, alpha fetoprotein; APOA/APOB, apolipoprotein A/B; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CAD, coronary atherosclerotic disease; Cr, creatine; DBP/SBP, diastolic/systolic blood pressure; FIB, fibrinogen; HbA1c, glycosylated hemoglobin; HDL/LDL-C, high/low-density lipoprotein-cholesterol; HGB, hemoglobin; PLT, platelet; TC, total cholesterol and TG, triglyceride.
Measurement values that conform to normal distribution are described as mean ± SD, others as median (interquartile range). Enumeration data was expressed as n (%).

associated with combined CAD and cancer, while an HGB level ≥ 131 g/L was significantly associated with a low risk of combined CAD and cancer.

THE FORMULA PREDICTION FOR CANCER

Our data showed that CAD was closely related to cancer. We explored a way to monitor the occurrence of cancer among CAD patients. Having confirmed that age and HGB, ALT and FIB levels were independent risk factors for cancer, we tried to give them weighting coefficients and generate a dependent variable Y through MATLAB software (version 7.0):

$$Y = 0.205 \times 10^{-1} \text{ age} - 0.595 \times 10^{-2} \text{ HGB} - 0.116 \times 10^{-1} \text{ ALT} + 0.135 \text{ FIB}$$

We calculated Youden's index to identify the optimal cutoff point, which was 0.6044, with a clinical sensitivity/specificity of 0.617/0.711, respectively. The maximum area under the curve (AUC) was 0.720 (95% CI, 0.688–0.752, **Figure 1**).

DISCUSSION

Coronary atherosclerotic heart disease and cancer share similar epidemiological characteristics. And in our clinical work, we found a very interesting phenomenon in which patients who had been diagnosed with cancer were more susceptible to

TABLE 3 | Logistic regression analysis for influencing factors for CAD.

	OR	95% CI	*p*
Gender: Female	1		
Male	2.558	1.812–3.610	< 0.001
Age (years) < 45	1		
45–60	2.010	1.211–3.336	0.007
60–75	3.834	2.257–6.515	< 0.001
>75	9.791	4.524–21.192	< 0.001
Hypertension	1.464	1.114–1.924	0.006
Diabetes	4.879	3.052–7.800	< 0.001
Cancer	2.024	1.475–2.778	< 0.001
Cancer types			
No cancer	1		
Respiratory system [a]	1.981	1.236–3.175	0.005
Digestive system [b]	1.899	1.177–3.064	0.009
Urogenital system [c]	3.595	1.696–7.620	0.001
Superficial organ [d]	1.407	0.696–2.843	0.342
Drinkers	3.333	2.186–5.082	< 0.001
HGB (g/L) ≥ 131	0.636	0.455–0.889	0.008
LDL-C (mmol/L) < 1.8	1		
1.8–2.6	1.382	1.005–1.902	0.047
≥2.6	1.641	1.148–2.345	0.007
AST (U/L) < 20	1		
20–40	0.999	0.734–1.358	0.993
≥40	3.053	1.976–4.716	< 0.001
FIB (g/L) < 2.5	1		
2.5–3.5	1.301	0.972–1.742	0.077
≥3.5	2.599	1.631–4.143	< 0.001

[a] *Respiratory system cancer includes nasopharyngeal and pulmonary cancers.*
[b] *Digestive system cancer includes esophageal, gastric, colon, liver, gallbladder and pancreatic cancers.*
[c] *Urogenital system cancer includes uterine, ovarian, renal, ureteral, bladder, prostatic and penile cancers.*
[d] *Superficial organ cancer includes thyroid, breast and other superficial organ cancers.*
AST, aspartate aminotransferase; BP, blood pressure; CAD, coronary atherosclerotic disease; FIB, fibrinogen and HGB, hemoglobin.

TABLE 4 | Logistic regression analysis for influencing factors for cancer.

	OR	95% CI	*p*
Age (years) < 45	1		
45–60	5.359	1.908–15.048	0.001
60–75	15.193	5.434–42.478	< 0.001
≥75	18.179	6.053–54.599	< 0.001
CAD	2.157	1.603–2.902	< 0.001
Smokers	1.651	1.247–2.184	< 0.001
HGB (g/L) ≥ 131	0.743	0.560–0.986	0.040
ALT (U/L) < 20	1		
20–40	0.849	0.630–1.143	0.279
≥40	0.490	0.333–0.722	< 0.001
FIB (g/L) < 2.5	1		
2.5–3.5	1.354	1.013–1.809	0.041
≥3.5	1.926	1.315–2.821	0.001

ALT, alanine aminotransferase; CAD, coronary atherosclerotic disease; FIB, fibrinogen and HGB, hemoglobin.

CAD. Therefore, we tried to determine whether there is any relationship between CAD and cancer. We reviewed the raw data of 1,600 patients with/without CAD and cancer in Tangdu Hospital, which led us to the following findings. (1) We discovered that CAD was an independent risk factor for cancer and vice versa. (2) Digestive, respiratory and urogenital cancers were independent risk factors for CAD. (3) We created a formula for the prediction of

TABLE 5 | Logistic regression analysis for influencing factors for combined CAD and cancer.

	Model 1*		Model 2#		Model 3†	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Age (years) < 45	1		1		1	
45–60	8.164 (2.203–30.252)	0.002	0.589 (0.057–6.049)	0.656	3.626 (1.061–12.389)	0.040
60–75	41.179 (11.011–153.998)	< 0.001	0.598 (0.060–5.996)	0.662	8.256 (2.437–27.967)	0.001
>75	90.253 (20.792–391.762)	< 0.001	4.862 (0.324–72.907)	0.252	12.362 (3.399–44.964)	< 0.001
HGB (g/L) ≥ 131	0.475 (0.298–0.758)	0.002	0.414 (0.231–0.741)	0.003	0.618 (0.441–0.865)	0.005
FIB (g/L) < 2.5	1		1		1	
2.5–3.5	1.751 (1.135–2.702)	0.011	1.904 (1.071–3.385)	0.028	1.508 (1.058–2.150)	0.023
≥3.5	5.054 (2.651–9.634)	< 0.001	3.025 (1.380–6.631)	0.006	2.038 (1.317–3.152)	0.001

**Model 1 represents comparison between the CAD-/cancer- and CAD + /cancer + groups.*
#Model 2 represents comparison between the CAD-/cancer + and CAD + /cancer + groups.
†Model 3 represents comparison between the CAD + /cancer- and CAD + /cancer + groups.
CAD, coronary atherosclerotic disease; FIB, fibrinogen and HGB, hemoglobin.

cancer among CAD patients. (4) ALT, usually considered a risk factor, was proven to be a protective factor for cancer in this study.

To the best of our knowledge, this was the first study to verify that CAD and cancer are independent risk factors for each other. CAD and cancer were originally thought of as two separate diseases. Currently, accumulating studies have focused on the relevance of these two diseases. In the 1980s and 1990s, Smith GD and Kagan A reported that the mortality and morbidity of CAD increased with increasing cholesterol concentration, while the mortality and morbidity of cancer showed an inverse trend with cholesterol levels (3, 12). These early epidemiological studies may indicate a negative correlation between the two diseases. With the study of the relationship between them, this view has changed greatly. The most recent studies showed that the risk of recurrence and cancer-specific death in patients with early cardiovascular events after cancer diagnosis has increased. In addition, a number of studies have shown that aspirin and statins, the drugs that benefit CAD patients, also have protective effects on patients with cancer (13–15). All these studies may suggest a possible connection between cancer and CAD. Our study creatively pointed out that CAD and cancer are independent risk factors for each other. Then, we explored the distribution of cancer in patients with or without CAD to determine which specific kind of cancer makes patients more susceptible to CAD. Several studies have involved this question, but have not offered a firm conclusion for that. In 2006 and 2007, Chan et al. delivered two publications reporting that patients with colorectal neoplasms suffered a higher prevalence of CAD than patients without colorectal neoplasms, while colorectal cancer was not significantly associated with CAD by multivariate logistic regression (4, 16). In 2021, a retrospective study showed that lung, colorectal, gastric, breast, and thyroid cancer patients suffered from a relatively high prevalence of CVD, but this study did not include non-cancer patients (17). In our study, respiratory system, digestive system and urogenital system cancers were significantly associated with a higher risk of CAD compared with no cancer, with ORs of 1.981, 1.899 and 3.595, respectively (all $p < 0.01$), providing evidence for enhancing the prevention and screening of CAD in these patients.

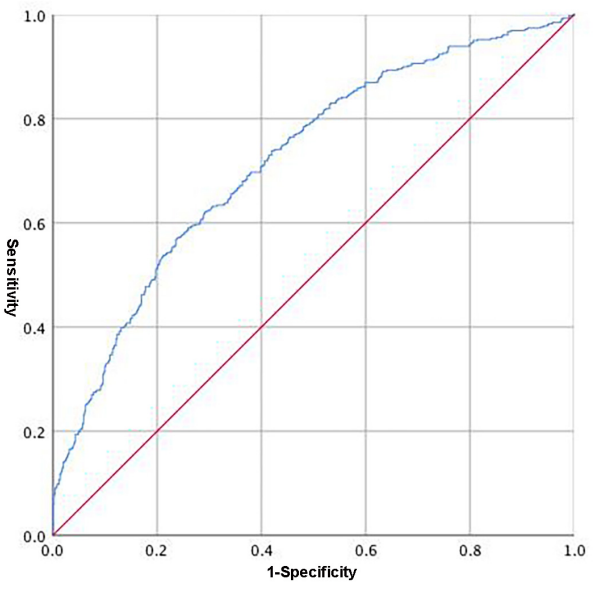

FIGURE 1 | Receiver operating characteristic (ROC) of the integrated variable ($Y = 0.205 \times 10^{-1}$ age $- 0.595 \times 10^{-2}$ HGB $- 0.116 \times 10^{-1}$ ALT + 0.135 FIB) predicting the occurrence of cancer among CAD patients.

Coronary atherosclerotic heart disease is mostly attributed to the occurrence of cardiovascular risk factors (e.g., hypertension, diabetes, smoking, age, hypercholesterinemia, and adiposity) (18). These are not solely risk factors for cardiovascular diseases, but also increase the risk of cancer (19). Our study applied single-factor and multivariate logistic analyses and found that CAD, cancer and combined CAD and cancer shared a common risk factor, FIB levels (20, 21), and a common protective factor, HGB levels (22, 23). The phenomenon that CAD and cancer

possess various similarities and interactions suggests a shared biology for the two diseases. Inflammation appears to be a major unifying factor in the etiology and progression of these diseases (24, 25). Common conditions such as a high level of FIB induce inflammation (26), which may, in part, explain why CVD and cancer share several similarities. Except for the common influencing factors, we also concluded that sex, age (7), diabetes (27), drinking (28) and AST levels (29) were risk factors for CAD, and age (30) and smoking (8) were risk factors for cancer, which is similar to the findings of previous studies. ALT levels were found to be significantly higher in patients with cancer (31), and elevated levels of ALT were reported to be correlated with death in cancer patients (32). This study showed an opposite result, with the conclusion that ALT is a protective factor for cancer. However, the underlying mechanism is unclear. We need larger-scale studies to further clarify this.

Cancer is usually diagnosed at an advanced stage, leading to a poor prognosis. The reason is that there are no specific symptoms or effective diagnostic means in the early stage of cancer. Patients with CAD were more susceptible to cancer than non-CAD patients. Therefore, we attempted to predict the probability of cancer in CAD patients. The traditional method of most cancer diagnoses is tissue biopsy or imaging examination (33, 34). However, these are invasive, demanding, and time-consuming. A biomarker for cancer prediction would have tremendous clinical benefits in reducing the rate of invasive procedures, the time to diagnosis and costs. Therefore, study intended to use the most common items of blood examinations to address these problems. We gave a specific weighting coefficient to independent influencing factors of cancer(including age and HGB, ALT and FIB levels) and obtained an integrated parameter named Y, with a clinical sensitivity/specificity of 0.617/0.711, respectively: $Y = 0.205 \times 10^{-1}$ age $- 0.595 \times 10^{-2}$ HGB $- 0.116 \times 10^{-1}$ ALT + 0.135 FIB. The study proposed a new non-invasive method for the prediction of cancer among CAD patients. We still need more data to demonstrate the accuracy and effectiveness of this formula. This is the direction of our future clinical work and research.

Limitations

The present study has several limitations. First, as a retrospective study, some laboratory examinations, such as the tumor biomarkers of the patients, were incomplete. Further cohort studies are needed to verify the conclusions of this study. Second, this was a descriptive study of patients admitted to a single center; therefore, this study is not representative of the entire population. Finally, the sample size was relatively small. Subsequent studies are needed to provide additional explanations.

CONCLUSION

(1) We discovered that CAD was an independent risk factor for cancer and vice versa. (2) Digestive, respiratory and urogenital cancers were independent risk factors for CAD. (3) We created a formula for the prediction of cancer among CAD patients. (4) ALT, usually considered a risk factor, was proven to be a protective factor for cancer in this study.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Tangdu Hospital of the Fourth Military Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JJL, XL, and LS designed the study and drafted the manuscript. MC, XW, JL, and XY extracted and verified the data. JZ, YL, RC, and LS analyzed the data. JZ, YC, and PL revised the manuscript. JJL and XL incorporated comments from the co-authors and finalized the manuscript. All authors approved the final version of the paper.

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SYSTEMATIC REVIEW
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Higher Mortality Associated With New-Onset Atrial Fibrillation in Cancer Patients: A Systematic Review and Meta-Analysis

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Aim: This research was conducted to evaluate the mortality outcome of cancer patients with new-onset atrial fibrillation. We also aimed to assess if there was any confounding relation between the mortality of these patients and surgical intervention.

Materials and Methods: A systemic search was conducted from electronic databases (PubMed/Medline, Cochrane Library, and Google Scholar) from inception to 7 February 2022. All statistical analyses were conducted in Review Manager 5.4.1. Studies meeting inclusion criteria were selected. Only those studies that involved cancer patients without pre-existing atrial fibrillation were selected, and mortality rate was compared between the patients who developed atrial fibrillation and those who did not. A random-effect model was used when heterogeneity was seen to pool the studies, and the result was reported in the odds ratio (OR) and the corresponding 95% confidence interval (CI).

Results: Eighteen studies were selected for meta-analysis. Statistical analysis showed that the cancer patients who subsequently developed atrial fibrillation had a significantly higher mortality rate as compared to those who did not (OR = 1.90 [1.65, 2.19]; $p < 0.00001$; $I^2 = 100\%$). We also separately analyzed the mortality risk in the surgery group and the non-surgery group. Statistical analysis showed that there was significantly higher mortality rate associated with new-onset atrial fibrillation in cancer patients in the surgery group (OR= 3.68 [2.29, 5.94]; $p < 0.00001$; $I^2 = 61\%$) as well as in the non-surgery group (OR = 1.64 [1.39, 1.93]; $p < 0.00001$; $I^2 = 100\%$).

Conclusion: Cancer patients, who subsequently developed atrial fibrillation, had a higher mortality rate as compared to those cancer patients who did not develop atrial fibrillation. A higher mortality rate was seen in both surgical and non-surgical subgroups. This implies that extra care and specific measures must be taken in the management of cancer patients with new-onset atrial fibrillation.

Keywords: new-onset atrial fibrillation, cancer, mortality, cardio-oncology, meta-analysis

INTRODUCTION

Cancer is among the most terrible diseases in the world, and its incidence is constantly on the rise (1). Considering the prevalence of known risk factors such as aging, family history, obesity, and radiation exposure, and increased adoption of unhealthy lifestyles such as tobacco smoking, alcohol, physical inactivity, and unhealthy diet, the rate of occurrence of almost all types of cancer is expected to further increase (2).

Lung cancer and breast cancer were found to be the most frequently diagnosed cancers and the leading cause of death among males and females, respectively, in 2012. Esophageal cancer notably varies in incidence rates internationally, and is found to be higher in men. Colorectal cancer is the third most frequently diagnosed cancer in males, and the second-most in females. Non-Hodgkin lymphoma is more common in more developed areas, with the highest incidence rates found in Australia, Western and Northern Europe, and Northern America (2).

Atrial fibrillation is the most frequently encountered cardiac arrhythmia that has great clinical importance. It causes significant morbidity and mortality by increasing the incidence of cardiomyopathies and their subsequent complications (3). It is well established that cancer patients undergoing surgery have an increased risk of developing atrial fibrillation in the perioperative and postoperative periods (4, 5).

In a meta-analysis conducted by Yuan et al., it was demonstrated that there is a higher risk of developing atrial fibrillation in cancer patients as compared to non-cancer patients. Cancer patients have a 47% higher risk of atrial fibrillation (6). The risk of atrial fibrillation varies in different types of cancer. For instance, colorectal cancer patients have a 54% higher risk and breast cancer patients have double the risk of developing atrial fibrillation as compared to people with no cancer (6).

Conversely, an increased risk of subsequent diagnosis of cancer in patients with atrial fibrillation has also been reported (7).

It has been found that cancer-related atrial fibrillation occurs more often after cancer surgery. Many risk factors have been implicated in the development of postoperative atrial fibrillation. These include advanced age, male gender, and advanced cancer stage (8). Moreover, a study reported 19.6% incidence rate of postoperative atrial fibrillation in patients undergoing an operation for malignant pulmonary disease, compared to the 3.1% incidence rate in those operated for benign pulmonary disease. This suggests that atrial fibrillation is not merely a complication of surgery, but it has a strong link with cancer itself (9).

In the case of surgical patients, pre-operative cardiac symptoms and echocardiogram (ECG) abnormalities, operative parameters, and post-operative clinical findings may be responsible for the development of atrial fibrillation, but their causal role could not be demonstrated (9).

Cancer could not be demonstrated as an independent predictor of atrial fibrillation. However, the elevated levels of C-reactive protein (CRP) associated with cancer, and the remodeling of the atrial structure due to the presence of an inflammatory state suggest inflammation as the causal intermediary link between the two (10).

Increased incidence of atrial fibrillation in cancer patients may also be related to autonomic disturbances, atrial inflammation due to autoimmune paraneoplastic syndromes, or cancer therapy (10, 11).

The previous meta-analysis conducted by Yuan et al. determined the relationship between cancer and the risk of developing atrial fibrillation, but it did not evaluate the impact of atrial fibrillation on cancer mortality (6). There has been inconsistent evidence on whether the new-onset atrial fibrillation in cancer patients significantly affects mortality outcomes. Therefore, this systematic review and meta-analysis was conducted to establish a conclusive relationship between new-onset atrial fibrillation and mortality in cancer patients.

TABLE 1 | Search strategy.

Search engine	Search strategy
Pubmed/medline	("cancer*"[All Fields] OR ("carcinoma*"[All Fields] AND "malignanc*"[All Fields])) AND ("atrial fibrillation"[MeSH Terms] OR ("atrial"[All Fields] AND "fibrillation"[All Fields]) OR "atrial fibrillation"[All Fields] OR "AF"[All Fields] OR "a-fibrillation"[All Fields]) AND ("mortality"[MeSH Terms] OR "mortality"[All Fields] OR "mortalities"[All Fields] OR "mortality"[MeSH Subheading] OR ("death"[MeSH Terms] OR "death"[All Fields] OR "deaths"[All Fields]) OR ("mortality"[MeSH Subheading] OR "mortality"[All Fields] OR "survival"[All Fields] OR "survival"[MeSH Terms] OR "survivability"[All Fields] OR "survivable"[All Fields] OR "survivals"[All Fields] OR "survive"[All Fields] OR "survived"[All Fields] OR "survives"[All Fields] OR "surviving"[All Fields]))
Cochrane	(cancer* OR carcinoma* malignanc*) AND (atrial fibrillation OR AF OR a-fibrillation) AND (mortality OR death OR survival)
Google scholar	(cancer* OR carcinoma* malignanc*) AND (atrial fibrillation OR AF OR a-fibrillation) AND (mortality OR death OR survival)

MATERIALS AND METHODS

Data Sources and Search Strategy

This systematic review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines (12). An electronic search from PubMed/Medline, Cochrane Library, and Google Scholar was conducted from their inception to 7 February 2022 (detailed strategy is provided in **Table 1**), with only English language-based literature, using the search string: (cancer* OR carcinoma* malignancy*) AND (atrial fibrillation OR AF OR a-fibrillation) AND (mortality OR death OR survival). In addition, we manually screened the cited articles of previous meta-analyses, cohort studies, and review articles to identify any relevant studies.

Study Selection

All studies were included, which met the following eligibility described as PECOS: (1) P (Population): cancer patients; (2) E

TABLE 2 | Quality assessment of cohorts using New Ottawa scale (NOS).

Studies	Selection (maximum 4)				Comparability (maximum 2)	Outcome (maximum 3)			Total score
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts	
Amar et al. (14)	1	0	1	1	1	1	1	1	7
Amioka et al. (15)	1	1	1	1	1	1	1	1	8
Cardinale et al. (16)	1	1	1	1	1	1	1	1	8
Chin et al. (17)	1	1	1	1	2	1	1	1	9
Constantin et al. (18)	1	1	1	1	2	1	1	1	9
Imperatori et al. (19)	1	1	1	1	1	1	1	1	8
Ishibashi et al. (20)	1	1	1	1	2	1	1	1	9
Kotova et al. (21)	1	1	1	1	1	1	1	1	8
McComrack et al. (22)	1	1	1	1	2	1	1	1	9
Murthy et al. (23)	1	1	1	1	1	1	1	1	8
Rao et al. (25)	1	1	1	1	2	1	1	1	9
Roselli et al. (26)	1	0	1	1	1	1	1	1	7
Stawicki et al. (27)	1	1	1	1	2	1	1	1	9
Wang et al. (28)	1	1	1	1	2	1	1	1	9
Ammad Ud Din et al. (29)	1	1	1	0	2	1	1	1	8
Han et al. (30)	1	1	1	1	2	1	1	1	9
Zubair Khan et al. (31)	1	1	1	1	2	1	1	1	9

TABLE 3 | Quality assessment of clinical trials using Cochrane Collaboration's risk of bias tool.

Study	Adequate sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Free of other bias	Net risk
Ojima et al. (24)	High risk	Unclear risk	High risk	Unclear risk	Low risk	High risk	High risk	High risk

(Exposure): atrial fibrillation; (3) C (Control): cancer patients without atrial fibrillation; (4) O (Outcome): mortality; (5) S (Studies): human-based randomized controlled trials and cohort studies published in English only.

Case series, case reports, literature reviews, editorials, and studies not meeting the inclusion criteria were excluded.

Data Extraction and Quality Assessment of Studies

Two reviewers independently searched electronic databases. Studies searched were exported to the EndNote Reference Library software version 20.0.1 (Clarivate Analytics), and duplicates were screened and removed.

Data extraction and quality assessment of included studies were done simultaneously and independently by two reviewers. Newcastle-Ottawa Scale (NOS) was used to assess the quality of the cohort studies. NOS score <6 was considered high risk for bias, 6–7 was moderate, and score >7 was considered low risk of bias (details of scoring is provided in **Table 2**). The modified Cochrane Collaboration's risk of bias tool for randomized controlled trials was used to assess the quality of published trials (details are provided in **Table 3**).

Statistical Analysis

Review Manager (version 5.4.1; Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2020) was used for all statistical analyses. The data from studies were pooled using a random-effects model. Analysis of results was done by calculating the odds ratio (OR) with respective 95% confidence intervals (CI). The chi-square test was performed to assess any differences between the subgroups. Sensitivity analysis was done to see if any individual study was driving the results and to implore reasons for high heterogeneity. As per Higgins et al., scale for heterogeneity was considered as follows: I^2 =

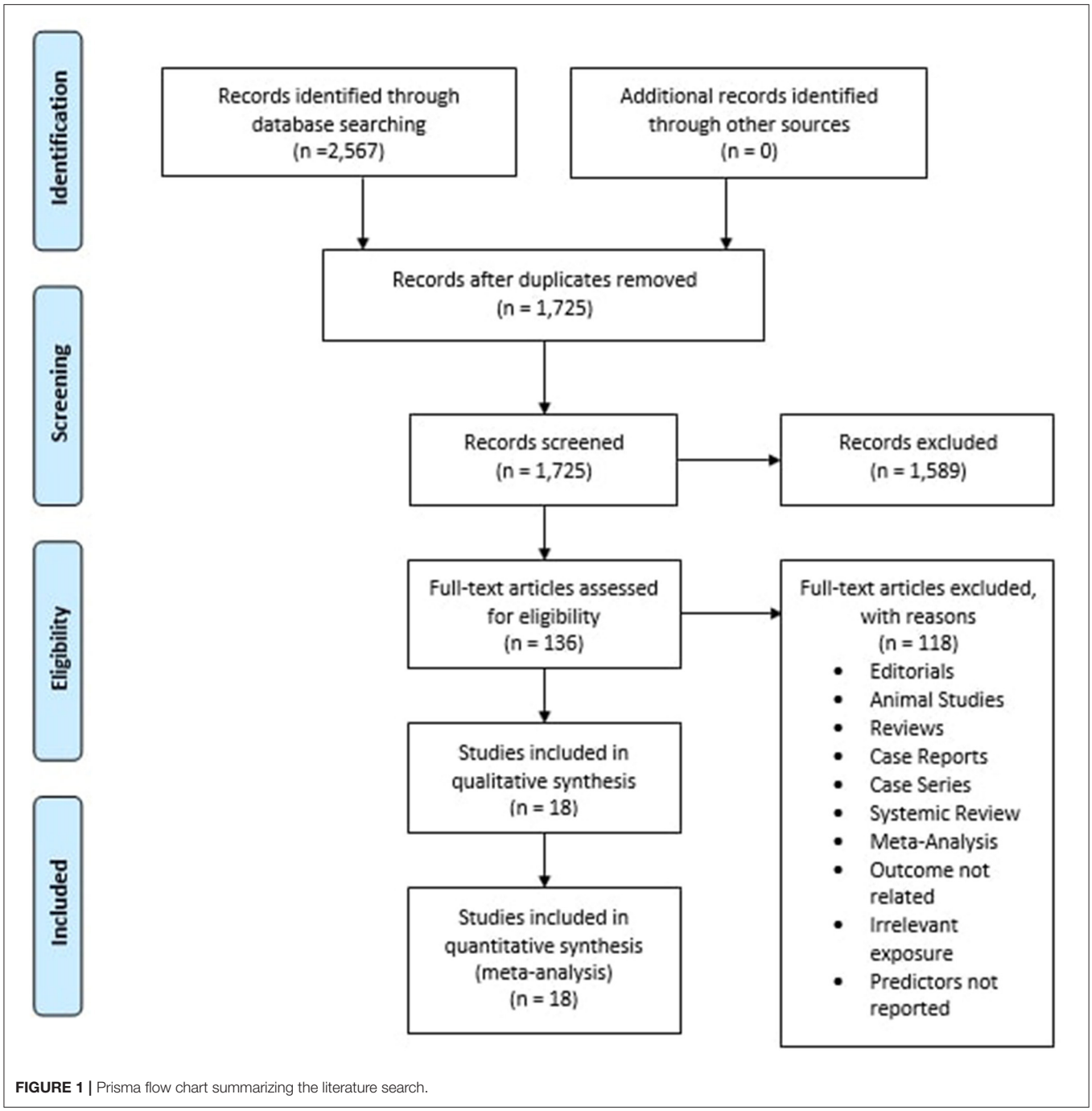

FIGURE 1 | Prisma flow chart summarizing the literature search.

25–60%–moderate; 50–90%–substantial; 75–100%–considerable heterogeneity, and $p < 0.1$ indicated significant heterogeneity (13). A value of $p < 0.05$ was considered significant for all analyses.

RESULTS

Literature Search Results

The initial search of the three electronic databases yielded 2,567 potential studies. After exclusions based on titles and abstracts, the full texts of 136 studies were read for possible inclusion. A total of 18 studies remained for quantitative analysis. **Figure 1** summarizes the results of our literature search.

Study Characteristics

Table 4 provides the basic characteristics of included studies (14–31). Our analysis included 18 published studies. Among these, there are 17 observational studies and 1 randomized controlled trial. The studies were conducted in different regions of the world, i.e., USA, Japan, Italy, Korea, Romania, Ireland, China,

TABLE 4 | Characteristics of included studies.

Study	Year	Study design	Duration	Country	Total cancer patients (n)	Males (n)	Mean age (years)	Incident AF (n)	Cancer type	Mortality attestation	Major causes of death	Surgery or non-surgery group	Risk of Bias
Amar et al. (14)	2002	Cohort	1990–1999	USA	527	325	68 (AF) 62 (non-AF)	79	Lung cancer, Esophageal cancer	Medical record	Surgery-related complications	Surgery	Moderate Risk
Amioka et al. (15)	2016	Cohort	2009–2013	Japan	249	121	67	15	Hodgkin, Non-Hodgkin lymphoma	Medical record	Primary disease, solid cancer, hepatitis, sepsis	Non-surgery	Low Risk
Cardinale et al. (16)	1999	Cohort	1995–1997	Italy	233	170	59.3	28	Lung cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Chin et al. (17)	2016	Cohort	2005–2012	Korea	583	548	67 (AF) 62 (non-AF)	63	Esophageal cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Constantin al (18)	2020	Cohort	2008–2017	Romania	391	N/A*	N/A*	27	Colorectal cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Imperatori et al. (19)	2012	Cohort	1996–2009	Italy	454	369	65.4	45	Lung cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Ishibashi et al. (20)	2020	Cohort	2010–2019	Japan	947	626	69.2	49	Lung cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Kotova et al. (21)	2017	Cohort	2005–2014	USA	933	426	72 (AF) 65.4 (non-AF)	113	Lung cancer	Medical record	Surgery-related complications	Surgery	Low Risk
McComrack et al. (22)	2014	Cohort	2006–2013	Ireland	473	344	63	96	Esophageal cancer, Junctional cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Murthy et al. (23)	2003	Cohort	1982–2000	China	288	235	66.8 (AF) 67 (non-AF)	144	Esophageal cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Ojima et al. (24)	2020	RCT*	2014–2016	Japan	57	77	N/A*	13	Esophageal cancer	Medical record	Surgery-related complications	Surgery	High Risk
Rao et al. (25)	2012	Cohort	1991–2009	UK	997	709	67	209	Esophageal cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Roselli et al. (26)	2005	Cohort	1998–2002	USA	183	N/A*	N/A*	91	Lung cancer	Medical record	Surgery-related complications	Surgery	Moderate Risk
Stawicki et al. (27)	2011	Cohort	1996–2007	USA	156	145	63.7 (AF) 59.8 (non-AF)	32	Esophageal cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Wang et al. (28)	2021	Cohort	2013–2018	China	324	291	58.4	75	Lung cancer	Medical record	Surgery-related complications	Surgery	Low Risk
Ammad Ud Din et al. (29)	2021	Cohort	2009–2018	USA	14,530	8,569	81.80 (AF) 81.76 (non-AF)	7,265	chronic lymphocytic leukemia	Medical record	Acute Myocardial Infarction, heart failure, cardiac arrest, stroke	Non-surgery	Low Risk
Han et al. (30)	2021	Cohort	2003–2014	China	2,478,598	1,104,903	N/A	216,737	Various cancers	Medical record	Not specified	Non-surgery	Low Risk
Zubair Khan et al. (31)	2021	Cohort	2005–2015	USA	46,030,380	7,673,063	N/A	6,731,310	Various cancers	Medical record	Not specified	Non-surgery	Low Risk

N/A, Not available; RCT*, Randomized Controlled Trial.*

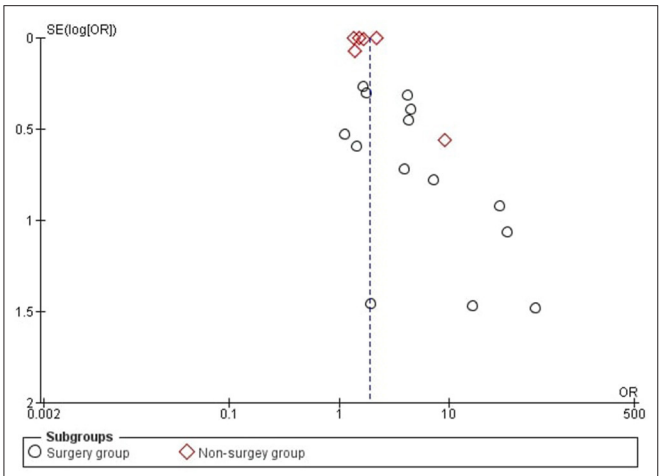

FIGURE 2 | Funnel plot which is used to demonstrate publicaion bias.

and the UK. A total of 6 studies evaluated lung cancer, 5 studies evaluated esophageal cancer, and 6 studies evaluated various other cancers including lymphoma, leukemia, colorectal cancer, prostate cancer, and breast cancer. The mean age of patients was 67.12 years.

Publication Bias and Quality Assessment

The visual inspection of the funnel plot (**Figure 2**) did indicate that there is publication bias in our meta-analysis.

Out of the 18 studies, 15 studies have a low risk of bias (15–23, 25, 27–31), two studies have a moderate risk of bias (14, 26), and one study has a high risk of bias (24).

Results of Meta-Analysis

Detailed forest plots, outlining the effect size of overall mortality outcome of atrial fibrillation in cancers, are shown in **Figure 3**. A forest plot outlining the effect size based on surgery and the non-surgery group is also presented in **Figure 3**.

Overall Mortality Outcome of Atrial Fibrillation in Cancers

Eighteen studies evaluated the mortality outcome for cancer patients who develop atrial fibrillation. **Table 5** provides further details of the studies selected for this objective. Pooled results (**Figure 3**) showed a significantly higher mortality rate in cancer patients who subsequently develop atrial fibrillation as compared to those in the control group (OR = 1.90 [1.65, 2.19]; $p < 0.00001$; $I^2 = 100\%$).

Surgery Group

Out of 18 studies, 14 studies reported data of patients undergoing cancer-related surgeries. Statistical analysis showed that there was a significantly higher mortality rate in cancer patients who subsequently develop atrial fibrillation as compared to those in the control group (OR = 3.68 [2.29, 5.94]; $p < 0.00001$; $I^2 = 61\%$).

Non-Surgery Group

Out of 18 studies, 4 studies reported data of non-surgical patients having cancer. Statistical analysis showed that there was a significantly higher mortality rate in cancer patients who subsequently develop atrial fibrillation as compared to those in the control group (OR = 1.64 [1.39, 1.93]; p <0.00001; I^2 = 100%).

Sensitivity Analysis

A sensitivity analysis was conducted to assess the influence of each study on the overall effect, by excluding one study at a time, followed by the generation of pooled OR for the rest of the studies. No significant change was observed after the exclusion of any individual study, suggesting that the results were robust.

We also removed the study by Amioka et al. (15) to check its effect on our results, as it was the only study with a high risk of bias, but there was no statistically significant change. The overall result was OR = 1.85 [1.61, 2.14]; $p < 0.0001$; I^2= 100%.

DISCUSSION

Our systematic review and meta-analysis of 18 published studies suggest that cancer patients who subsequently develop atrial fibrillation have an increased rate of mortality as compared to those who do not (OR = 1.90 [1.65, 2.19]; $p < 0.00001$; I^2 = 100%). We also separately analyzed the mortality risk in the surgery group and the non-surgery group. This subgroup analysis shows that new-onset atrial fibrillation associated with cancer increases the mortality rate, irrespective of any surgical intervention. Mechanisms contributing to death included surgery-related complications, sepsis, hepatitis, pneumonia, heart failure, myocardial infarction, and stroke.

Previous studies have evaluated this relationship in specific cancer patients. However, the results are inconsistent, and the studies are limited by their sample size and geographical location. We have pooled data from studies performed in different regions of the world and with different cancer types to provide better and more reliable evidence.

Cancer continues to be one of the deadliest diseases worldwide. In the year 2020, an estimated 19.3 million new cancer cases and about 10 million cancer deaths occurred globally (32). Death in cancer patients may occur due to index cancer, non-index cancer, or non-cancer causes. Patients with cancers of the colorectum, prostate, breast, genitourinary tract, tonsils, melanoma, and lymphomas are more likely to die due to non-cancer causes. Heart disease is the most common non-cancer cause of death (33). Many mechanisms can lead to the death of cancer patients, including infections (36%), hemorrhagic and thromboembolic phenomena (18%), and respiratory failure (19%) (34).

It is well recognized that there is an increased risk of developing atrial fibrillation in cancer patients (6). Cancer itself is a high-risk and life-threatening condition. Concomitant development of atrial fibrillation brings additional risks including infections, stroke, and bleeding among others (35). Atrial fibrillation alone is associated with four times increased risk of all-cause mortality as compared to the general population (36).

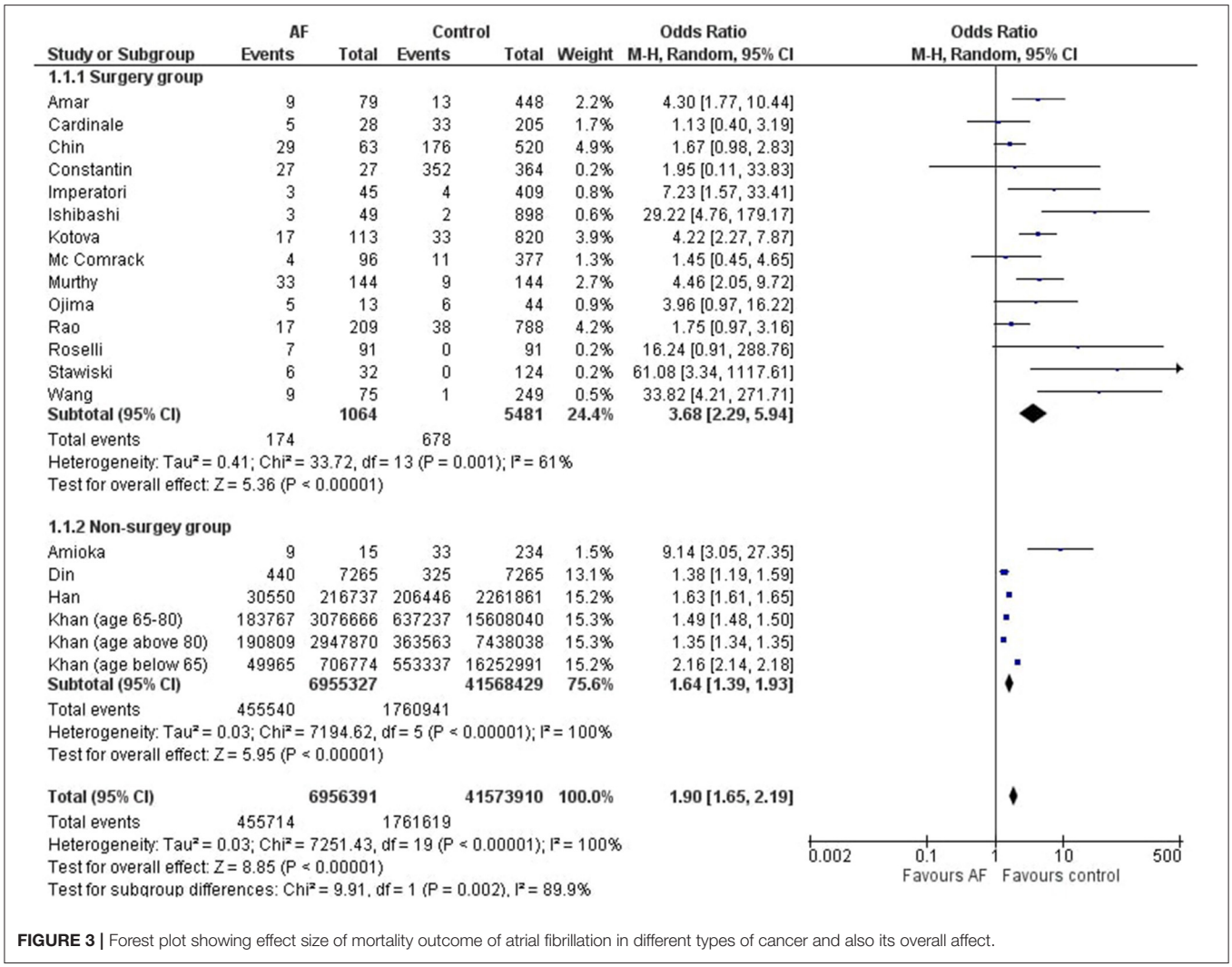

FIGURE 3 | Forest plot showing effect size of mortality outcome of atrial fibrillation in different types of cancer and also its overall affect.

Atrial fibrillation has been demonstrated as a risk factor for stroke (37). A five-fold increased risk of stroke has been reported to be associated with chronic atrial fibrillation (38).

The underlying mechanism leading to the development of atrial fibrillation in cancer patients has been a matter of concern. Atrial fibrillation may be a co-morbid state considering the common predisposing factors for both conditions, and it might involve specific etiologies (10).

Inflammation of the atria due to autoimmune paraneoplastic syndromes, the abnormal release of some hormones by cancer cells, and imbalances between the sympathetic and parasympathetic autonomic control may predispose the patient to atrial fibrillation (10). These mechanisms support our findings that new-onset atrial fibrillation can increase the risk of mortality in cancer patients.

Cancer therapy may be responsible for the development of atrial fibrillation. However, it has been observed that the risk of developing atrial fibrillation persists even if no cancer-specific treatment has been given (11).

Although the exact mechanism responsible for increased mortality in cancer patients with atrial fibrillation remains undetermined, it can be speculated from the following findings. In atrial fibrillation, the perfusion of tissues becomes inadequate, and due to this, oxidative injury can occur (38, 39). The hypoxic conditions not only favor tumor survival and growth but also cause resistance to radiation therapy (40, 41). Moreover, endothelial dysfunction, inflammatory conditions, and prothrombic state associated with atrial fibrillation may also be responsible for the worsened outcomes (42). The inflammatory state may be further aggravated by cancer surgery. The increase in in-hospital mortality can also be explained by the fact that patients with atrial fibrillation are susceptible to hospital-acquired pneumonia, which can become the cause of death in these patients (43).

Most of the studies included in our analysis enrolled patients undergoing cancer surgery. It is highly likely that the surgery may play an important role in the worsened outcomes. Various processes and mediators involved in surgical wound healing may accelerate tumor growth, invasion, and metastasis (44). Tumor

TABLE 5 | Analytical details of the studies that were selected.

Study name and year		Deaths in AF		Deaths in control		Odds ratio [95% CI]	*P* value
		Events (n)	Total (n)	Events (n)	Total (n)		
Amar et al. (14)	2002	9	79	13	448	4.30 [1.77, 10.44]	0.0013
Amioka et al. (15)	2016	9	15	33	234	9.14 [3.05, 27.35]	0.0001
Cardinale et al. (16)	1999	5	28	33	205	1.13 [0.40, 3.19]	0.8132
Chin et al. (17)	2016	29	63	176	520	1.67 [0.98, 2.83]	0.0576
Constantin et al. (18)	2020	27	27	352	364	1.95 [0.11, 33.83]	0.6463
Imperatori et al. (19)	2012	3	45	4	409	7.23 [1.57, 33.41]	0.0113
Ishibashi et al. (20)	2020	3	49	2	898	29.22 [4.76, 179.17]	0.0003
Kotova et al. (21)	2017	17	113	33	820	4.22 [2.27, 7.87]	0.0000
McComrack et al. (22)	2014	4	96	11	377	1.45 [0.45, 4.65]	0.5352
Murthy et al. (23)	2003	33	144	9	144	4.46 [2.05, 9.72]	0.0002
Ojima et al. (24)	2020	5	13	6	44	3.96 [0.97, 16.22]	0.0559
Rao et al. (25)	2012	17	209	38	788	1.75 [0.97, 3.16]	0.0652
Roselli et al. (26)	2005	7	91	0	91	16.24 [0.91, 288.76]	0.0576
Stawicki et al. (27)	2011	6	32	0	124	61.08 [3.34, 1,117.61]	0.0056
Wang et al. (28)	2021	9	75	1	249	33.82 [4.21, 271.71]	0.0009
Ammad Ud Din et al. (29)	2021	440	7,265	325	7,265	1.38 [1.19, 1.59]	0.0000
Han et al. (30)	2021	30,550	2,16,737	2,06,446	2,261,861	1.63 [1.61, 1.65]	0.0000
Zubair Khan et al. (31) (age 65-80)	2021	1,83,767	30,76,666	6,37,237	15,608,040	1.49 [1.48, 1.50]	0.0000
Zubair Khan (31) (age below 65)	2021	1,90,809	29,47,870	3,63,563	74,38,038	1.35 [1.34, 1.35]	0.0000
Zubair Khan (31) (age above 80)	2021	49,965	7,06,774	5,53,337	1,62,52,991	2.16 [2.14, 2.18]	0.0000

outgrowth may be caused by activation of epithelial, endothelial, and inflammatory cells; platelets, and fibroblasts; and production of growth factors and cytokines during the healing of surgical wounds (45).

The efficacy of different prophylactic approaches to prevent atrial fibrillation after lung surgery was evaluated in a meta-analysis by Zhang et al. It was found that amiodarone was the most effective in preventing postoperative atrial fibrillation (46). The use of oral anticoagulants is associated with a lower risk of mortality in patients with atrial fibrillation (36). It has been demonstrated that the patients who have concomitant cancer and atrial fibrillation can be benefited from anticoagulation with nonvitamin K antagonist oral anticoagulants (also known as direct oral anticoagulants (DOACs)) as well as warfarin (47). However, DOACs have a safer profile and greater effectiveness as compared to vitamin K antagonists (VKAs) (48). Moreover, CHADS2 and CHA2DS2-VASc scores can be used as tools to predict the risk of stroke and mortality in patients with cancer and atrial fibrillation and guide in decision-making accordingly (49).

Although it is suggested that atrial fibrillation after cancer surgery tends to be transient with few clinical outcomes and does not require prolonged monitoring and intensive care (5), recent data suggest a relationship between atrial fibrillation and increased mortality in cancer patients. Cancer patients who subsequently develop atrial fibrillation may need closer surveillance during follow-up, and specific strategies to manage atrial fibrillation should be included in the cancer management plan.

Limitation

Our study is limited in several ways: (a) The majority of the studies were observational, the results of which can have some bias. (b) Most of the studies enrolled patients undergoing surgery, so our outcome, i.e., increased mortality, may not solely be due to the atrial fibrillation but there might be some role of the after-effects of surgery. (c) High heterogeneity was seen in our results because we pooled different studies containing different cancer types. These studies were pivotal in forming analysis, but more studies with the community and random controls should be conducted.

CONCLUSION

Cancer patients who subsequently developed atrial fibrillation had a higher mortality rate as compared to those cancer patients who did not develop atrial fibrillation. A higher mortality rate was seen in both surgical and non-surgical subgroups. This implies that cancer patients who subsequently develop atrial fibrillation need to be followed up with careful monitoring of the arrhythmia, and specific measures should be taken to minimize the adverse outcomes brought on by it.

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/s.

AUTHOR CONTRIBUTIONS

MB worked alongside MM in all steps from literature search till manuscript preparation. JA reviewed and actively participated in the analysis, manuscript preparation and reviewing, and is designated as the senior author. LS and SB played role in final analysis and reviewing of the manuscript. All authors contributed to the article and approved the submitted version.

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Cardiac Monitoring and Heart Failure in Advanced Breast Cancer Patients Treated With Trastuzumab in Ontario, Canada

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Background: Trastuzumab has improved patient outcomes in HER2 + breast cancer (BC) but carries a risk of cardiotoxicity. Routine cardiac imaging is recommended for advanced breast cancer (aBC) patients during trastuzumab treatment despite a lack of evidence that this improves patient outcomes. This study was conducted to understand predictive factors for cardiac events and determine the impact of cardiovascular monitoring in aBC.

Methods: This retrospective population-based cohort study included aBC patients treated with trastuzumab (all lines), in Ontario, Canada from 2007 to 2017. The overall cohort was divided into two groups; those who developed a cardiac event (CE) vs. those who did not. Patients with pre-existing heart disease were excluded. Logistic regression was performed to identify patient characteristics associated with an increased risk of CE.

Results: Of 2,284 patients with HER2 + aBC treated with trastuzumab, 167 (7.3%) developed a CE. Median age at first dose of trastuzumab was 57 (IQR 49–66); 61 (IQR 51–70) for patients with a CE. Median number of cycles was 16 (IQR 7–32); 21 (IQR 8–45) for patients with a CE ($p < 0.01$). Twelve (0.5%) patients died of cardiac causes; all had a prior CE. Increased risk of CEs was associated with age > 60 (OR 5.21, 95% CI 1.83–14.84, $p = 0.05$) and higher number cycles of trastuzumab (OR 1.01; 95% CI 1–101, $p = 0.028$).

Conclusion: This is the first population-based study to report on CEs and cardiac monitoring in HER2 + aBC patients during trastuzumab-based therapy. Older age and longer treatment with trastuzumab were associated with an increased risk of a CE.

Keywords: breast cancer, cardio-oncology, cardiotoxicity, trastuzumab, heart failure

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INTRODUCTION

Trastuzumab, a monoclonal antibody against human epidermal growth factor 2 (HER2) is the cornerstone of modern therapy for HER2 positive (+) advanced breast cancer (aBC). HER2 is overexpressed in approximately 15% of breast cancers (1–3) and therapies that target this pathway have significantly improved breast cancer outcomes for this population. Trastuzumab

is generally a well-tolerated drug but comes with a risk of cardiotoxicity (4, 5). Trastuzumab induced-cardiotoxicity (TIC) presents as either asymptomatic drops in left ventricular ejection fraction (LVEF) or overt heart failure (HF) (6–8). The initial observation of TIC led to the inclusion of routine cardiac monitoring in all subsequent clinical trials with anti-HER2 targeted therapies, as well as international consensus guidelines and FDA recommendation for routine cardiac monitoring every 3 months in all patients treated with trastuzumab-based therapy (9–11).

The risk of trastuzumab induced cardiotoxicity has been well-studied in the adjuvant setting. A meta-analysis of adjuvant trastuzumab trials found a 2.5% incidence of heart failure (HF) in trastuzumab treated patients vs. 0.4% in those treated with chemotherapy alone (RR 5.11 90% CI 3.00–8.72) (12, 13). The 2014 Cochrane review reported that 4.7% of patients with aBC had a severe cardiac event vs. 1.1% in the chemotherapy arm (RR 3.49, 95% CI 1.88–6.47, $p < 0.001$) (14). The use of sequential anti-HER2 targeted therapies has led to patients living longer with HER2 + aBC (15). In the end-of-study analysis of the CLEOPATRA trial, patients in the experimental arm (trastuzumab, pertuzumab, docetaxel) received a median of 24 cycles with a mean overall survival of 57.1 months (95% CI 50–72) and the 8-year landmark analysis reported an overall survival of 37% (31–42) (16). Only 4.4% of patients were found to meet criteria for cardiotoxicity in the dual anti-HER2 arm and 1.2% experienced symptomatic drops in LVEF (17).

While well-intentioned, the high frequency of cardiac monitoring recommended by the FDA in this patient population has led to an increased detection of asymptomatic drops in LVEF (18), the clinical significance of which is unknown. The American Society of Clinical Oncology has made a moderate strength recommendation based on low-quality evidence to perform routine echocardiographic surveillance indefinitely in patients with aBC who are receiving trastuzumab with the frequency determined by healthcare providers (11). However, population-based studies have reported that detection of early (asymptomatic) cardiotoxicity in the adjuvant setting places patients at risk of not completing their intended anti-HER2 targeted therapy, and thus increasing their risk of cancer recurrence and death (19). In Ontario, Canada, cancer care is provided through a single-payer health care system which requires LVEF monitoring in aBC HER2 + patients on anti-HER2 targeted therapy every 3–6 months (20). The objective of this study was to examine patients with HER2 + aBC to determine the incidence of cardiac events and cardiac death as well as examine clinical factors that may impact risk of cardiac complications.

MATERIALS AND METHODS

Study Design

This is a retrospective population-based cohort study of adult aBC patients (age > 18) treated with trastuzumab-based therapy in Ontario, Canada between January 1, 2007 and December 31, 2017. Patients with a history of heart failure or cardiomyopathy were excluded. The main exposure was treatment with trastuzumab given for palliative intent in any treatment line. In this provincial database with a single-payer system, access to anti-HER2 targeted therapies is only available to patients who overexpress HER2, therefore this was a surrogate for the presence of HER2 + status. Cases were defined by development of a cardiac event (CE). A CE was defined as new onset heart failure, pulmonary edema, or cardiomyopathy between initiation of trastuzumab and 90 days after the last dose, or death from any cardiovascular cause. The cohort was divided into two groups for comparison: group A who had a CE vs. group B who had no CEs during the study period. Ethics approval was obtained from the Ottawa Health Sciences Research Ethics Board, Ottawa, Canada.

Endpoints

The primary outcome of this study was the incidence of a CE in aBC patients treated with trastuzumab-based therapy. The secondary outcomes were to assess the incidence of cardiac death and to examine clinical factors associated with increased odds of cardiac events in this population.

Data Sources

Patients were identified using health administrative databases in Ontario, Canada that contain patient-level information on cancer diagnosis, cancer drug administration, inpatient and outpatient data, cancer registry data, and demographics. De-identified databases were accessed through the Institute for Clinical and Evaluative Sciences (ICES), and all data sources were linked through a unique encrypted identifier and analyzed at ICES. ICES is an independent, non-profit research institute funded by an annual grant from the Ontario Ministry of Health and Long-Term Care. As a prescribed entity under Ontario's privacy legislation, ICES is authorized to collect and use health care data for the purposes of health system analysis, evaluation, and decision support. Secure access to this data is governed by policies and procedures that are approved by the Information and Privacy Commissioner of Ontario.

The Ontario Health Insurance Plan (OHIP) is a publicly funded provincial health insurance plan that covers medical costs for all residents in Ontario. The OHIP database was used to identify outpatient visits, physician visits, echocardiogram, and multigated acquisition scan (MUGA) testing through billing codes in our patients. The Ontario Cancer Registry (OCR) was used to confirm breast cancer diagnosis, cancer staging, estrogen receptor (ER) status, and progesterone receptor (PR) status information. HER2 status was not available but implied based on treatment with trastuzumab. Patients treated with trastuzumab were identified through the New Drug Funding Program (NDFP), a public health program which captures information on trastuzumab administration. The Canadian Institute for Health Information (CIHI) Discharge Abstract Database (DAD), CIHI same-day surgery, the National Ambulatory Care Reporting System (NACRS) database, and ICES-derived cohorts for pre-treatment Charlson co-morbidity index scores, treatment location (community vs. teaching hospital), ambulatory and emergency department visits and hospitalizations for heart failure, pulmonary edema and cardiomyopathy, income

demographics, and cause of death were collected. Chemotherapy information was accessed through the cancer activity level reporting (ALR) database. We did not have access to LVEF values or clinic notes for this study.

Data collected included: age at diagnosis, *de novo* vs. recurrent disease, ER/PR status, history of anthracycline treatment, number cycles of trastuzumab, frequency of cardiac imaging, concurrent pertuzumab use, year of treatment, cause of death, CEs, Charlson co-morbidity index, and community vs. academic hospital setting. Cardiology visits were included in the descriptive analysis but not as a variable in the logistic regression model.

Statistical Methods

Descriptive statistics were performed to report the baseline characteristics of patients in each group [CE (or group A) vs. no CE (or group B)]. Categorical variables were compared using a chi-square test or a Fisher exact test. Means for continuous variables were compared using Student's *t*-test. Statistical significance was tested at an alpha of 0.05.

Logistic regression was carried out on the two groups to calculated odds ratios (OR) for factors predictive of CEs in this population. Patients who developed a CE (group A) were compared to patients who did not develop a CE (group B). Results are presented as odds ratios (ORs) with 95% confidence intervals (CI). The analyses were performed with SAS Software (version 9.4.3.0).

RESULTS

There were 2,284 patients with HER2 + aBC included in this study. Overall, the median age at first treatment was 57.0 (IQR 49.0–66.0) and patients received a median of 16.0 cycles of trastuzumab every 3 weeks (IQR 7.0–32.0). In our cohort, 25% (n = 572) of patients received concurrent pertuzumab therapy for aBC. Similarly, 25.9% (n = 592) had received anthracyclines in the previous 10 years and only 5.2% (n = 118) had received previous trastuzumab. The majority of patients in this study (62.6%, n = 1430) had stage IV disease (*de novo*) at the time of diagnosis.

For the primary endpoint, the incidence of CEs was 7.3% (n = 167) in what will herein be referred to as group A, whereas 92.3% (n = 2117) did not have any CEs (group B). **Table 1** contains baseline characteristics for both the study population overall as well as each study group individually. Heart failure was observed in 6.9% (n = 158) of the study population, representing 94.6% (158/167) of the CEs. Less than 1% of patients died of cardiac causes (n = 12, 0.5%) while 34.1% (n = 779) died of their cancer, and 14.8% died for other reasons (n = 339). Group A had an older median age of 61 (IQR 51–70) vs. 57 (48–66) in group B, $p < 0.01$. Patients in group A also received a greater number of trastuzumab cycles, 21.0 (IQR 8–45) vs. 16.0 (IQR 7–31), $p < 0.01$. **Figure 1** contains boxplots showing the distribution of age and trastuzumab cycles in groups A and B. The remaining baseline characteristics were well-balanced between groups A and B with no significant differences found for neighborhood income quintiles, pertuzumab use, prior anthracycline use, prior trastuzumab use, cancer related deaths, baseline Charlson co-morbidity index, estrogen receptor status, progesterone receptor status, or initial stage at diagnosis (I-III vs. IV). In total, the Charlson index was 0 in 28.2% (n = 644), 1–2 in 10.7% (n = 244) and $\geq$3 in 50.9% (1,163).

Risk Factors for Cardiac Events

Logistic regression was performed to determine if there were clinical factors that increased the odds of developing CEs during or immediately after trastuzumab. Age, neighborhood income quintile, concurrent pertuzumab use, Charlson co-morbidity index, prior anthracycline use, recurrent or *de novo* metastatic disease, hospital setting (teaching vs. community), and number of cycles of trastuzumab were included in the model. Older age (analyzed in 10-year blocks) vs. age < 40 was the most significant factor increasing the odds of CE: age 60–69, OR = 5.21 (95% CI 1.83–14.84, p = 0.05); age 70–79, OR = 6.23 (95% CI 2.09–18.51, p = 0.02); age > 80, OR = 7.24 (95% CI 2.26–23.18, p = 0.01). When treated as a continuous variable, each additional cycle of trastuzumab lead to a statistically significant increase in the odds of a CE (OR 1.01, 95% CI 1.00–1.01, p = 0.03). **Figure 2** depicts the Forrest Plot with the complete results of logistic regression presented in **Table 2**.

Cardiac Monitoring

The final outcome of interest was to understand cardiac monitoring patterns in HER2 + aBC. The overall study population had a median of 2 (IQR 1–3) cardiac tests per year. Patients with CEs had more cardiac imaging ($p < 0.01$) and a median three tests per year (IQR 2–5) vs. 2 (IQR 1–3) in those with no CEs. Overall, patients in group A had a median of 5 cardiac tests (2–11) vs. 3 (1–6) in group B, $p < 0.01$. Group A was also more likely to have seen a cardiologist during the time they were on trastuzumab 65.9 vs. 12.9% ($p < 0.01$). Of note, 14% (n = 307) of patients had no captured cardiac imaging at all, 7.8% (n = 13) in this group had CEs.

DISCUSSION

In patients with HER2 + breast cancer, anti-HER2 targeted therapy has dramatically improved cancer outcomes. Trastuzumab-based therapy is associated with an increased risk of cardiotoxicity in patients with early-stage disease with most cases observed within 18 months of completing treatment. There is a paucity of information on cardiotoxicity of anti-HER2 targeted agents in patients with aBC. Our study is the largest to report cardiac outcomes in aBC treated with trastuzumab and/or pertuzumab in a non-clinical trial setting. In this retrospective population-based study of HER2 + aBC, the incidence of cardiac events during or after trastuzumab-based therapy was 7.3%, (n = 167) with heart failure representing the majority of these events (6.9%, n = 158). These findings are similar to the 3–7% reported in the literature (6, 8, 14), but lower than a Dutch study of 429 patients likely given the reported combined incidence of severe and non-severe cardiotoxicity based on LVEF parameters of 11.7% and 9.1% after 1 and 2 years of trastuzumab

TABLE 1 | Baseline characteristics.

Variable	Value	Overall study population	Group A: incidence of cardiac event	Group B: no cardiac event	*P*-value
	N	**2,284**	**167**	**2,117**	
Year of treatment*	2007	202 (8.8%)	11 (6.6%)	191 (9.0%)	0.004
	2008	221 (9.7%)	24 (14.4%)	197 (9.3%)	
	2009	189 (8.3%)	19 (11.4%)	170 (8.0%)	
	2010	174 (7.6%)	15 (9.0%)	159 (7.5%)	
	2011	211 (9.2%)	17 (10.2%)	194 (9.2%)	
	2012	235 (10.3%)	16 (9.6%)	219 (10.3%)	
	2013	252 (11.0%)	27 (16.2%)	225 (10.6%)	
	2014	231 (10.1%)	16 (9.6%)	215 (10.2%)	
	2015	245 (10.7%)	14 (8.4%)	231 (10.9%)	
	2016	220 (9.6%)	8 (4.8%)	212 (10.0%)	
	2017	104 (4.6%)	0 (0.0%)	104 (4.9%)	
Age at first treatment	Mean ± SD	57.67 ± 12.94	61.10 ± 12.54	57.40 ± 12.94	<0.001
	Median (IQR)	57.00 (49.00–66.00)	61.00 (51.00–70.00)	57.00 (48.00–66.00)	0.001
	<40	197(8.6%)	≤5 (2.4%)	193(9.1%)	0.008
	40–49	491 (21.5%)	35 (21.0%)	456 (21.5%)	
	50–59	691 (30.3%)	44 (26.3%)	647 (30.6%)	
	60–69	517 (22.6%)	45 (26.9%)	472 (22.3%)	
	70–79	269 (11.8%)	25 (15.0%)	244 (11.5%)	
	≥80	119(5.2%)	14 (8.4%)	105 (5.0%)	
Charlson co-morbidity index	0	644 (28.2%)	57 (34.1%)	587 (27.7%)	0.317
	1–2	244 (10.7%)	18 (10.8%)	226 (10.7%)	
	≥3	1,163 (50.9%)	75 (44.9%)	1,088 (51.4%)	
	missing	233 (10.2%)	17 (10.2%)	216 (10.2%)	
ER status	Negative	444 (19.4%)	30 (18.0%)	414 (19.6%)	0.611
	Positive	556 (24.3%)	37 (22.2%)	519 (24.5%)	
	Missing	1,284 (56.2%)	100 (59.9%)	1,184 (55.9%)	
PR status	Negative	566 (24.8%)	40 (24.0%)	526 (24.8%)	0.538
	Positive	434 (19.0%)	27 (16.2%)	407 (19.2%)	
	Missing	1,284 (56.2%)	100 (59.9%)	1,184 (55.9%)	
Stage at diagnosis	Previous stage I-III	854 (37.4%)	61 (36.5%)	793 (37.5%)	0.811
	Stage IV	1,430 (62.6%)	106 (63.5%)	1,324 (62.5%)	
Trastuzumab in previous 5 years	No	2,166 (94.8%)	154 (92.2%)	2,012 (95.0%)	0.112
	Yes	118 (5.2%)	13 (7.8%)	105 (5.0%)	
Concurrent pertuzumab	No	1,712 (75.0%)	134 (80.2%)	1,578 (74.5%)	0.102
	Yes	572 (25.0%)	33 (19.8%)	539 (25.5%)	
Received anthracyclines in previous 10 years	No	1,692 (74.1%)	120 (71.9%)	1,572 (74.3%)	0.496
	Yes	592 (25.9%)	47 (28.1%)	545 (25.7%)	
Received anthracyclines in previous 5 years	No	1,762 (77.1%)	126 (75.4%)	1,636 (77.3%)	0.588
	Yes	522 (22.9%)	41 (24.6%)	481 (22.7%)	
Heart failure during treatment	No	2,126 (93.1%)	9 (5.4%)	2,117 (100.0%)	<0.001
	Yes	158 (6.9%)	158 (94.6%)	0 (0.0%)	
Deceased	No	1,154 (50.5%)	78 (46.7%)	1,076 (50.8%)	0.305
	Yes	1,130 (49.5%)	89 (53.3%)	1,041 (49.2%)	

(Continued)

TABLE 1 | Continued

Variable	Value	Overall study population	Group A: incidence of cardiac event	Group B: no cardiac event	*P*-value
	N	**2,284**	**167**	**2,117**	
Cause of death: breast cancer	No	1,505 (65.9%)	117 (70.1%)	1,388 (65.6%)	0.238
	Yes	779 (34.1%)	50 (29.9%)	729 (34.4%)	
Cause of death: cardiac	No	2,272 (99.5%)	155 (92.8%)	2,117 (100.0%)	<0.001
	Yes	12 (0.5%)	12 (7.2%)	0 (0.0%)	
Cardiac tests/year	Mean ± SD	2.26 ± 1.67	3.22 ± 2.17	2.18 ± 1.60	<0.001
	Median (IQR)	2.00 (1.00–3.00)	3.00 (2.00–5.00)	2.00 (1.00–3.00)	<0.001
Any cardiac testing	Cardiac testing	1,964 (86.0%)	154 (92.2%)	1,810 (85.5%)	0.016
	No cardiac testing	320 (14.0%)	13 (7.8%)	307 (14.5%)	
Total number of cardiac tests	Mean ± SD	4.54 ± 5.33	7.78 ± 7.71	4.29 ± 5.01	<0.001
	Median (IQR)	3.00 (1.00–6.00)	5.00 (2.00–11.00)	3.00 (1.00–6.00)	<0.001
Number of cardiologist visits	One or more cardiac visits	383 (16.8%)	110 (65.9%)	273 (12.9%)	<0.001
	No cardiac visits	1,901 (83.2%)	57 (34.1%)	1,844 (87.1%)	
Hospital type	Community	1,248 (54.6%)	84 (50.3%)	1,164 (55.0%)	0.242
	Teaching	1,036 (45.4%)	83 (49.7%)	953 (45.0%)	
Neighborhood income quintile	Lowest income, Q1	395 (17.3%)	24 (14.4%)	371 (17.5%)	0.357
	Q2	446 (19.5%)	31 (18.6%)	415 (19.6%)	
	Q3	435 (19.0%)	27 (16.2%)	408 (19.3%)	
	Q4	508 (22.2%)	47 (28.1%)	461 (21.8%)	
	Highest income, Q5	490 (21.5%)	38 (22.8%)	452 (21.4%)	
	Missing	10 (0.4%)	0 (0.0%)	10 (0.5%)	
Number of cycles trastuzumab	Mean ± SD	24.82 ± 27.66	33.81 ± 36.21	24.11 ± 26.75	<0.001
	Median (IQR)	16.00 (7.00–32.00)	21.00 (8.00–45.00)	16.00 (7.00–31.00)	0.001

**Each year provides a percentage of the total study population.*

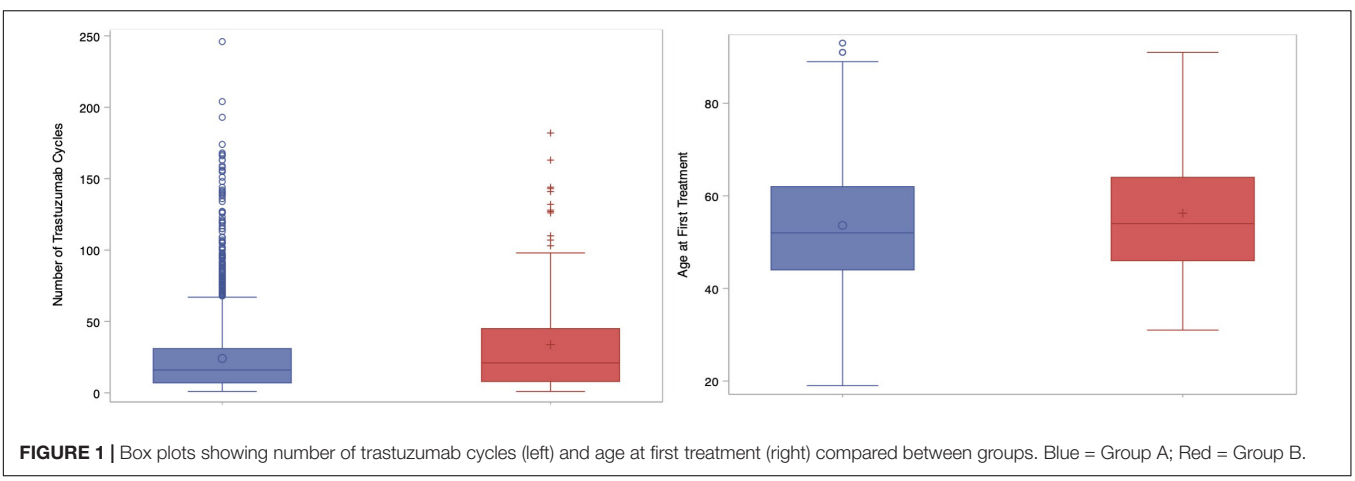

FIGURE 1 | Box plots showing number of trastuzumab cycles (left) and age at first treatment (right) compared between groups. Blue = Group A; Red = Group B.

respectively (21); after 4 years of treatment, the cumulative incidence of severe cardiotoxicity (LVEF < 40%) was 3.1%.

Although our study demonstrates a higher incidence of CEs in the community than reported in prior clinical trial studies (7.3 vs. 1–2%), there were very few deaths from cardiac causes (1%) in our study population while more than a third of all deaths were attributable to breast cancer (69%). During the timeframe of our data collection period, 49.4% of patients were still alive which is consistent with a near 5-year median overall survival seen in this population (16). These are important results to consider when weighing the risks and benefits of treatment for aBC. The risk of cardiotoxicity related to anti-HER2 targeted

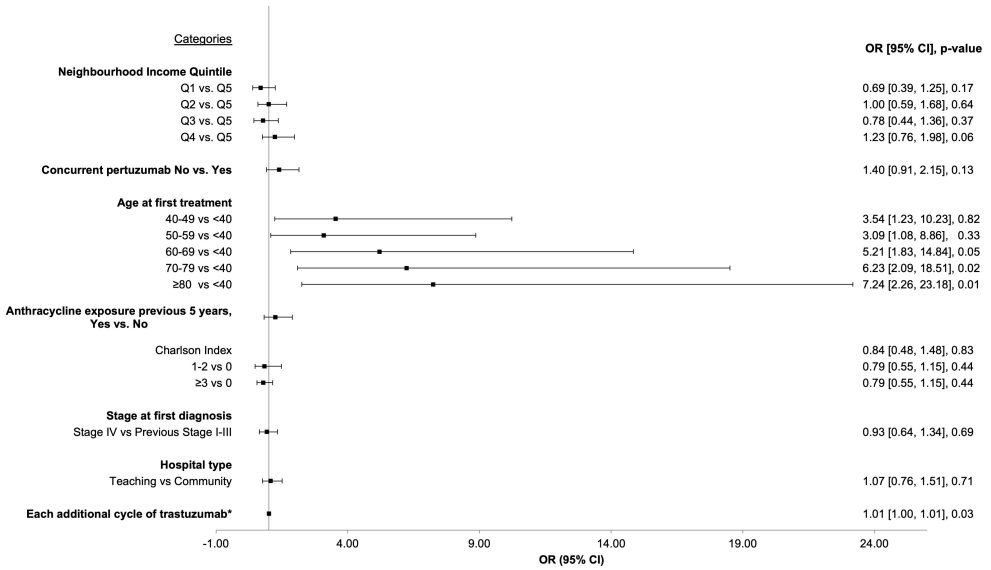

FIGURE 2 | Forrest plot depicting results from logistic regression for factors associated with increased odds of cardiac event.

therapy rarely translates into a greater mortality risk to the patient then their cancer. However, holding or delaying these agents precludes patients from life-prolonging therapy. In our study population, there were no differences in the incidence of CEs in patients with routine cardiac imaging as opposed to those with no cardiac imaging. Therefore, clinicians may decide that only patients with the presence of reliable predictors of CEs should undergo interval cardiac evaluations. In our cohort, older age and longer duration of trastuzumab-based therapy were predictive of CEs however given the borderline statistical significance (HR = 1.01) these results should be interpreted with caution when considering frequency of cardiac monitoring. Our results are consistent with findings from the Dutch group which observed that older age and a baseline reduced LVEF increased the risk of severe cardiotoxicity (21).

The results of our study challenges the practice of routine cardiac imaging in aBC patients treated with anti-HER2 targeted therapy (9–11) as per the current FDA and Cancer Care Ontario recommendations. In Ontario, the current cost of an echocardiogram is $2000 CAD (22). This would translate to more than $6,000 CAD in echocardiogram costs per individual as per a cohort-based analysis in the United States where patients treated with trastuzumab in the aBC setting had an average of 3.1 echocardiograms over the course of their treatment (23).

In our study, cardiac imaging was not available in a minority of patients (n = 307); these patients experienced a similar CE rate (7.8%, n = 13) compared to those patients with regular cardiac monitoring (group A: CE = 7.3%, n = 167). Future guidelines on cardiac monitoring in HER2 + aBC should consider the clinical benefit of anti-HER2 therapy in the context of the low incidence of CEs. Targeted LVEF monitoring should focus on those considered to be at higher risk of developing cardiotoxicity; patients over 60 or those who are treated for more than 1 year with anti-HER2 targeted therapy, while all other patients should be monitored clinically for cardiotoxicity. This approach would reduce the number of cardiac investigations and focus clinical decision making on quality of life and efficacy of anti-HER2 treatment. Another potential method suggested by the European Society of Medical Oncology is monitoring of

TABLE 2 | Results from logistic regression showing factors associated with incidence of cardiac events in advanced HER2 + breast cancer patients, 2007–2017.

Categories	OR (95% CI)	*p*-Values
Neighborhood income quintile		
Q1 vs. Q5	0.69 (0.39–1.25)	0.17
Q2 vs. Q5	1 (0.59–1.68)	0.64
Q3 vs. Q5	0.78 (0.44–1.36)	0.37
Q4 vs. Q5	1.23 (0.76–1.98)	0.06
Concurrent pertuzumab no vs. yes	1.4 (0.91–2.15)	0.13
Age at first treatment		
40–49 vs. <40	3.54 (1.23–10.23)	0.82
50–59 vs. <40	3.09 (1.08–8.86)	0.33
60–69 vs. <40	5.21 (1.83–14.84)	0.05
70–79 vs. <40	6.23 (2.09–18.51)	0.02
≥80 vs. <40	7.24 (2.26–23.18)	0.01
Anthracycline exposure previous 5 years, yes vs. no	1.25 (0.82–1.9)	0.31
Charlson index		
1–2 vs. 0	0.84 (0.48–1.48)	0.83
≥3 vs. 0	0.79 (0.55–1.15)	0.44
Stage at first diagnosis		
Stage IV vs. previous stage I–III	0.93 (0.64–1.34)	0.69
Hospital type		
Teaching vs. community	1.07 (0.76–1.51)	0.71
Each additional cycle of trastuzumab*	1.01 (1–1.01)	0.03

**Cycles of trastuzumab treated as continuous variable.*

cardiac biomarkers such as NT-pro-BNP and troponin (9). NT-proBNP is a known prognostic and predictive biomarker in heart failure (24). Although its predictive use in the context of patients treated with cardiotoxic cancer therapy has been inconsistent, a recent large prospective study of 323 patients observed consistent associations between increased in NT-proBNP and LVEF declines particularly notable in the sequential anthracycline and trastuzumab group (25). Therefore, the use of a dynamic risk prediction model combining clinical risk factors and serial biomarkers should be an avenue of future research. Population-based registries provide valuable information on the incidence of cardiotoxicity in patients with HER2 + aBC exposed to multiple lines of anti-HER2 targeted therapy, facilitating the development of risk stratification models to determine the optimal cardiac imaging strategy for individual patients.

There are some limitations to our study. Firstly, it is limited by its nature of being a retrospective database review. While the source of data from ICES is rich, it is only as strong as the data entered into the system. Within the provincial database, we did not have access to patient level data including LVEF. Furthermore, the database does not permit access to patients prescribed oral medications or details about participation in clinical trials. We were also not able to clearly discern which line of therapy patients received trastuzumab due to differences in recording this data. There is a possibility of misclassification of the outcome in our study due to the outcome definition being based on diagnostic codes and hospital visits. Using this approach, we likely overestimate the incidence of severe or clinically significant cardiotoxicity in our cohort as it is conceivable that patients were referred to cardiology for asymptomatic changes in left ventricular function. The administrative data used in this study may not capture care that is continued and provided at other hospitals. Furthermore, our study excluded patients with a prior history of heart failure and therefore we were not able to assess the risk associated with that comorbidity. We also acknowledge that the proportion of *de novo* stage IV cancer patients was higher than expected which may reflect timing and limitations within the database. In comparison, the MA.31 trial also had a large portion of patients (43%) with *de novo* stage IV HER2 + disease. This however is unlikely to impact our primary endpoints. Given that this is a population-based study which relies on reporting, there is a selection bias toward patients with clinical heart failure and although recent guidelines recommend baseline imaging, these were not standard of care during the chosen study period. In terms of therapy, this study was limited to trastuzumab and pertuzumab and did not include other anti-HER2 targeted therapies (e.g., lapatinib, neratinib) used in the advanced setting which limits its generalizability. Finally, we did not capture treatments received subsequent to trastuzumab and thus cannot comment on the impact of those agents.

In conclusion, this is the largest population-based study to report CEs in aBC patients treated with trastuzumab-based therapy. The incidence of CEs was low and cardiac death was very rare. More than a third of deaths were attributable to breast cancer. Routine cardiac imaging was performed per provincial guidelines in most patients, but the rates of cardiac events were comparable regardless of whether or not patients had surveillance LVEF assessments. Our results combined with other recent cohort studies challenge the requirement for routine cardiac monitoring in this patient population. Given the chronicity of this disease, patients may be on anti-HER2 targeted therapy for several years with exposure to serial cardiac monitoring with little clinical benefit. We recommend using a personalized risk-based approach that incorporates clinical risk factors and cardiovascular health. Further research on defining the best cardiac imaging techniques with incorporation of cardiac biomarkers is needed to optimize cardiac surveillance strategies in this patient population.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was reviewed and approved by the Ottawa Health Sciences Research Ethics Board, Ottawa, Canada.

AUTHOR CONTRIBUTIONS

MR contributed to the analysis and interpretation of the results, the design of the tables and figures, and writing of the manuscript. CK contributed to the design of the tables and figures and writing of the manuscript. SD contributed to organization and supervision of the research. MR, CK, IL, MT, KP, SH, and SD contributed to the literature review, writing of the manuscript, and critical review. All authors contributed to the article and approved the submitted version.

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A Nomogram for Predicting Survival in Patients With Colorectal Cancer Incorporating Cardiovascular Comorbidities

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Background: Cardiovascular comorbidities (CVCs) affect the overall survival (OS) of patients with colorectal cancer (CRC). However, a prognostic evaluation system for these patients is currently lacking.

Objectives: This study aimed to develop and validate a nomogram, which takes CVCs into account, for predicting the survival of patients with CRC.

Methods: In total, 21,432 patients with CRC were recruited from four centers in China between January 2011 and December 2017. The nomogram was constructed, based on Cox regression, using a training cohort (19,102 patients), and validated using a validation cohort (2,330 patients). The discrimination and calibration of the model were assessed by the concordance index and calibration curve. The clinical utility of the model was measured by decision curve analysis (DCA). Based on the nomogram, we divided patients into three groups: low, middle, and high risk.

Results: Independent risk factors selected into our nomogram for OS included age, metastasis, malignant ascites, heart failure, and venous thromboembolism, whereas dyslipidemia was found to be a protective factor. The c-index of our nomogram was 0.714 (95% *CI*: 0.708–0.720) in the training cohort and 0.742 (95% *CI*: 0.725–0.759) in the validation cohort. The calibration curve and DCA showed the reliability of the model. The cutoff values of the three groups were 68.19 and 145.44, which were also significant in the validation cohort ($p < 0.001$).

Conclusion: Taking CVCs into account, an easy-to-use nomogram was provided to estimate OS for patients with CRC, improving the prognostic evaluation ability.

Keywords: colorectal cancer, comorbidity, cardiovascular disease, prognosis, nomogram

Abbreviations: CVCs, cardiovascular comorbidities; CRC, colorectal cancer; OS, overall survival; C-index, Harrell's concordance index; DCA, decision curve analysis; CI, confidence interval; HR, hazard ratio.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the fifth most common cause of cancer-related deaths (8.6%) in China, which imposes a heavy burden on the healthcare system (1, 2). Increasing evidence suggests that CRC and cardiovascular comorbidities (CVCs) are not separate disease entities, and studies have described the shared pathophysiology between CRC and CVCs (3). Patients with CRC are at 2–4 times increased risk of developing cardiovascular diseases (4), and CVCs are the leading cause of death among patients with CRC (5), which due to the fact that CVCs not only increase the non-cancer mortality but also restrict the options for treatment. Therefore, the OS of patients with CRC is not only influenced by cancer-related factors but also closely related to CVCs. However, the prognostic models combined with CVCs factors for patients with CRC are still lacking.

The TNM staging system of the 8th edition of the American Joint Committee on Cancer (AJCC) is widely used in prognosis prediction for patients with CRC. However, it may be inapplicable to patients with CVCs because it only includes cancer-related variables (6). To integrate CVCs and cancer-related prognostic factors for providing more individualized risk estimates, a nomogram, which could estimate numerical probabilities for individual patients by incorporating prognostic factors (7, 8), can be used to develop a more individual and more accurate prognostic tool.

In this study, we aimed to develop and validate a prediction model, which was visualized as a nomogram, for the OS of patients with CRC and CVCs, and stratify patients into three risk groups. This nomogram may provide a more individualized prognosis for these patients and guide the selection of treatment regimens.

PATIENTS AND METHODS

Patients Selection and Predictor Variables

Data present in this study were collected through a computer-assisted personal interview system from January 2011 to December 2017. We collected information on newly diagnosed primary CRC inpatients from four cancer specialized hospitals in China (Yunnan Cancer Hospital, Jiangxi Cancer Hospital, Chongqing Cancer Hospital, and Yuncheng Central Hospital). The data for birthdays, gender, diagnosis dates, and the information of diagnosis were abstracted from hospital discharge records. Each record had information on up to 30 diagnoses, which were used to identify comorbidities of patients. The follow-up information was collected by clinic visit, hospitalization, or telephone call.

The exclusion criteria were as follows: (1) patients who were under the age of 18 or over the age of 90; (2) pathologically confirmed benign cancers or cancer-like diseases; (3) patients with more than one cancer; and (4) patients who had incomplete data. A total of 21,432 patients were selected in the final dataset. We divided the dataset into a training cohort (19,102 patients, from Yunnan, Jiangxi, and Yuncheng) and a validation cohort (2,330 patients from Chongqing). The study was conducted in accordance with the Institutional Review Board of the participating hospitals and informed consent was waived because the data were de-identified.

The CVCs in our study defined as the pre-existing cardiovascular diseases when cancer was diagnosed included: hypertension, diabetes, coronary artery disease, heart failure, dyslipidemia, atrial fibrillation, cerebrovascular disease, pericardial effusion, and venous thromboembolism. The cancer-related variables in our study included: age of diagnosis, gender, metastasis (M stage in TNM staging system, M1a denotes metastasis to one distant site or organ, M1b denotes metastasis to more than one, and M1c for peritoneal metastasis), malignant pleural effusion, and malignant ascites. Continuous variables were translated into categorical variables.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (IBM, Chicago, IL, United States) and R version 4.4.0.[1] Overall survival (OS) was defined as the time from the cancer diagnosis to the patient's death or censored at the time of the last follow-up. Variables were screened by univariate Cox regression analysis ($p < 0.10$), and every variable of CVCs and cancer was included in the process of variables screening. Variables selected from the screening process were subjected to a multivariable Cox regression analysis model ("nomogram model") which was used to develop the nomogram. Furthermore, the backward stepwise and forward stepwise methods were used to identify the combination of variables, and the two methods were compared by the value of the Akaike information criterion (AIC). We examined the proportional hazard assumption by plotting the log minus log survival curves and found it to hold. A decision curve analysis (DCA) was conducted to determine the clinical usefulness of the prediction model by quantifying the net benefits at different threshold probabilities (9). To evaluate the significance of adding CVCs into the CRC prognostic scoring system, the multivariable Cox regression model which only included cancer-related variables ("cancer model") was developed, it was used in DCA to compare with the nomogram model.

The discrimination and calibration of the nomogram model were assessed by the concordance index (c-index) and calibration curve which were subjected to 1,000 bootstrap resamples for internal validation, and the validation cohort was used in external validation. The clinical utility of the "nomogram model" and "cancer model" was compared by DCA. We calculated the nomogram score of each patient and used the x-tile software (a bioinformatics tool for outcome-based cut-off value optimization) (10) to divide patients into three groups: low risk, middle risk, and high risk. The Kaplan–Meier method and log-rank test were used for survival analysis. All statistical tests were two-tailed, and the value of $p < 0.05$ was considered to be statistically significant.

[1] http://www.r-project.org/

TABLE 1 | Baseline clinical features.

Characteristics	Training cohort (*n* = 19,102)	Validation cohort (*n* = 2,330)
Age, year	59.06 ± 12.33	61.01 ± 13.06
Age stratification, year		
<50	4,106 (21.5%)	477 (20.5%)
50–59	5,478 (28.7%)	477 (20.5%)
60–69	5,497 (28.8%)	732 (31.4%)
70–79	3,298 (17.3%)	487 (20.9%)
≥80	723 (3.8%)	157 (6.7%)
Gender		
Male	10,925 (57.2%)	1,368 (58.7%)
Female	8,177 (42.8%)	962 (41.3%)
T stage[a]	*n* = 3,180	*n* = 545
T1	80 (2.5%)	27 (5.0%)
T2	359 (11.3%)	118 (21.7%)
T3	1,090 (34.3%)	204 (37.4%)
T4	1,650 (51.9%)	196 (36.0%)
N stage[a]	*n* = 3,180	*n* = 545
N0	1,608 (50.6%)	258 (47.3%)
N1	773 (24.3%)	174 (31.9%)
N2	799 (25.1%)	113 (20.7%)
Metastasis		
M0	12,984 (68.0%)	1,357 (58.2%)
M1a	4,225 (22.1%)	743 (31.9%)
M1b	1,654 (8.7%)	203 (8.7%)
M1c	239 (1.3%)	27 (1.2%)
Pleural effusion	222 (1.2%)	44 (1.9%)
Malignant ascites	446 (2.3%)	93 (4.0%)
CVCs	4,148 (21.7%)	739 (31.7%)
Hypertension	2,614 (13.7%)	383 (16.4%)
Diabetes	1,282 (6.7%)	219 (9.4%)
Coronary artery disease	388 (2.0%)	124 (5.3%)
Dyslipidemia	34 (0.2%)	92 (3.9%)
Heart failure	108 (0.6%)	114 (4.9%)
Atrial fibrillation	69 (0.4%)	20 (0.9%)
Cerebrovascular disease	789 (4.1%)	118 (5.1%)
Venous thromboembolism	120 (0.6%)	48 (2.1%)
Pericardial effusion	46 (0.2%)	4 (0.2%)

Data are presented as mean ± SD or No (%).
CVCs, cardiovascular comorbidities.
[a] *3,725 patients had records of T stage and N stage.*

RESULTS

Basic Characteristics

A total of 19,102 patients with CRC were included in the training cohort, the mean age was 59.06 ± 12.33 years with 57.2% male, and 7,124 (37.3%) patients died of all causes (**Table 1**). The validation cohort included 2,330 patients and 815 (35.0%) patients died during the follow-up (**Table 1**). Patients in the validation cohort were older (61.01 ± 13.06) with a higher proportion of men (58.7%) and a higher prevalence of CVCs.

Variables Selection and Prediction Model Development

All variables except gender were selected using the univariable Cox regression analysis (**Table 2**) and were subjected to the multivariable Cox regression analysis. The backward stepwise method performed better than forward stepwise method in AIC (AIC for backward stepwise was 131,341.6, which was smaller than 131,355.2 for the forward stepwise). The result showed that age, metastasis, malignant ascites, dyslipidemia, heart failure, and venous thromboembolism had important predictive value for the prognosis of patients with CRC (**Table 2**). Interestingly, dyslipidemia, as a traditional risk factor for cardiovascular disease, was found to be a protective factor. In addition, patients with at least one kind of CVC have a higher risk of death than patients without (hazard ratio [*HR*] = 1.194 (1.130–1.261), $p < 0.001$, **Table 2**). The nomogram (**Figure 1**) was developed based on multivariable Cox regression analysis. By calculating the total point of a patient, and drawing a vertical line from the total point axis to three outcome axes, estimated 1-, 3-, and 5-year survival probabilities could be obtained.

Validation of the Prediction Model

The c-index of the training cohort was 0.714 (95% *CI*: 0.708–0.720) while in the validation cohort was 0.742 (95% *CI*: 0.725–0.759), which indicated acceptable discrimination. The calibration curves in the training cohort and validation cohort were closely aligned with the 45 degrees diagonal. It revealed good concordance between the nomogram predicted probabilities and the observed probabilities (**Figure 2**).

Decision Curve Analysis

In DCA (**Figure 3**), the net benefit of the decision curves for the "nomogram model" is higher than all patient dead scheme or no patient dead scheme. Furthermore, the "nomogram model" was constantly higher in net benefit compared with the "cancer model." The net benefit was comparable. It demonstrated that our nomogram in predicting OS is more beneficial than that of only including cancer-related variables.

Risk Stratification of Overall Survival by the Prediction Model

We divided patients into three groups: low risk (0–68.19), middle risk (68.19–145.44), and high risk (>145.44) according to their nomogram scores by x-tile software (10). The survival curve of the training cohort and validation cohort is shown in **Figure 4**. To verify the reliability of the cutoff value, log-rank tests were used to compare survival between three groups ($p < 0.001$, the Bonferroni-corrected level of significance in this analysis was $p < 0.0167$).

DISCUSSION

The information of 21,432 patients with CRC cancers was collected in our study. We developed and validated a nomogram, which combined cancer and CVC variables, for survival prediction. Compared with only focusing on cancer-related variables, the addition of CVCs variables could provide individual prognostic information and guide clinical decision-making. To our knowledge, this is the first nomogram assessing the prognostic impact of CVCs in patients with CRC.

TABLE 2 | Univariate and multivariate cox regression analysis between characteristics and overall survival (OS).

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Age stratification, year		<0.001		<0.001
<50	Reference		Reference	
50–59	1.002 (0.934–1.075)	0.957	1.076 (1.003–1.154)	0.041
60–69	1.129 (1.054–1.209)	0.001	1.283 (1.197–1.375)	<0.001
70–79	1.489 (1.384–1.602)	<0.001	1.887 (1.753–2.032)	<0.001
≥ 80	2.300 (2.065–2.562)	<0.001	3.252 (2.916–3.627)	<0.001
Gender				
Male	Reference			
Female	1.006 (0.959–1.054)	0.818		
Metastasis		<0.001		<0.001
M0	Reference		Reference	
M1a	4.068 (3.861–4.286)	<0.001	4.234 (4.015–4.464)	<0.001
M1b	4.789 (4.477–5.122)	<0.001	4.956 (4.626–5.309)	<0.001
M1c	5.612 (4.833–6.517)	<0.001	5.147 (4.397–6.025)	<0.001
Pleural effusion	3.187 (2.740–3.706)	<0.001		
Malignant ascites	3.664 (3.291–4.080)	<0.001	1.686 (1.505–1.890)	<0.001
Hypertension	1.096 (1.026–1.172)	0.006		
Diabetes	1.085 (0.991–1.188)	0.078		
Coronary artery disease	1.248 (1.071–1.455)	0.005		
Dyslipidemia	0.414 (0.186–0.921)	0.031	0.418 (0.188–0.932)	0.033
Heart failure	5.513 (4.532–6.705)	<0.001	2.572 (2.111–3.135)	<0.001
Atrial fibrillation	1.372 (0.970–1.942)	0.074		
Cerebrovascular disease	1.303 (1.168–1.454)	<0.001		
Venous thromboembolism	2.072 (1.640–2.617)	<0.001	1.308 (1.034–1.653)	0.025
Pericardial effusion	3.000 (2.152–4.182)	<0.001		
CVCs[a]	1.194 (1.130–1.261)	<0.001	–	

[a]*Patients have at least one kind of cardiovascular comorbidities.*

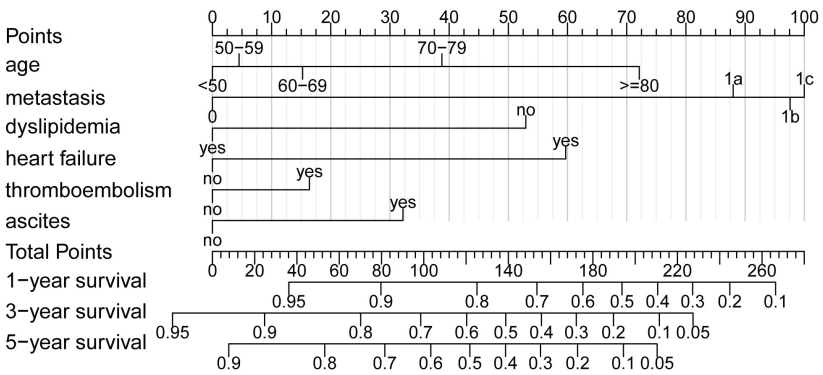

FIGURE 1 | Nomogram. Nomogram to predict the probability of 1-, 3-, and 5-year overall survival (OS) in patients with colorectal cancer (CRC). In addition, 1-, 3-, and 5-year OS could be obtained by adding up the points of each corresponding variable.

With the differences in comorbidities definitions, study populations, and cancer types, it is difficult to state with certainty how common the CVCs are (5). However, our result showed that the prevalence of CVCs in patients with CRC was higher than in the general population (**Table 1**) (11). Although the effects of different CVCs on patients with CRC are different, CVCs make the risk of death in patients with CRC rise by 19.4% relatively (**Table 2**). We found heart failure and venous thromboembolism were the risk factors for the prognosis of patients with CRC, whereas dyslipidemia was a protective factor.

In recent years, various prognostic models of CRC have been described (12–15). Despite these models being used in different clinical scenes, age and metastasis (or M stage) can be found in almost all models. It is known that the OS

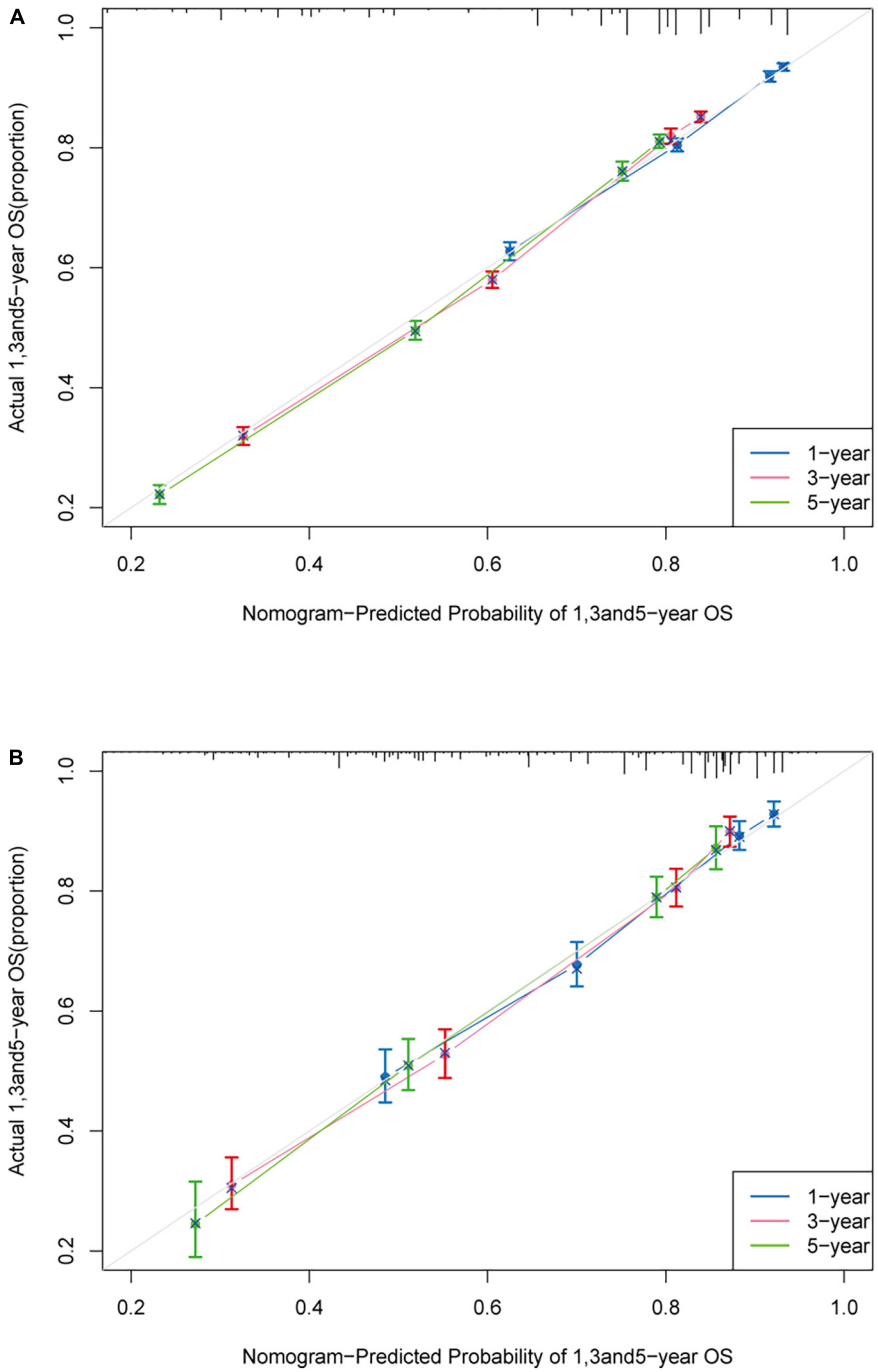

FIGURE 2 | Calibration plot. Calibration curve of the nomogram both in the training and validation cohort. Predicted survival probability produced by nomogram is *x*-axis, and actual survival is *y*-axis, close alignment with 45 degrees diagonal represents the good estimation. **(A)** 1-, 3-, and 5-year OS of the training cohort; **(B)** 1-, 3-, and 5-year OS of the validation cohort.

of patients with CRC declines with age (16), and that is consistent with our study. The effect of age on the prognosis is increasing with age, which can be seen in the increasing score interval of age stratification in our nomogram. The peritoneal metastasis of patients with CRC, which has a poor prognosis, is often viewed as a preterminal state reflecting widespread cancer dissemination (17). For this reason, the M stage has been expanded in the 8th edition of the AJCC TNM staging system (adding M1c for peritoneal metastasis) (8). However, an analysis showed that in 72 clinical trials of metastatic CRC, only seven trials include peritoneal metastasis (18). Peritoneal metastasis as a prognostic indicator has rarely been included

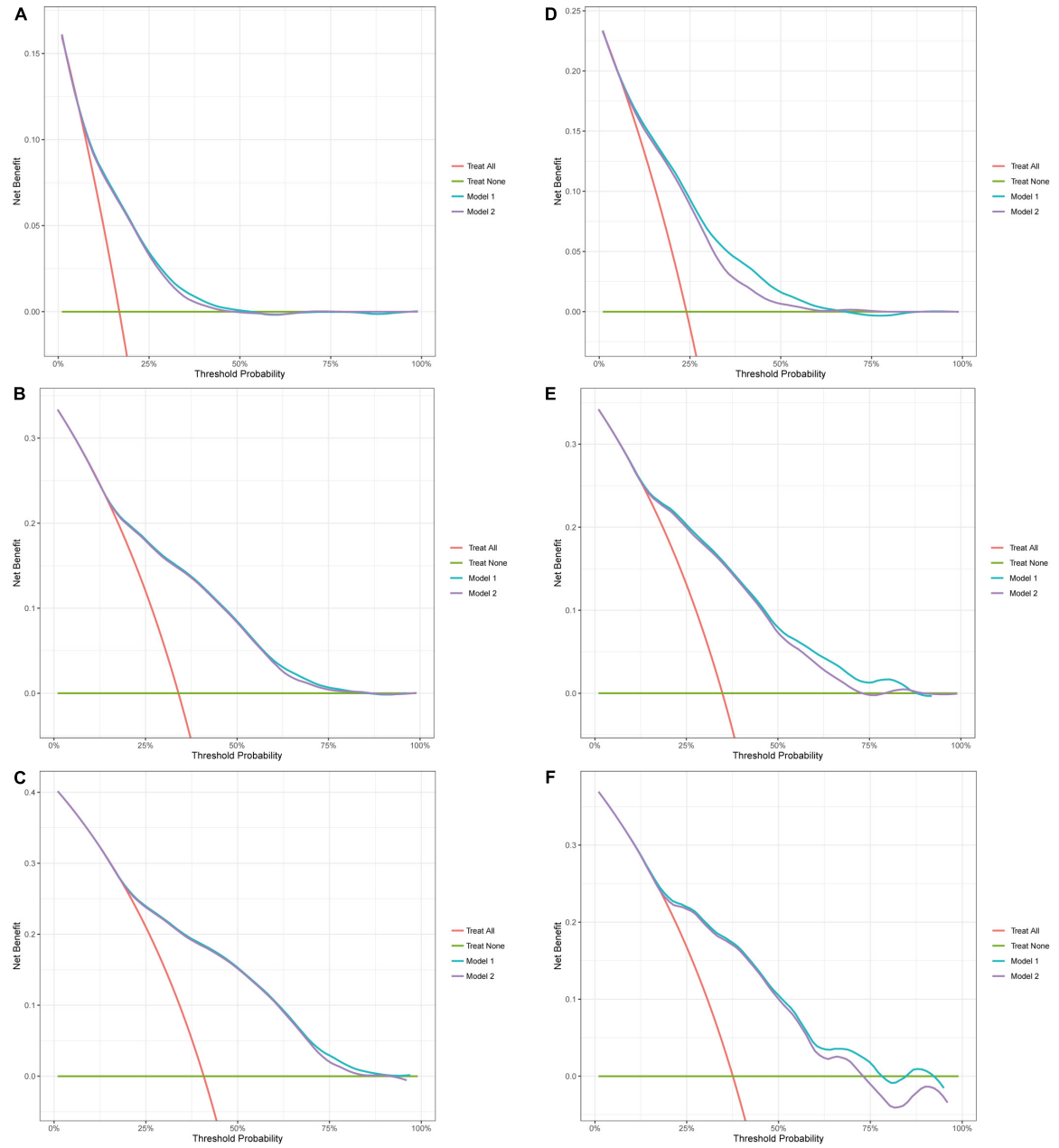

FIGURE 3 | Decision curve analysis (DCA). Decision curve analysis for OS. Red line (Treat all): "all patient dead scheme." Green line (Treat none): "no patient dead scheme." Blue line (Model 1): "nomogram model." Purple line (Model 2): "cancer model." **(A)** 1-year DCA in the training cohort; **(B)** 3-year DCA in the training cohort; **(C)** 5-year DCA in the training cohort; **(D)** 1-year DCA in the validation cohort; **(E)** 3-year DCA in the validation cohort; **(F)** 5-year DCA in the validation cohort.

in previous studies. We extracted the metastatic sites from the diagnostic information in our database and demonstrated that the prognosis of patients becomes poor with the rise of the M stage. It should be noted that the gold standard to assess peritoneal metastasis is operative exploration, and for patients who did not undergo surgery, conventional imaging examinations, such as computerized tomography (CT) lack the resolution to detect early peritoneal metastasis (19), thus the incidence of peritoneal metastasis may be underestimated. Furthermore, the most common cause of malignant ascites is peritoneal dissemination, which accounts for approximately 53% of cases (20), although the presence of malignant ascites is an apparent poor prognostic factor (21), previous prognostic prediction models rarely incorporate this variable. Our study showed that malignant ascites as a predictor of prognosis cannot be neglected.

Heart failure, which has the highest score among CVCs in our nomogram, is one of the most significant risk factors for

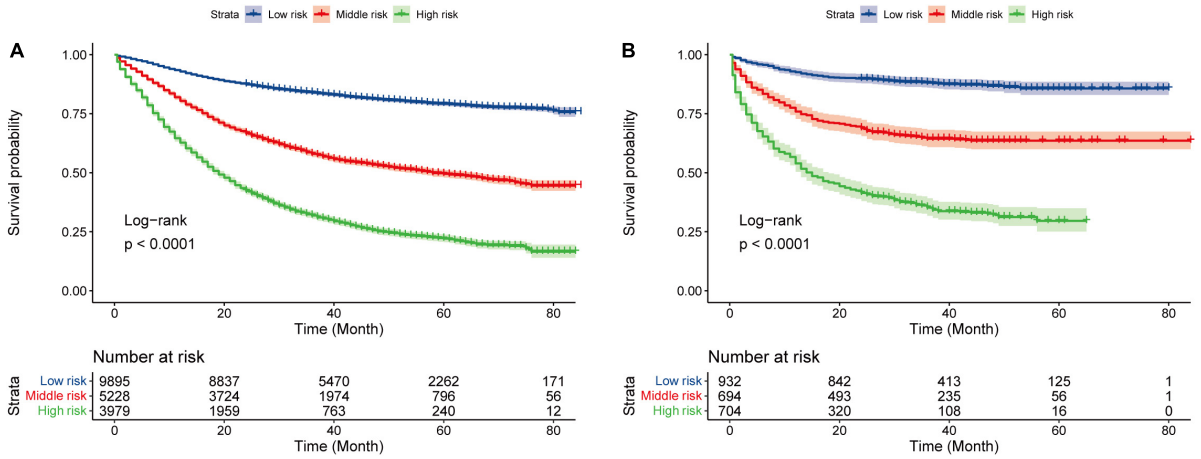

FIGURE 4 | Kaplan–Meier curves for three groups. Kaplan–Meier curves for three groups in the training cohort **(A)** and the validation cohort **(B)**.

prognosis in patients with CRC. It is consistent with the study of Gross et al., which showed that about 9% of patients with stage I–III colorectal cancer died due to heart failure, and heart failure was the most significant comorbidity affecting the OS (22). Heart failure can not only increase the non-cancer mortality for patients with CRC (23) but also restrict the options for treatment and reduce patients' compliance (24, 25). Despite advances in management, heart failure still has a worse prognosis than some of the common cancers in both men and women (26). Furthermore, our study shows that 85.2% of heart failure patients entered the high-risk group, and the OS of patients with heart failure is significantly shorter than that of patients without heart failure [median: 10.0 (Q1: 1.0, Q3: 30.2) vs. median: 36.0 (Q1: 21.0, Q3: 52.0), $p < 0.001$].

Cancer is a significant risk factor for venous thromboembolism (VTE). Thrombotic events sometimes may become the first manifestation of cancer (27). Research shows that the 2-year cumulative incidence rate of VTE after cancer diagnosis was 3.1% (28). However, autopsy studies have confirmed the occurrence of pulmonary embolism in patients with CRC was as high as 28% (29). This means that many of the thrombotic events in patients with CRC are not detected because of the accuracy of examination and the unremarkable clinical manifestations. The relatively low incidence of thrombotic events in our cohort is also related to this reason. VTE is the second leading cause of death in patients with cancer after cancer progression (30). The development of VTE in patients with cancer reflects their enhanced cancer-associated thrombin generation, which demonstrates that the cancer is biologically more aggressive (31). Therefore, for patients with VTE or with high thrombotic risk based on predictive models [such as, the Khorana risk scoring model (32) and the COMPASS-CAT risk assessment model (33)], rational intervention for the VTE could improve these patients' outcomes (34).

However, the interaction between CVCs and cancer is sometimes protective. Although some studies on the relationship between dyslipidemia and cancer considered dyslipidemia as a protective factor (35, 36), the results of different CRC studies conducted to date have been inconsistent (37, 38). Our study identified that dyslipidemia was a protective factor. Research showed that serum lipids, especially cholesterol, are involved in many processes of cancer development (39) and make up the lipid rafts in the cancer cell membrane, which are involved in the transduction of signaling pathways related to the cancer cell survival (40). To meet the increasing need for cholesterol, the process of cholesterol absorption is enhanced in cancer cells, which may lead to a decreased cholesterol concentration in patients with CRC (41). Furthermore, the changed concentration of serum lipid might be related to the malignancy of CRC. Zhang et al. have found that with the progression of the TNM stage in patients with CRC, the concentration of serum total cholesterol and triglyceride were reduced significantly (42). These results showed the negative correlation between the concentration of serum lipid and the severity of CRC, and dyslipidemia could thus reflect the prognosis of patients with CRC indirectly. However, more studies are still needed to validate the impact of various lipid biomarkers on the prognosis of CRC.

The TNM staging system is still the "golden standard" for CRC prognosis in clinics. However, as a classifier that groups patients into ordered risk strata, the lack of CVCs factors caused its inability to deal with heterogeneity within risk groups caused by CVCs. Thus, the risk calculators (such as nomograms), which could utilize multiple prognostic factors to provide more individualized risk estimates, gained increasing popularity. To improve the quality and acceptability of the risk models for patients with cancer, AJCC has put forward the acceptance criteria for risk models for individualized prognosis (43). However, of the 29 published risk calculators for colon or rectal cancer, only 3 have been endorsed by the AJCC (8), and the models focused on CVCs are still lacking. Therefore, in this study, we followed these acceptance criteria and developed an easy-to-use nomogram, which included the variables of cancer and CVCs that are readily available in clinical work and most relevant to the prognosis for patients with CRC and CVCs.

In the following work, we will work to compare the availability of our nomogram and TNM staging system in guiding the choice of patients' treatment options.

Limitation

Although our study had a large sample size, there were still some limitations. First, we could not be sure of the causal relationship between CVCs and CRC because the time of CVCs emerging was not recorded, so the impact of CVCs on the prognosis of patients with CRC was our point. Second, the therapeutic regimens of CRC developed rapidly during the data collection period, but the database lacked detailed treatment regimens. Considering that some anticancer therapies potentially have cardiotoxicity, adding more treatment-related variables to the nomogram or developing different nomograms based on different therapies is meaningful. Third, our nomogram did not include molecular or genetic biomarkers that had prognostic value. However, considering the high cost of biomarkers tests, our nomogram may also be easier to apply in the clinic.

CONCLUSION

In this study, two CVCs and three cancer-associated characteristics were identified as risk factors for the prognosis of patients with CRC, whereas dyslipidemia exerted a protective effect. Taking CVCs into account, we developed and validated a nomogram that could estimate OS for patients with CRC. It may improve the prognostic evaluation ability and facilitate individualized patient management.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was reviewed and approved by and conducted in accordance with the Institutional Review Boards of Yunnan Cancer Hospital, Jiangxi Cancer Hospital, Chongqing Cancer Hospital, and Yuncheng Central Hospital. The requirement of written informed consent was waived because the data were deidentified.

AUTHOR CONTRIBUTIONS

HW and DL: conception of design and analysis, and interpretation of data. HW, DL, JSY, WY, YQ, ZY, WG, MZ, HA, XZ, SQ, XG, JGY, HX, JW, TW, TT, HL, ZB, and YM: data collection and drafting of the manuscript or revising it critically for important intellectual content. JSY and WY: final approval of the manuscript submitted. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Report: Multimodal Imaging Guides the Management of an Eosinophilic Leukemia Patient With Eosinophilic Myocarditis and Intracardiac Thrombus

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Background: Eosinophilic leukemia (EL) is a rare, serious and potentially life-threatening condition characterized by the overproduction of eosinophils leading to tissue eosinophilic infiltration and damage. Although multiple organ systems may be involved, progressive eosinophilic myocarditis (EM) is the most common cause of morbidity and mortality. Early diagnosis and follow-up surveillance combined with multimodal imaging are crucial for appropriate treatment of EM.

Case Summary: It's a rare case of EL with EM and intracardiac thrombus in a 59-year-old patient who presented with asthenia for 3 weeks. Full blood count analysis indicated significant eosinophilia. Bone marrow aspirate revealed dysplastic eosinophilia and a FIP1L1-PDGFRA fusion gene (4q12) was detected, confirming EL. Echocardiography revealed EM with intracardiac thrombus. This was later confirmed by cardiac magnetic resonance imaging. The patient was commenced on imatinib and prednisolone and good clinical response was obtained. Through 18F-FAPI PET/CT imaging, we obtained *in vivo* visualization of fibroblast activation changes in the early stage of cardiac structure remodeling. With anti-fibrotic therapy after heart failure, the patient achieved a good clinical response.

Conclusion: This case demonstrates *in vivo* visualization of fibroblast activation after EM. Multimodality imaging can provide early diagnosis and may guide tailored antifibrotic therapy in early stage of EM.

Keywords: 18F-FAPI, eosinophilic leukemia, thrombotic, fibroblast activation protein, PET/CT

INTRODUCTIONS

Eosinophilic myocarditis (EL) is a neoplastic condition with persistent eosinophilia as the main hematological abnormality and with the eosinophils being part of the neoplastic clone (1). These eosinophils have a clonal molecular genetic abnormality that excludes PDGFRA, PDGFRB, and FGFR1 rearrangements and PCM1-JAK2, ETV6-JAK2, and BCR-JAK2 fusion genes, especially

a FIP1L1–PDGFRA fusion gene resulting from a cryptic deletion of part of the long arm of chromosome 4 (2). Reducing eosinophil levels both in peripheral blood and tissue, preventing end-organ damage and avoiding adverse thrombotic events are the three primary goals for the management of EL (3). EM, a common cardiac complication of EL and the major cause of morbidity and mortality which can occur in about 40–50% of patients (4), is an inflammatory disorder of the myocardium characterized by eosinophilic infiltration (5). EM usually undergoes acute necrotic, thrombotic, and fibrotic stages, which may overlap. Early diagnosis and follow-up surveillance combined with multimodal imaging are crucial for appropriate treatment of EM.

CASE PRESENTATION

A 56-year-old male presented with asthenia for 3 weeks. He had no food or drug allergies, no laboratory evidence of parasite, fungal or virus infection. Vital signs were normal with blood pressure of 130/80 mmHg, heart rate of 80 beats/min and respiratory rate of 12 breaths/min on presentation. Complete blood examination revealed hemoglobin 95 g/dL, total leukocyte count 27.74 $\times$ 10^9/L with an absolute eosinophil count of 12.94 $\times$ 10^9/L, accounting for 59.8% of cells, platelet count 55 $\times$ 10^9/L, elevated N-terminal pro-brain natriuretic peptide (NT-proBNP) level (604.6 ng/L, normal range <450 ng/L) and C-reactive protein (11 mg/L, reference: <8 mg/L). High Sensitivity Cardiac Troponin I (hs-cTnI) level was within the normal range. Other laboratory results were unremarkable, including anti-nuclear antibody screen. The electrocardiogram (ECG) revealed sinus rhythm (**Figure 1A**). Neither coronary computed tomographic angiography nor encephalic computed tomography showed significant lesions. Echocardiography demonstrated slightly increased chamber size, normal left ventricular systolic function (left ventricular ejection fraction of 57%), and abnormal diastolic function of grade III. There was a large, heterogeneous cardiac mass (39 mm $\times$ 22 mm, arrow) in the left ventricle (LV). The most probable diagnosis was Löffler's endocarditis (LE; cardiac involvement in hypereosinophilia) with intracardiac thrombus (**Figures 2A,B**). A cardiac magnetic resonance (CMR) short-axis image showed a curved line of late gadolinium enhancement (LGE) located subendocardial in the apical-middle heart of LV. The arc-shaped unenhanced area in the left ventricular cavity and the medical history were consistent with eosinophilic endocarditis with subendocardial thrombosis (3) (**Figure 3**). Because of the drastically increased eosinophils, we suspected a myeloproliferative disorder. We did a bone marrow examinations including aspiration cytology, biopsy, cytogenetic, and gene rearrangement analysis. While waiting for the results of the examinations, on day 3 of his admission, the patient complained of progressive development of chest pain. The electrocardiographic examination showed a new ST segment depression with T-wave inversion in precordial leads (**Figure 1B**). Laboratory findings demonstrated an elevated hs-cTnI and NT-proBNP of 2.72 and 1,789 ng/L, respectively, indicating ongoing myocardial damage. After treatment with nitrates to dilate the coronary arteries, the chest pain disappeared. As the symptoms were related to EM, the cause might be coronary artery spasm or a small thromboembolism obstructing the microcirculation, but the patient refused to undergo coronary angiography. Subsequently, bone marrow aspirate revealed dysplastic eosinophilia and eosinophilic promyelocytes. A normal karyotype (46, XY) was present and a FIP1L1-PDGFRA fusion gene (4q12) was detected. The marrow B- and T-cell receptor rearrangement analysis and flow cytometry showed no B- or T-cell clone, confirming EL. The patient was treated with glucocorticoid. The initial medication prescribed was intravenous methylprednisolone (60 mg per day for 3 days and then 40 mg per day for 4 days), then which was switched to oral prednisolone (50 mg per day), with a gradual tapering of the doses and imatinib mesylate (a tyrosine kinase inhibitor) of 100 mg/day was commenced. After only 4 days on imatinib and prednisolone, and the patient's eosinophil cell levels returned to normal (the lowest level was 0.01 $\times$ 10^9/L). After diagnosis of intracardiac thrombus, he was initially treated with low-molecular-weight heparin (1 mg/kg/dose q12 h) for 7 days. The heparin was gradually switched to warfarin. Warfarin was adjusted to a PT INR of 2–3. Before discharge, the hs-cTnI and NT-proBNP levels had significantly decreased to 0.032 and 617.8 ng/L, respectively. Furthermore, an ECG showed the normalization of ST segment and T wave (**Figure 1C**).

The patient underwent twice 18F-FAPI PET/CT for the visualization of cardiac fibroblast activation during treatment. Before discharge, on whole-body 18F-FAPI PET imaging, heterogeneously increased accumulations of 18F-FAPI were seen in endomyocardial. No other suspected fibrosis with 18F-FAPI accumulation was found (**Figures 4A,B**). Given the presence of myocardial fibrosis, he was treated for myocardial fibrosis and heart failure with angiotensin receptor-neprilysin inhibitor (ARNI), β-blockers and spironolactone. Good clinical response was obtained and after 2 months of treatment, follow-up PET/CT revealed that accumulations of 18F-FAPI in endomyocardial were significantly reduced (**Figures 4C,D**).

The patient was closely followed up in both cardiology and hematology outpatient clinics over a course of 2 months. The eosinophil counts remained within the normal range, when the dose of prednisolone was gradually tapered to 5 mg per day. Peripheral blood examination gave the following results: hemoglobin, 124 g/dL; total leukocyte count 8.66 $\times$ 10^9/L with 6.71% eosinophils; and platelet count, 177 $\times$ 10^9/L. While remaining on warfarin, imatinib mesylate, prednisolone and heart failure therapy, he had no specific symptoms on evaluation in the outpatient clinic. Follow-up echocardiogram revealed normal left ventricular size and rapid regression of the left ventricular mass (6 mm $\times$ 13 mm, arrow) (**Figure 2C**).

During a 6-month follow-up, there was no recurrence of hypereosinophilia. The disease remained clinically stable. Echocardiogram showed that left ventricular thrombus had resolved (**Figure 2D**).

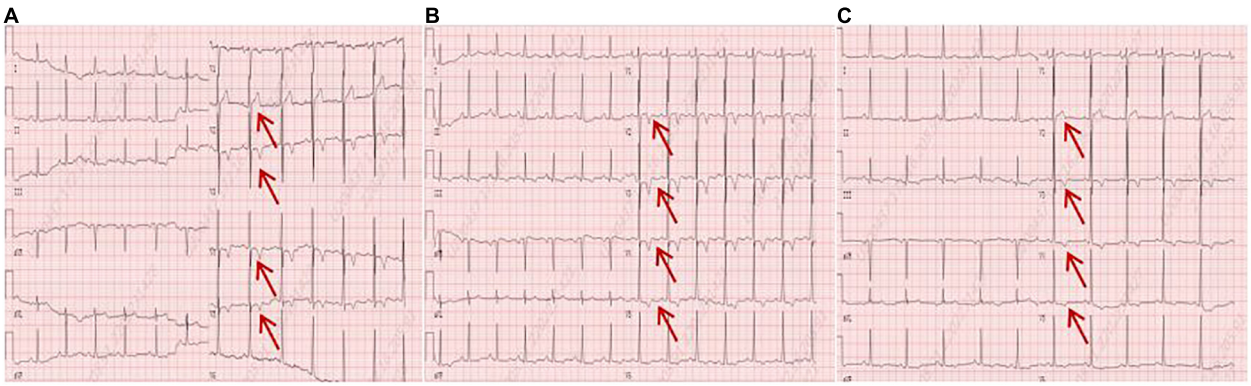

FIGURE 1 | (A) An electrocardiogram on admission. **(B)** An electrocardiogram revealed a new ST segment depression with T-wave inversion in the precordial leads (arrows). **(C)** An electrocardiogram before discharge showed the normalization of ST segment and T wave (arrows).

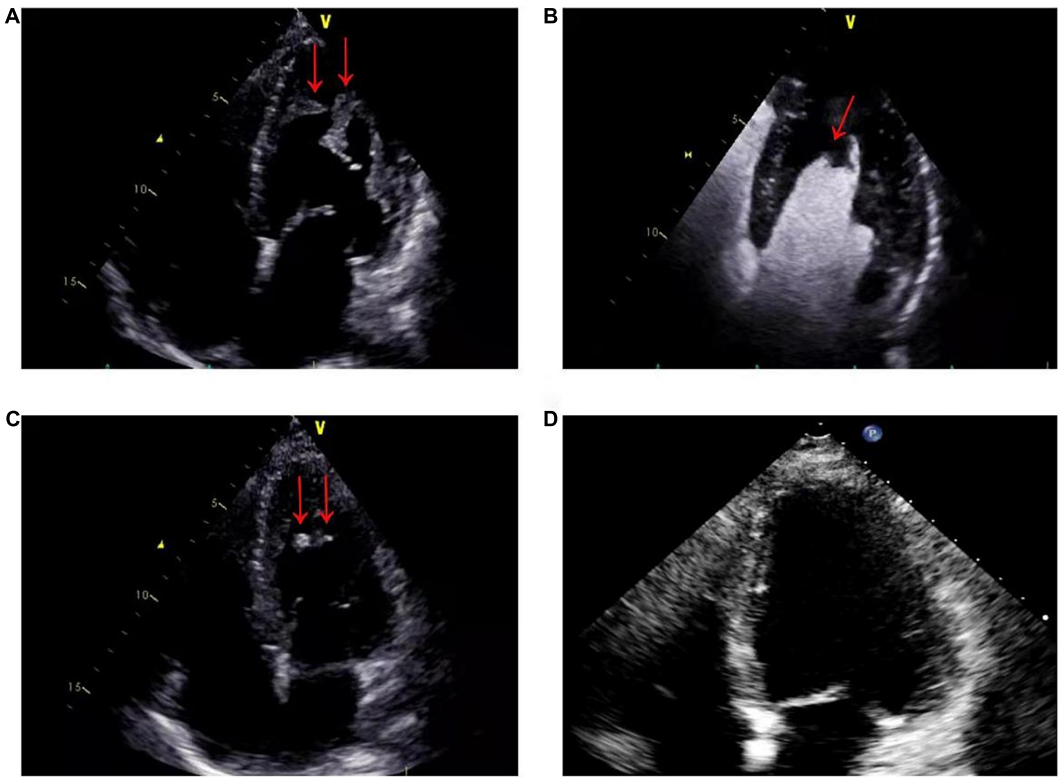

FIGURE 2 | (A) On admission, echocardiography showed that the apex of the left ventricle was filled with a thrombotic mass measuring 3.9 cm × 2.2 cm (arrows). **(B)** On admission, echocardiographic view after venous injection of an ultrasound contrast agent: thrombus (arrow) at left ventricle apex. **(C)** Two months after therapy, echocardiography showed a marked reduction in the size of the mass, with a thrombotic mass measuring 6 mm × 13 mm (arrows). **(D)** Six months after therapy, echocardiography showed that left ventricular thrombus had resolved.

DISCUSSION

The diagnostic features of EL include (1) persistent proliferation of eosinophil precursors and (2) marked eosinophilia in peripheral blood (eosinophils $>1.5 \times 10^9$/L) and bone marrow. Eosinophils play a role in host defense and immune regulation by secreting intracytoplasmic granules containing cytotoxic molecules, cytokines and chemokines, which can lead to end-organ injury. EM is a common cardiac complication of hypereosinophilia. The pathophysiology of EM progresses through three stages, namely acute necrotic stage, thrombotic stage and fibrotic stage, which may overlap (6). The prognosis for EM is often poor, with a 5-year mortality rate of up to 50% (7). Besides, endomyocardial exhibits various cardiac manifestations

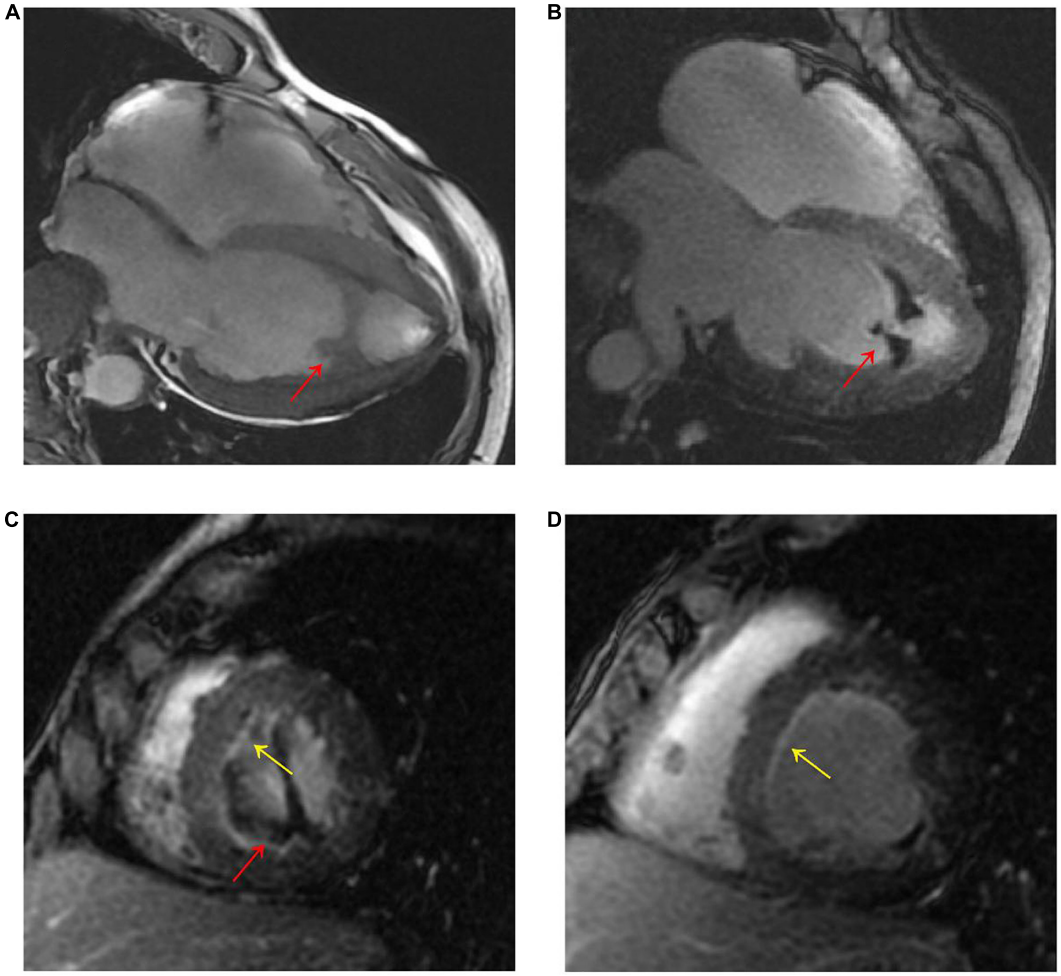

FIGURE 3 | (A) CMR four-chamber cine sequence exhibited arc-like region in the LV apex (red arrow). **(B–D)**, Late gadolinium enhancement imaging showed LV apex arc-like unenhanced region (red arrow), and diffuse subendocardial enhancement in LV mid- to apical parts (yellow arrow). The patient was diagnosed with Loeffler's endocarditis based on the typical clinical and imaging features.

depending on the severity and extent of the endomyocardial damage caused by eosinophilic infiltration (8). Therefore, EM often leads to the development of heart failure. Treatment of EM to prevent further deterioration includes halting of eosinophilic infiltration, counteraction of inflammation reaction, and prevention of myocardial fibrosis/intracardiac thrombus formation, in addition to symptomatic treatment of heart failure. If left untreated, serious complications such as valvular regurgitation or restrictive cardiomyopathy may occur at an advanced stage of EM. However, early and proper diagnosis is particularly challenging due to the subclinical presentation and the lack of characteristic signs in early stages of disease. In our case, the EM was most likely in the second or early third stage due to the presence of LV thrombus and fibroblast activation; echocardiography and PET-CT follow-up revealed improved LV function and suppressed fibroblast activation suggesting that the prompt treatment was protective against permanent cardiac dysfunction.

In this report, we describe a case of EL with EM and LV thrombus. Our case may offer several valuable clinical lessons. Imatinib therapy is indicated in all patients with either a chronic myeloid leukemia or a myeloproliferative disorder with PDGFRB and PDGFRA abnormalities (9). Corticosteroids are used as a cornerstone of myocarditis treatment, but its mechanism of action is not fully understood. It is thought that corticosteroids effectively inhibit the proliferative, developmental maturation pathway of eosinophils, thereby promoting redistribution of peripheral blood eosinophils (10). So a very effective treatment with imatinib and corticosteroids was possible in this case with FIP1L1–PDGFRA fusion gene, and early treatment with imatinib and corticosteroids did show favorable results. In the case described, the patient developed chest pain during treatment, elevated leukocytosis, eosinophilia, hs-cTnI, and NT-proBNP were evident and this was explained by infiltration of eosinophils into the myocardium. This infiltration leads to mitochondrial dysfunction, myocardial cell damage and necrosis

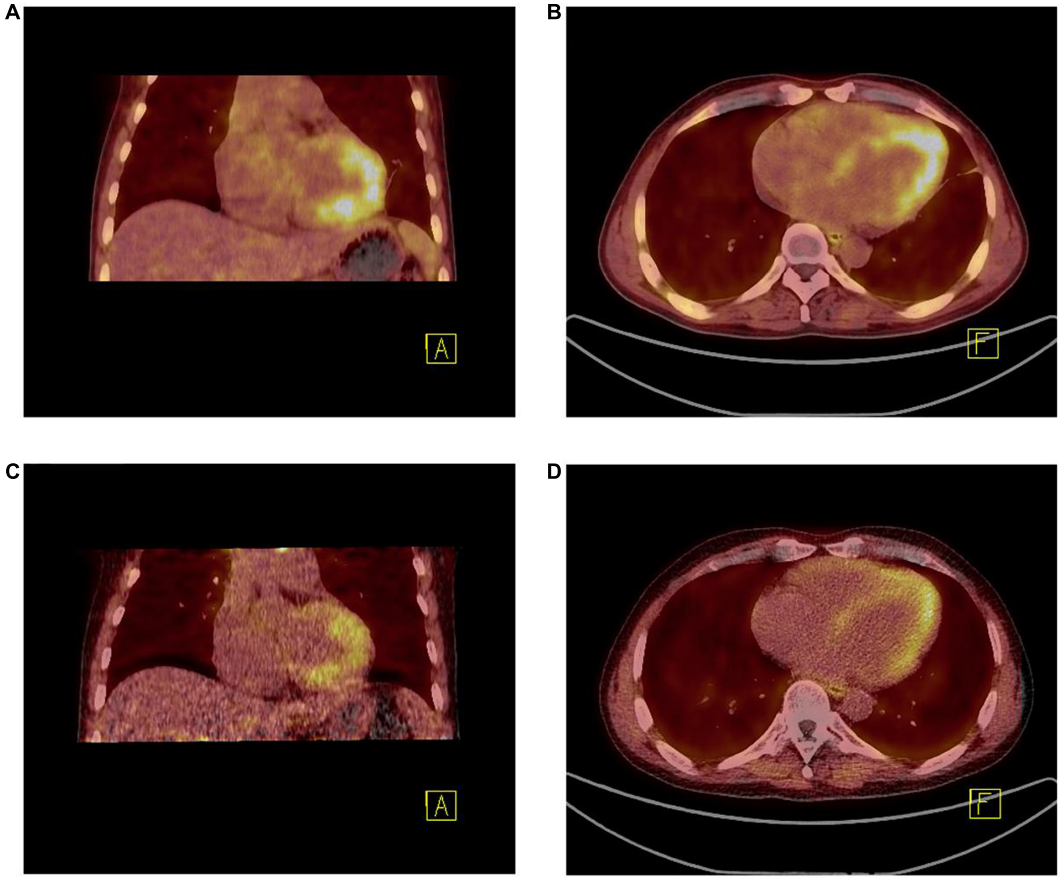

FIGURE 4 | (A,B) 18F-FAPI PET/CT fused image demonstrated the uptake [10.10 (SUVmax), 8.75 (SUVpeak), and 1.92 (SUVmean)] was much higher than that after 2 months of treatment **(C,D)** [8.40 (SUVmax), 6.91 (SUVpeak), and 1.32 (SUVmean)].

through the release of toxic granules, cationic proteins, pro-inflammatory cytokines and oxygen free radicals. Additionally, eosinophil infiltration can lead to coronary vasculitis and the release of cytokines that might cause coronary artery spasm (11). Therefore, in our case with chest pain, pronounced eosinophilia levels, and normal coronary computed tomography angiography, the diagnosis of EM should be considered. In some individuals, EM masquerades as acute coronary syndrome, with severe chest pain, electrocardiographic changes, and elevated serum levels of troponins (12). If patients who have hypereosinophilia presenting as acute coronary syndrome only receive stent implantation and anti-angina pectoris, coronary spasm will occur repeatedly and may even lead to sudden death. However, if the glucocorticoid or immunosuppressor is administered their condition will be improved (13, 14). In addition to first-line therapy (corticosteroids), early and aggressive anticoagulation therapy shall be ensured (15). While there is no standard recommendation for the use of anticoagulation for EL in the presence of thrombosis, anticoagulation should be considered in patients at high risk (i.e., with evidence of intracardiac thrombosis, deep venous thrombosis or recurrent thromboembolic events) with a view to treating this potentially fatal complication (16). In our case, after anticoagulation treatment, the patient achieved good therapeutic response.

CMR allows the visualization of myocardial inflammation, mural thrombus and fibrosis, with high sensitivity, specificity and accuracy, and the differentiation of inflammation from fibrosis based on the enhancement on LGE imaging (8, 17). EM is often associated with diffuse or patchy subendocardial LGE, which was shown in our patient. However, CMR is not specific for assessing early cardiac structural remodeling.

The newly developed PET tracer FAPI specifically targets fibroblast activation protein, which allows *in vivo* visualization of fibroblast activation during an early stage of cardiac structural remodeling (18). Currently, there are no other imaging studies of human fibroblast activation using this novel biosignal for EM. Given the limited treatment approach in an irreversible stage, earlier diagnosis and therapeutic intervention in EM progression is essential. Although endomyocardial biopsy (EMB) is the cornerstone of EM histological diagnosis, it is an invasive procedure and inflammatory cell infiltration is heterogeneous distributed, resulting in low diagnostic accuracy (19). Additionally, EMB is not indicated in all patients with

suspected EM (5). In our case, because the patient remained in good condition after starting corticosteroid therapy, biopsy was not performed, and the presence of left ventricular thrombosis was also an influencing factor. PET/CT imaging can safely provide valuable information about the location, extent, and pattern of endomyocardial fibrosis. Moreover, excessive fibrosis development is suggested to cause a progressive decline in ventricular diastolic and systolic function, which may lead to the development of chronic heart failure (20). Our case illustrates how EM due to EL could cause endomyocardial fibrosis, diastolic dysfunction, and thrombus (21). The FAPI uptake peaks on day 6 following ischemia (22), in the present case, the patient underwent FAPI imaging 16 days after chest pain, and the FAPI uptake area also did not conform to the patterns of myocardial ischemia. Thus, we excluded myocardial ischemia and considered that the FAPI imaging changes were caused by EM. Affected myocardium showed a partial to complete match between tracer uptake and confirmed lesion by contrast echocardiography and CMR. Pathologic features of EM fibrosis were identified. As fibroblast activation protein expression is low in most normal organs, it provides an interesting target for PET imaging to assess the severity of fibrosis (23). 18F-FAPI may represents a highly promising, novel biosignal for monitoring short-term structural remodeling processes and guiding tailored antifibrotic therapy in hypereosinophilia (24, 25). In our case, 18F-FAPI PET/CT, CMR and Echocardiography together present synergies that go beyond the limitations that each examination presents separately.

In conclusion, this case illustrates how EM due to EL could cause endomyocardial fibrosis, diastolic dysfunction, and thrombus. Advances in diagnostic imaging techniques may aid in the diagnosis of EM. Multimodality imaging can provide early diagnosis and potentially guide tailored antifibrotic therapy in EM.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the First Affiliated Hospital of Dalian Medical University. The patients/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 for the publication of this case report.

AUTHOR CONTRIBUTIONS

YL, XD, and TC contributed to the conception of the case report. JS and XZ contributed to the management of the patient and manuscript writing. NC contributed to the manuscript preparation and clinical data collection. FS, ZL, and DT contributed to the imageological examination. PD, GS, and XS contributed to the eosinophilic leukemia, eosinophilic myocarditis, and intracardiac thrombus treatment of the patient. All authors contributed to the article and approved the submitted version.

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REVIEW
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Relevance of Ferroptosis to Cardiotoxicity Caused by Anthracyclines: Mechanisms to Target Treatments

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Anthracyclines (ANTs) are a class of anticancer drugs widely used in oncology. However, the clinical application of ANTs is limited by their cardiotoxicity. The mechanisms underlying ANTs-induced cardiotoxicity (AIC) are complicated and involve oxidative stress, inflammation, topoisomerase 2β inhibition, pyroptosis, immunometabolism, autophagy, apoptosis, ferroptosis, etc. Ferroptosis is a new form of regulated cell death (RCD) proposed in 2012, characterized by iron-dependent accumulation of reactive oxygen species (ROS) and lipid peroxidation. An increasing number of studies have found that ferroptosis plays a vital role in the development of AIC. Therefore, we aimed to elaborate on ferroptosis in AIC, especially by doxorubicin (DOX). We first summarize the mechanisms of ferroptosis in terms of oxidation and anti-oxidation systems. Then, we discuss the mechanisms related to ferroptosis caused by DOX, particularly from the perspective of iron metabolism of cardiomyocytes. We also present our research on the prevention and treatment of AIC based on ferroptosis. Finally, we enumerate our views on the development of drugs targeting ferroptosis in this emerging field.

Keywords: ferroptosis, doxorubicin, iron, treatment, mechanism, cardiotoxicity

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Abbreviations: ANT, anthracyclines; RCD, regulated cell death; ROS, reactive oxygen species; PUFA, polyunsaturated fatty acids; GSH, glutathione; GPX4, glutathione peroxidase 4; FSP1, ferroptosis suppressor protein 1; CoQ10, coenzyme Q10; NADPH, nicotinamide adenine dinucleotide phosphate; GCH1, GTP cyclohydrolase-1; BH4, tetrahydrobiopterin; AIC, anthracycline-induced cardiotoxicity; DIC, doxorubicin-induced cardiomyopathy; DOX, doxorubicin; CVDs, cardiovascular diseases; DFO, deferoxamine; Fe^{3+}, ferric iron; Tf, transferrin; TfR1, transferrin receptor 1; Fe^{2+}, ferrous iron; DMT1, divalent metal transporter 1; FPN, ferroportin; LIP, labile iron pool; LOX, lipoxygenase; FTH, ferritin heavy chain; IRP, iron regulatory protein; IREs, iron-responsive elements; HO-1, heme oxygenase 1; $O2^{\bullet-}$, active oxygen; $OH^{\bullet}$, hydroxyl radical; $PL^{\bullet}$, phospholipid radical; $PLOO^{\bullet}$, phospholipid peroxyl radical; PLOOH, phospholipid hydroperoxide; MDA, malondialdehyde; Cys, cysteine; Nrf2, nuclear factor (erythroid-derived 2)-like 2; AMPK, AMP-activated protein kinase; HMGB1, high mobility group box 1; DXZ, dexrazoxane; ABCB8, ABC protein-B8; RTA, radical-trapping antioxidant; α-TOH, α-tocopherol; Fer-1, ferrostatin-1; SOD, superoxide dismutase; Trx, thioredoxin; TrxR, thioredoxin reductase; HETE, hydroxyeicosatetraenoic acid; PE, phosphatidylethanolamine; PRMT4, protein arginine methyltransferase 4.

INTRODUCTION

With the advancements in medical technology, while the survival time of cancer patients has been prolonged, cardiovascular toxicity has become one of the most severe complications of cancer treatment (1, 2). Studies have shown that cancer survivors are at an eight-times higher risk of developing cardiovascular disease (CVD) than the general population (3). Anthracyclines (ANTs) are a class of chemotherapy drugs commonly used in clinical practice that significantly improve the survival rate of patients. However, the use of ANTs is restricted due to their cardiotoxic effects (2, 4). The incidence of left ventricular dysfunction, which is up to 48%, is positively correlated with dose (2). In some cancer survivors, the death rate of CVDs even exceeds that of their primary cancers (5). Therefore, it is necessary to explore the mechanism of cardiotoxicity caused by cancer therapy. The mechanisms of ANTs-induced cardiotoxicity (AIC) involve oxidative stress, inflammation, topoisomerase 2β inhibition, pyroptosis, immunometabolism, autophagy, apoptosis, etc. (6–8). Besides, in recent years, more and more studies have shown that ferroptosis plays a vital role in AIC (9, 10). Inhibiting the ferroptosis of cardiomyocytes can reduce AIC, which may be a novel prevention and treatment strategy in cardio-oncology.

Ferroptosis is a new form of regulated cell death (RCD) different from apoptosis, necrosis, necroptosis, pyroptosis, and autophagy. It is characterized by iron overload and reactive oxygen species (ROS) accumulation, resulting in lipid peroxidation of cell membranes (11, 12). A few decades ago, it was demonstrated that glutamate could inhibit the uptake of cystine, leading to a decrease in glutathione (GSH) levels within cells, thereby causing oxidative death of cells, and termed this process as "oxytosis" (13, 14). We believe that doxorubicin (DOX) can induce ferroptosis in cardiomyocytes through the following mechanisms: firstly, by regulating iron homeostasis-related proteins and iron-responsive elements (IREs)/iron regulatory proteins (IRPs), leading to increased iron levels in cardiomyocytes; secondly, DOX can increase ROS, thereby causing cell membrane lipid peroxidation. The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling pathway plays an important role. As the central organelle for ROS generation and the site where iron accumulation may occur, mitochondria are crucial for developing doxorubicin-induced cardiomyopathy (DIC). Further, we summarize the current treatments to prevent and treat AIC by inhibiting the ferroptosis of cardiomyocytes. Finally, we provide our future perspectives on this emerging field.

MECHANISMS OF FERROPTOSIS

Ferroptosis is an iron-dependent lipid peroxidation induced novel RCD, caused by redox imbalances between the oxidant and antioxidant systems. Antioxidant systems include the Cyst(e)ine-GSH-glutathione peroxidase 4 (GPX4) pathway, the ferroptosis suppressor protein 1 (FSP1)-coenzyme Q10 (CoQ10)-nicotinamide adenine dinucleotide phosphate (NADPH) pathway, the GTP cyclohydrolase-1 (GCH1)-tetrahydrobiopterin (BH4) pathway, etc. (15, 16). Intracellular iron overload is a necessary condition for ferroptosis. Therefore, lipid peroxidation is the most common cause of ferroptosis (15) (**Figure 1**).

Oxidation System

Iron Overload

Iron overload is a prerequisite of ferroptosis. The erastin-induced ferroptosis was inhibited by deferoxamine (DFO, an iron chelator), evidenced by increased cell viability and decreased lipid ROS production in HT-1080 cells. In contrast, the erastin-induced ferroptosis was triggered by incubation with three different exogenous iron supplements (11). In intestinal ischemia/reperfusion-induced acute lung injury model of C57BL/6 mice, the injection of Fe (15 mg/kg) aggravated lung injury and pulmonary edema, while the injection of ferrostatin-1 (Fer-1, 5 mg/kg) rescued this injury (17). Iron transport involves import, storage, and export (18, 19). Circulating iron exists in the form of ferric iron (Fe^{3+}) by binding to transferrin (Tf). Fe^{3+} enters the endosome through membrane protein transferrin receptor 1 (TfR1). Then, Fe^{3+} is reduced to ferrous iron (Fe^{2+}) by the iron reductase activity of the six-transmembrane epithelial antigen of the prostate 3. The divalent metal transporter 1 (DMT1, also known as SLC11A2) releases Fe^{2+} from the endosome into the cytoplasm. While part of the Fe^{2+} in the cytoplasm is stored as ferritin, part is oxidized to Fe^{3+} and transported outside the cell by the membrane protein iron transporter ferroportin (FPN, an iron efflux pump, also known as SLC11A3), and the rest is stored in the labile iron pool (LIP) of the cytoplasm or mitochondria (20). The iron in LIP spontaneously undergoes redox reactions, namely, Fenton and Harber Weiss reactions, to generate ROS, which in turn leads to lipid peroxidation (21). Moreover, iron and iron derivatives, such as heme or [Fe-S] clusters, also affect ferroptosis as they act on the active centers of ROS producing enzymes, such as lipoxygenase (LOX), cytochrome P450, NADPH oxidase and so on (22). Therefore, iron overload is essential for ferroptosis. Maintaining the LIP within a relatively narrow concentration range is crucial for preventing ferroptosis. Using DFO could prevent cell death caused by erastin and RSL3 (11, 23).

In this process, the core negative regulators of ferroptosis are ferritin heavy chain (FTH) (24–27) and FPN (28), and the core positive regulators of ferroptosis are Tf (29–31), TfR1 (24, 26, 31–33), and DMT1 (24, 34, 35). These iron homeostasis proteins involved in iron uptake, storage, utilization, and efflux from cells are regulated by IREs/IRPs (36, 37). In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced PD mice models, apoferritin inhibited ferroptosis by downregulating the iron importers DMT1 and FSP1, and conversely upregulating long-chain acyl-CoA synthetase 4 (38). The lipopolysaccharide then increased the expression of nuclear receptor co-activator 4, which directly interacted with ferritin and degraded ferritin in a ferritin phagocytosis-dependent manner. It then released a large amount of iron (39). In addition, heme oxygenase 1 (HO-1) mediates the release of free iron from heme, resulting in the accumulation of Fe^{2+} in LIP, which also exacerbates ferroptosis (40, 41).

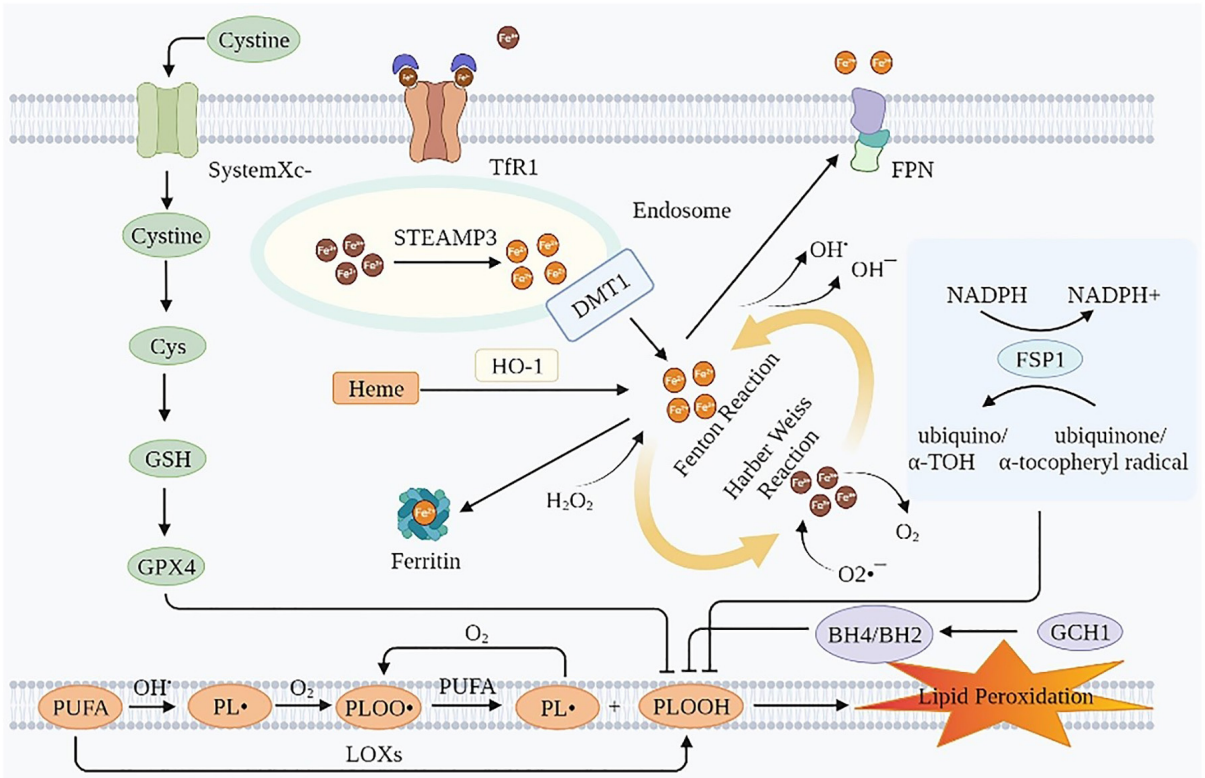

FIGURE 1 | Mechanisms of ferroptosis. Ferroptosis is essentially an iron-dependent lipid peroxidation. Intracellular iron overload is a necessary condition for ferroptosis, and lipid peroxidation is the presentation form of ferroptosis. TfR1, transferrin receptor 1; FPN, ferroportin; Cys, cysteine; GSH, glutathione; GPX4, glutathione peroxidase 4; STEAP3, six-transmembrane epithelial antigen of the prostate 3; DMT1, divalent metal transporter 1; HO-1, heme oxygenase 1; NADPH, nicotinamide adenine dinucleotide phosphate; FSP1, ferroptosis suppressor protein 1; PUFA, polyunsaturated fatty acids; GCH1, GTP cyclohydrolase-1; BH4/BH2, tetrahydrobiopterin/dihydrobiopterin; LOXs, lipoxygenases; PL•, phospholipid radical; PLOO•, phospholipid peroxyl radical; PLOOH, phospholipid hydroperoxide; α-TOH, α-tocopherol.

Lipid Peroxidation

Fe^{2+} in LIP can spontaneously undergo redox reactions to produce ROS, including both Fenton and Harber-Weiss reactions. The chemical equations are as follows (42):

$$\text{The Fenton reaction is: } Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}.$$

$$\text{The Harber} - \text{Weiss reaction is: } Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2.$$

$$\text{The overall reaction ROS is: } O_2^{\bullet-} + H_2O_2 \rightarrow OH^{\bullet} + OH^{-} + O_2.$$

The electron transport system of mitochondria is the primary source of H_2O_2 and active oxygen ($O_2^{\bullet-}$) (43). ROS, generated by the above mechanism, causes damage to the biomembrane in two ways. One is an enzyme-independent way, that is: Firstly, the hydroxyl radical ($OH^{\bullet}$) combines with polyunsaturated fatty acids (PUFA) on the biomembrane to generate the phospholipid radical ($PL^{\bullet}$). Secondly, $PL^{\bullet}$ reacts with O_2 generating a phospholipid peroxyl radical ($PLOO^{\bullet}$). Thirdly, $PLOO^{\bullet}$ reacts with PUFA to generate phospholipid hydroperoxide (PLOOH) and $PL^{\bullet}$, which can react again with O_2, forming a vicious circle. The other is the enzymatic way, that is, PUFA generates PLOOH under the action of LOXs. However, the detailed mechanism of PLOOH resulting in ferroptotic lipid peroxidative cell death remains obscure. Continued oxidation and consumption of PUFA may alter the structure of lipid pores, ultimately leading to compromised membrane integrity. In addition, PLOOH may be decomposed into active toxic aldehydes, such as 4-hydroxy-2-nonenal or malondialdehyde (MDA), causing cytotoxic effects (44). The hallmark of ferroptosis is the iron-dependent accumulation of lipid hydroperoxides to cell-lethal levels, especially peroxidized phosphatidylethanolamine (PEox). However, only a few studies detected and quantified these directly. In heart transplantation mice models, hydroperoxy-arachidonoyl-phosphatidylethanolamine (HOO-C20:4/C18:0-PE) was elevated and the resulting ferroptosis triggered early inflammation by recruiting neutrophils. In IRI mice models, the abundance of several hydroxyeicosatetraenoic acids (HETE) (such as 5-HETE, 11-HETE, 12-HETE, and 15-HETE) and epoxyeicosatrienoic acid species were increased (45). Besides, in RSL3-induced ferroptosis in H9C2 cardiomyocytes, *via* LC/MS, three significant species of hydroperoxy-PE were found to be up-regulated, namely, PE(36:4)-OOH, PE(38:4)-OOH, and PE(40:4)-OOH (46).

Sparvero et al. used gas cluster ion beam secondary ion mass spectrometry imaging with a 70 keV (H_2O) $(_n)$ (+) (n > 28000) cluster ion beam to visualize them at the single-cell and subcellular levels (47). The ferroptosis inhibitor, Fer-1 inhibited ferroptosis by reducing PLOOH.

Anti-oxidation System

The Cyst(e)ine-Glutathione-Glutathione Peroxidase 4 Pathway

The cyst(e)ine-GSH-GPX4 pathway is considered the canonical pathway for restricting ferroptosis. System Xc^-, a heterodimeric 12-pass transmembrane cystine–glutamate anti-porter, consists of a xCT light chain (also known as SLC7A11) that mediates cystine transport specificity, and a 4F2 heavy chain (also known as SLC3A2) (48), plays an important role in this process. SLC7A11 transports extracellular cystine, which is rapidly reduced to cysteine (Cys) by an NADPH-consuming reduction process. Cys participates in the production of GSH, a fundamental component of GPX4 (49). GPX4 is the primary inhibitor of ferroptosis. It can prevent lipid peroxidation by reducing PLOOH to non-toxic phospholipid alcohols (49). Cardiac impairments were ameliorated in GPX4 Tg mice and exacerbated in GPX4 heterodeletion mice. In cultured cardiomyocytes, GPX4 overexpression prevented DOX-induced ferroptosis (50). Surprisingly, a recent study showed that in non-small-cell lung cancer cell lines, cystine starvation induces an unexpected accumulation of γ-glutamyl-peptides under the influence of glutamate-cysteine ligase catalytic subunit, which limits the accumulation of glutamate, thereby protecting against ferroptosis (51). In addition, methionine can be used as one of the sources of intracellular cystine through the trans-sulfuration pathway (49). GPX4 is a selenoenzyme and its biosynthesis relies on the co-translational incorporation of selenocysteine (49). Selenium augments GPX4 and other genes by enhancing adaptive transcription factors TFAP2c and Sp1 to protect the cells from ferroptosis (52).

The Ferroptosis Suppressor Protein 1-Coenzyme Q10-Nicotinamide Adenine Dinucleotide Phosphate Pathway

The FSP1-CoQ10-NADPH pathway exists as a GPX4-independent one. FSP1 catalyzes the transformation of CoQ10 into ubiquinol, which is an excellent radical-trapping antioxidant in phospholipids and lipoproteins (53–55). Furthermore, the pathway can reduce oxidized α-tocopheryl radical to its non-radical form, increasing antioxidant capacity (56). The MDM2-MDMX complex is a negative regulator of FSP1. It changes the activity of PPARα, resulting in a decrease in the level of FSP1 protein and an increase in the level of CoQ10 (57). MiR-4443, whose target gene is METLL3, inhibited FSP1-mediated ferroptosis induced by cisplatin treatment *in vitro* and enhanced tumor growth *in vivo* (58).

The GTP-GTP Cyclohydrolase-1-Tetrahydrobiopterin Signaling Pathway

The GTP-GCH1-BH4 pathway is also not dependent on GPX4. BH4/dihydrobiopterin synthesis by GCH1-expressing cells caused lipid remodeling, suppressing ferroptosis by selectively preventing depletion of phospholipids with two polyunsaturated fatty acyl tails (59). Using a co-culture model system, iNOS/NO (•) in M1 macrophages has been confirmed to inhibit the effect of NO (•) on epithelial cells by inhibiting phospholipid peroxidation, especially the generation of 15-HpETE-PE signal that promotes ferroptosis. It is an intercellular mechanism that distantly prevents the ferroptosis of epithelial cells stimulated by *Pseudomonas aeruginosa* (60).

Nuclear factor (erythroid-derived 2)-like 2 signaling is implicated in many molecular aspects of ferroptosis, including glutathione homeostasis, mitochondrial function, and lipid metabolism (61, 62). The Nrf2-Focad-Fak signaling pathway is closely related to ferroptosis caused by Cys deprivation. In non-small-cell lung carcinoma, brusatol (an Nrf2 inhibitor) was added based on ferroptosis inducer erastin or RSL3. The therapeutic effect based on ferroptosis was better than single treatment *in vivo* and *in vitro* (63). In immunocompetent mice and humanized mice, ZVI-NP, a dual-functional nanomedicine, enhanced the degradation of Nrf2 by GSK3/β-TrCP through AMP-activated protein kinase (AMPK)/rapamycin activation, leading to cancer-specific ferroptosis of lung cancer cells (64).

The Other Antioxidant Elements

The antioxidant system of the heart is very complex. In addition to the above three major systems, the antioxidant system of the heart also includes some other elements that inhibit ferroptosis. $O_2^{\bullet -}$, $OH^{\bullet}$, OH^-, H_2O_2, $PL^{\bullet}$, $PLOO^{\bullet}$, PLOOH, ROS, etc. play important roles in the occurrence and development of ferroptosis. Superoxide dismutase (SOD) and superoxide reductases can reduce $O_2^{\bullet -}$ to H_2O_2. Catalase catalyzes H_2O_2 to water and O_2. Water-soluble ascorbic acid (vitamin C), lipid-soluble vitamin E or α-tocopherol (α-TOH), and lipoic acid can reduce lipid hydroperoxide production and peroxyl radicals. Besides, ascorbic acid increases the vitamin E content by reducing vitamin E semiquinone (65). The thioredoxin system, consisting of the thioredoxin (Trx) and thioredoxin reductase (TrxR), is also an important antioxidant system (66). Ferroptocide causes an accumulation of lipid peroxidation by inhibiting this system, thereby inducing ferroptosis (67). TrxR and NADPH reduce the active site disulfide in Trx. Under the combination of Trx and TrxR, peroxides, including lipid hydroperoxides and H_2O_2 were observed to be reduced effectively. In addition, there are several crosstalks between these antioxidants. For example, the thioredoxin system promotes the regeneration of certain antioxidants. It reduces ascorbyl free radical to ascorbic acid and turns GSSG to GSH. The thioredoxin system increases the content of ascorbic acid by reducing dehydroascorbic acid (65).

FERROPTOSIS AND ANTHRACYCLINE-INDUCED CARDIOTOXICITY

Doxorubicin is one of the most cardiotoxic anticancer agents. Current studies on DIC based on ferroptosis mainly focus on DOX. DOX's anti-cancer activity is primarily mediated

by DNA intercalation and inhibition of the topoisomerase II enzyme in rapidly proliferating tumors. However, DOX causes cumulative and dose-dependent cardiotoxicity, resulting in increased mortality risks among cancer patients and thus limits its wide clinical applications (68). Ferroptosis is involved in DIC both *in vivo* and *in vitro* (50, 69–72). The survival rate of rats was markedly elevated with the ferroptosis inhibitor Fer-1 than with apoptosis inhibitor emricasan, necroptosis inhibitor necrostatin-1, and autophagy inhibitor 3-methyladenine (73, 74). Besides, compared with apoptosis-defective ($Ripk3^{-/-}$) mice and necroptosis-defective ($Mlkl^{-/-}$) mice, intraperitoneal injections of Fer-1 (20 mg/kg) followed by DOX in normal mice improved their survival rate remarkably (74). The mechanisms of DOX causing cardiac ferroptosis are as follows (**Figure 2** and **Table 1**):

Doxorubicin and Reactive Oxygen Species

The quinine moiety of DOX received electrons from NADPH oxidase and nitric oxide synthase (NOS) to become semiquinones, which was then accompanied by the ROS production such as $O_2^{\bullet-}$ and $OH^{\bullet}$ generation (75). In addition, DOX can turn Fe^{3+} into Fe^{2+}, thereby aggravating the Fenton reaction to produce more ROS (76). DOX can lead to downregulation of the antioxidant system. Several studies support the view that the levels of antioxidant substances (GPX4, SOD, and GSH) in DOX-treated rats and mice were significantly lower and the content of MDA was significantly higher than in control groups (50, 77, 78). One study suggested that ENPP2 overexpression enhances the expression levels of the ferroptosis-associated gene "GPX4" in H9C2 cells while FoxO4 regulates gene transcription negatively by the suppression of post-transcriptional coding mRNAs. In the H9C2 cells overexpressing ENPP2, DOX-induced increased Fe^{2+} activity, ROS and NOX4 production, while decreasing SLC7A11 and reversing GPX4 and FPN expression (79).

Doxorubicin and Iron

Iron Plays a Pivotal Role in Doxorubicin-Induced Cardiomyopathy

We know that ferroptosis is an iron-dependent lipid peroxidation process, and iron is essential in both the occurrence and

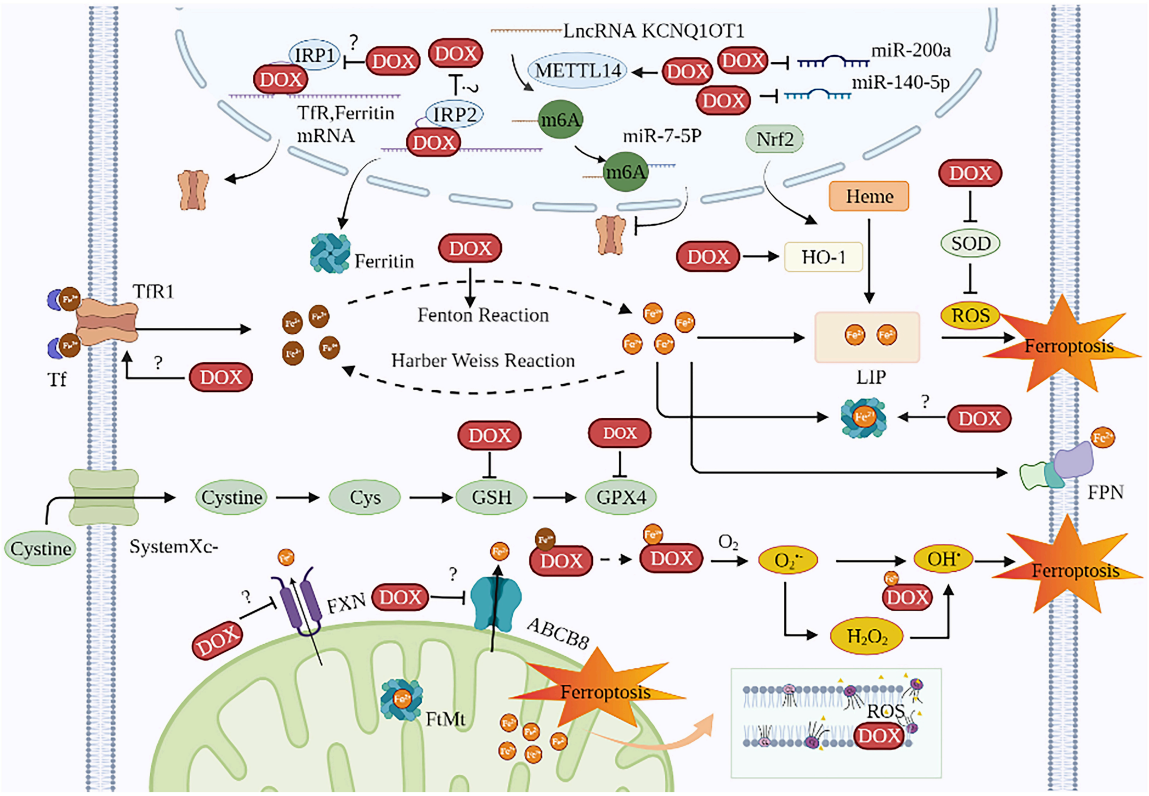

FIGURE 2 | The mechanisms of DIC based on ferroptosis. DOX induces ferroptosis in cardiomyocytes involves two major mechanisms: one is to disrupt iron homeostasis and the other is to promote lipid peroxidation. The targets of DOX on iron disorder are Tf, ferritin, HO-1, FXN, ABCB8, IRE, IRP, and KCNQ1OT1m6A. The targets of DOX for lipid peroxidation are ROS, SOD, GPX4, and GSH. The site of iron death in cardiomyocytes is probably the mitochondria. TfR1, transferrin receptor 1; Tf, transferrin; DOX, doxorubicin; IRP, iron regulatory protein; METTL14, methyltransferase-like 14; Nrf2, nuclear factor (erythroid-derived 2)-like 2; LIP, labile iron pool; HO-1, heme oxygenase 1; ROS, reactive oxygen species; SOD, superoxide dismutase; Cys, cysteine; GSH, glutathione; GPX4, glutathione peroxidase 4; FPN, ferroportin; FXN, frataxin; ABCB8, ABC protein-B8; O_2, oxygen; $O_2^{\bullet-}$, active oxygen; FtMt, mitochondrial ferritin; CL, cardiolipin; $OH^{\bullet}$, hydroxyl radical.

TABLE 1 | The main molecular mechanism of DOX-induced ferroptosis in cardiomyocytes.

Experimental model	DOX dose/Route of administration	Findings (mechanisms)	References
Lipid peroxidation			
H9C2 cells;Mice	2 μM (*in vitro*) 6 mg/kg, tail vein injection; on days 0, 2, and 4 (*in vivo*)	Mitochondrial GPX4↓	(50)
H9C2 cells;NRVMs	1 μM (H9C2 cells) 2 μM (NRVMs)	Nrf2 (nuclear)↓/GPX4↓	(102, 103)
HL-1 cells;Mice	2 μM (*in vitro*) 1 mg/kg, IP; every other day for 8 times (*in vivo*)	Acot1↓	(69)
H9C2 cells;Mice, rats	5 μM (*in vitro*); 15 mg/kg, IP; for 8 days (*in vivo*)	miR-140-5p ↑/Nrf2 ↓, Sirt2 ↓pathway	(77)
Iron metabolism			
H9C2 cells;Rats	2 μM (*in vitro*) 20 mg/kg, IP; a single dose (*in vivo*)	HMGB1 ↓	(73)
Mice	10 mg/kg, IP; a single dose	HO-1 ↑	(74)
H9C2 cells	5 μM DOX	FoxO4 ↑/Enpp2 ↓	(79)
H9C2 cells;Mice	10 μM (*in vitro*) 5 mg/kg, IP; one dose per week for 5 times (*in vivo*)	FXN ↓	(87)
BAEC	0.5 μM	TfR ↑	(88)
AC16 cells	2 μM	METTL14 ↑/KCNQ1OT1 ↑/miR-7-5p ↓/TfR ↑	(89)
H9C2 cells;HL-1 cells	5 μM, 10 μM (H9C2 cells) 5 μM (HL-1 cells)	Ferritin ↑ (especially FTH)	(91, 92)
H9C2 cells;Rats	1 μM (*in vitro*) 2.5 mg/kg, tail vein injection; once a week for 6 weeks (*in vivo*)	Nrf2 (nuclear) ↓/GPX4 ↓, HO-1 ↓, FTH1 ↓, FPN ↑	(105)
NRCMs;Mice	10 μM (*in vitro*) 6 mg/kg, IP; one dose every third day for 4 times OR 10 mg/kg, IP; one dose over 5 days for 3 times (*in vivo*)	ABCB8 ↓	(109)
Rat cardiomyocytes	5 μM	Inhibit Fe mobilization from ferritin	(119)
H9C2 cells;Mice	10 μM (*in vitro*); 5 mg/kg, IP; one dose per week for 5 weeks (*in vivo*)	FXN ↓	(87)
IREs/IRPs			
H9C2 cells;Rat primary cardiomyocytes	1, 2.5, 5, and 10 μM (H9C2 cells); 1, 5, 10, and 20 μM (rat primary cardiomyocytes)	Inactivate IRP1 and IRP2	(93, 96)
Mice	15 mg/kg, IP; a single dose	Inactive IRP2/ferritin ↑, TfR1 ↓, unchanged IRP1 activity	(97)

development of this process. It is generally believed that excessive iron can aggravate DIC. More than 20 years ago, research indicated that iron overload aggravated DIC (80). DOX reduced the viability of H9C2 cardiomyocytes, while ferric ammonium citrate aggravated it in a concentration-dependent manner (81). Male Sprague Dawley rats fed with iron-rich chow showed significantly higher DOX cardiotoxicity, accompanied with a significant weight loss and severe myocyte injury as evidenced through electron microscopy and light microscopy. However, feeding an iron-rich meal alone did not result in any cardiotoxicity (82). Paradoxically, in cultured H9C2 cardiomyocytes and male C57BL/6 mice, researchers concluded that pretreatment with dextran-iron (125–1,000 μg/mL) in combination with DOX did not potentiate DIC and even prevented some aspects of it (83). Therefore, the regulation of DIC by iron is a complicated process, which may involve the balance between iron dosage, protection, and damage.

The HFE gene encodes HFE protein, which binds to TfR1 and facilitates the uptake of iron-bound to Tf. The elevation of iron concentration in the heart was much more accentuated in DOX-treated $HFE^{-/-}$ mice. Mutations in the HFE gene led to iron overload in cardiomyocytes, which increased the susceptibility of cardiomyocytes to ferroptosis and exacerbated DIC (84). One study concluded that the mutation status of HFE RS1799945 H63D could be used as one of the critical markers to identify patients at high risk for AIC (85). Among survivors of high-risk acute lymphoblastic leukemia in children, patients with the C282Y mutation of the HFE gene had a more severe DIC, which was reflected in higher levels of cardiac troponin-T, lower left ventricular quality and thickness, and worsened left ventricular function in echocardiography (86). These studies on the iron-regulated gene HFE support the view that iron plays a pivotal role in DIC.

Changes in Iron Homeostasis Regulatory Proteins

Proteins associated with iron transport are considered significant indicators of cellular iron homeostasis and are mainly involved in cellular iron uptake (TfR1) and storage (ferritin). As for TfR1, most studies believe that DOX could elevate its expression (87–89). A study believed that TfR1 is critical for the DOX-induced

increase in iron uptake. After incubation with the specific anti-TfR antibody (12 μg/ml), DOX-induced increase of ^{55}Fe uptake in bovine aortic endothelial (BAEC) cells was reversed (88). The mechanism of increased TfR1 could be because DOX inhibited the expression of miR-7-5p by increasing the METTL14-mediated expression of KCNQ1OT1m6A, thus reducing the degradation of TfR1 (89). In AC16 cells, METTL14 knockdown, KCNQ1OT1 silencing, and miR-7-5p mimic attenuated the DOX-induced increase of Fe^{2+} and lipid ROS, and reduced DOX-induced decrease in mtDNA and MMP levels. In METTL14 shRNA mice models, DOX-induced increase in the levels of MDA and 4-HNE were also alleviated (89). However, one study suggested that TfR1 was reduced in heart lysates extracted from DOX-treated mice that received a single i.p. DOX dose (20 mg/kg) as observed in Western-blot and RT-PCR analysis (90). As for ferritin, DOX elevated it, and this change was accompanied by an increase in the level of iron bound to it (90). After being treated with 5 and 10 μM DOX for 24 h, the content of ferritin, especially ferritin heavy chain (FHC), in H9C2 cells increased, and was ROS-dependent. The increase in DOX-induced ferritin was reversed after clearing ROS using N-acetylcysteine (a known ROS scavenger) in H9C2 cells. Interestingly, the increased ferritin induced by DOX protected H9C2 cells from iron toxicity, as demonstrated by increased cell viability in 500, 750, and 1,000 μg/ml FAC measured by MTT assays (91). Similarly, ferritin mRNA and protein levels were also increased in an adult cell line of cardiomyocytes (HL-1) exposed to 5 μM DOX (92). In addition, elevated intracellular iron levels were also associated with high mobility group box 1 (HMGB1)-mediated heme degradation. Compared with the DOX group, the ferroptosis-related indexes (PTGS2, MDA, and 4-HNE) and cardiac injury-related indexes (Anp, Bnp, and Myh7) of the rat heart in the DOX + shHMGB1 group were significantly decreased (73). Therefore, the effect of DOX on iron homeostasis regulatory proteins is a complex process, possibly related to cell lines, drug dosage, and imbalances in protection and injury.

Changes in Iron-Responsive Elements/Iron Regulatory Proteins

At present, many studies have shown that DOX can act on IRPs, but the results are numerous. Some studies believed that DOX irreversibly inactivated IRP1 and IRP2. This study believed that the secondary alcohol doxorubicinol (DOXol) and certain products of DOX metabolism, converted cytoplasmic aconitase to the cluster-free IRP1 by removing iron from its catalytic [Fe-S] clusters. This eventually produced a null protein, which could not adapt the levels of TfR1 and ferritin, and IRP2 was inactivated only by DOX related ROS (93). Some scholars thought H_2O_2 activated IRP1, leading to the upregulation of TfR1 and cellular iron accumulation, which was considered an important molecular mechanism in DIC (94). One study demonstrated that the IRP1 activity of BAEC cells increased in a dose-dependent manner after being treated with 0.5 μM DOX (88). However, one study found that even exposing DOX-sensitive GLC4 cells to 12.5 μM DOX for 24 h did not change IRP activity. It can be seen that the effect of DOX on cellular IRP activity may be related to cell line and cell resistance (95). However, Juliana et al. believed that the effect of DOX on cardiomyocyte IRP activity was closely related to DOX concentration and incubation time. After 6 h of DOX administration, total IRP1 did not change significantly, but active IRP1 and active IRP2 decreased in a concentration-dependent manner (1 μM DOX, 5 μM DOX, 10 μM DOX, and 20 μM DOX). IRP2, in particular, dropped by more than 50% at 20 μM DOX. After 24 h of administration, while the total IRP1 level decreased, the active IRP1 and active IRP2 did not significantly reduce but even increased. Interestingly, they thought that the free radical scavengers, DOXol, and *cis*-aconitate had little effect on IRP-RNA-binding activity in SK-Mel-28 melanoma cells and cardiomyocytes. The Fe and Cu complexes of anthracyclines altered iron metabolism in cardiomyocytes (96). Gianfranca suggested that DOX had differential effects on the two IPRs, as evidenced by reduced IRP2 activity and unchanged IRP1 activity. The effect of DOX on IRP2 resulted in an upregulation of ferritin gene expression and a decrease in TfR1 expression. Surprisingly, this change reduced the iron content in LIP and protected cardiomyocytes from ferroptosis induced by DOX (97). Furthermore, one study concluded that DOX at low concentrations (≈1 μM) activated IRP1 in cardiomyocytes, while at higher concentrations (>5 μM), it irreversibly inactivated IRP1 in BAEC cells (88). Besides, according to one study, DOX can directly interact with IREs. DOX intercalated double-stranded RNA by recognizing the IREs hairpins located in the 50-UTR of ferritin mRNAs, thereby changing the tertiary structure of the RNA drastically altering the effectiveness of the IREs/IRPs interaction (98). Anyway, DOX could modify the expression of genes involved in iron metabolism by inactivating IRPs binding to IREs (99).

Changes in the Nuclear Factor (Erythroid-Derived 2)-Like 2 Signaling Pathway

Under normal physiological conditions, Nrf2 binds to Keap1 in the cytoplasm, and then Nrf2 is degraded by the ubiquitin-proteasome system. In the case of oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, where it binds to promoter regions (AREs), activates the transcription of a series of downstream genes, and exerts physiological functions (100). Notably, many of the genes associated with ferroptosis are target genes for Nrf2, but DIC-related studies mainly focus on GPX4 and HO-1 genes. Nrf2 up-regulates the expression of GPX4 and has an anti-ferroptosis effect (101–103). Nrf2 can be methylated by the protein arginine methyltransferase 4 (PRMT4), leading to its nuclear restriction and consequently decreased GPX4 expression. PRMT4 aggravated the expression of ferroptosis markers (ROS, MDA, NCO4, and Fe^{2+}) in the DOX-induced primary neonatal rat ventricular myocytes and C57BL/6 J mice cardiotoxicity models, and this influence can be mitigated by PRMT4 knockout (103). However, studies have also shown that Nrf2-mediated activation of HO-1 promotes ferroptosis. HO-1 mediates the release of Fe^{2+} from heme, which accumulates in cardiomyocytes and induces ferroptosis (74, 104). Through $Nrf2^{+/+}$ mice, $Nrf2^{-/-}$mice, Znpp (an HO-1 inhibitor), Hemin (an HO-1 activator), DOX has been proven to increase HO-1 by affecting Nrf2 and accelerating the degradation

of heme, leading to an increase in non-heme iron and myocardial ferroptosis. An increase in intestinal iron absorption did not accompany this increase of iron content. In this study, DOX was confirmed to be able to upregulate hepatic Hamp1 mRNA to increase hepcidin and reduce FPN degradation (74). In addition, Nrf2 can be activated by deacetylation of SIRT1, and Fisetin activated Nrf2 by up-regulating the expression of SIRT1, leading to the up-regulation of HO-1, FTH1, TfR1, and FPN, and exerting an anti-ferroptosis effect. After being transfected with SIRT1 and Nrf2 siRNA, the anti-ferroptosis effect of Fisetin was abolished in H9C2 cells (105).

The Role of Mitochondria in Doxorubicin-Induced Cardiomyopathy

Mitochondria Is the Major Source of Reactive Oxygen Species

The morphological changes of ferroptosis under the electron microscope were mainly observed in the mitochondria (11). The metabolic activity of mitochondria drives ferroptosis. Mitochondria are the main source of cellular ROS. When electrons are transferred to O_2, some escape from the ETC and react directly with O_2 to form $O_2^{\bullet -}$, a precursor to many other ROS such as $OH^{\bullet}$ and H_2O_2 (106). Energy stress inhibits ferroptosis by activating AMPK, while AMPK inactivation promotes ferroptosis (107). ETC complex inhibitors can inhibit ferroptosis, indicating that mitochondria play an important role in ferroptosis, and possibly through the activation of AMPK (108). The mitochondrial TCA cycle is involved in ferroptosis induced by Cys deprivation, for which glutaminolysis is essential. However, mitochondria are not essential for ferroptosis induced by GPX4 inhibition (108).

Mitochondria May Be the Site of Iron Accumulation

Doxorubicin can cause cell iron metabolism disorders, but there is much debate about where iron metabolic disorders occur. Some studies have suggested that the site where the DIC occurs is the mitochondria. Compared with cytoplasm, DOX and iron preferentially accumulated in mitochondria (109), especially in mitochondrial cardiolipin (110–112). After incubation with 10 μM DOX, the accumulation of DOX in the mitochondria of neonatal rat cardiomyocytes increased significantly compared with the cytoplasm, and DOX caused a significant increase in mitochondrial iron levels detected by ^{55}Fe colorimetric measurement of mitochondrial non-heme iron (109). In the presence of Fe^{2+}, DOX induced the activation of the mitochondrial permeability transition pore (113). Tadokoro et al. believed that ferroptosis was triggered by GPX4 deficiency in mitochondria for the following reasons: (1) DOX-induced lipid peroxidation occurred on mitochondria rather than other organelles; and (2) even though the cell viability was improved and lipid peroxidation indexes (MitoPeDPP, MDA) were reduced in both isolated neonatal rat ventricular cardiomyocytes cells transfected with Ad-cytoGPx4-FLAG and Ad-mitoGPx4-FLAG, electron microscopy revealed that mitochondrial GPX4 was almost exclusively localized to mitochondria during this process. In contrast, cytoplasmic GPX4 was transferred to mitochondria (50). Mitochondria-2,2,6,6-tetramethylpiperidin-N-oxyl, a mitochondrial superoxide scavenger, abolished DOX-induced lipid peroxidation and cardiac ferroptosis in DOX-treated mice models. In contrast, the non-mitochondrial targeted version only mildly rescued the DOX-induced effects (74). Dexrazoxane (DXZ) reduced iron in mitochondria. The poor impact of DFO in treating DIC compared with DXZ may be attributed to its inability to penetrate mitochondria and chelate iron in mitochondria specifically (109, 114). The most likely target of free radicals produced by ANTs through redox reactions was cardiolipin, a major phospholipid component of the inner mitochondrial membrane, known to be susceptible to peroxidative injury with abundant PUFA. Iron overload can aggravate the damage of ANTs to the mitochondria of cardiomyocytes (115).

The mechanism of DOX causing myocardial ferroptosis may be related to its effect on mitochondrial iron regulation-related proteins. Compared with wild-type mice, cardiotoxicity was more pronounced in $FtMt^{-/-}$ mice injected intraperitoneally with a single dose (15 mg/kg of body weight) of DOX, as manifested by higher mortality, more morphological changes (incomplete cristae, condensation, and fragmentation of most myofibril), more severe lipid peroxidation, and worse cardiac function (ATP and BNP) (116). In mice, neonatal cardiomyocytes, and H9C2 cardiomyoblasts, DOX led to the reduction of frataxin, a nuclear-encoded mitochondrial protein involved in maintaining mitochondrial iron homeostasis through the ubiquitin-proteasome system. In addition, the mitochondrial iron export protein ABC protein-B8 (ABCB8) is essential for maintaining mitochondrial iron homeostasis. The depletion of ABCB8 led to compromised systolic and diastolic functions, a significant accumulation of ^{55}Fe in the mitochondria, and higher lipid peroxidation levels both *in vivo* and *in vitro* (87, 109, 117). The levels of ABCB8 in explanted hearts from patients with end-stage cardiomyopathy were significantly reduced (117). Intriguingly, one study concluded that there was no effect on ABCB8 in the myocardium of the DIC mice models, and the silencing of ABCB8 did not increase the iron content in cultured cardiomyocytes after 30 h of exposure to 2 μM DOX (50).

On the contrary, another study showed that DOX affected more cytosolic than mitochondrial iron metabolism in murine hearts and human HeLa cells, as manifested by alterations in proteins associated with cytoplasmic iron transport proteins (ferritin, TfR1, and hepcidin). In contrast, mitochondrial iron-related proteins (aconitase, succinate-dehydrogenase, and frataxin) appear to be unaffected (90). In addition, Kwok thought that the mechanism of DOX-induced cardiomyocyte ferroptosis was related to lysosomes. It may be that ANTs act on lysosomes to inhibit ferritin degradation, thereby affecting the iron-dependent life activities of cells. However, this study was conducted in SK cells, not cardiomyocytes, so the conclusion is debatable (118). Interestingly, some studies believed that DOX affected neither total cellular Fe content nor total cellular ferritin protein levels (119). Furthermore, the myocardial iron content was not statistically different between the saline and the DOX groups (82). In the presence of NADPH-cytochrome p450 reductase, ANTs underwent redox cycling to generate superoxide, which

mediated a slow reductive release of iron from ferritin. More the cardiotoxic ANTs, more the rapid and extensive iron release it led to (120).

Doxorubicin and Lipid Peroxidation

As mentioned earlier, iron promotes ROS generation through the Fenton and Harber-Weiss reactions (42, 121, 122). PUFA undergoes lipid peroxidation under the action of ROS, which in turn leads to ferroptosis. Furthermore, DOX can also be combined with Fe^{3+} to form the DOX-Fe^{3+} complex (123, 124), which generates the DOX-Fe^{2+} complex through both enzymatic or non-enzymatic reactions. And this DOX-Fe^{2+} complex reacts with oxygen to form $O_2^{\bullet-}$, which is transformed into OH and H_2O_2 through disproportionation reaction (125). H_2O_2 can also react with DOX-Fe^{2+} complexes to generate $OH^{\bullet}$ (126). Thus, under the action of the DOX-iron complex, OH and O_2, PUFA undergoes lipid peroxidation. In addition, even without free Fe^{3+} and Fe^{2+}, DOX can extract Fe^{3+} directly from ferritin to form DOX-Fe^{3+} complexes, resulting in lipid peroxidation (127). In this process, it is not the free Fe^{3+}, free Fe^{2+}, or DOX-Fe^{3+} iron complexes, but the DOX-Fe^{2+} complexes that induce ferroptosis in cardiomyocytes. The use of specific Fe^{2+} chelators like Mito-FerroGreen and bathophenanthroline effectively attenuate cardiomyocyte lipid peroxidation, measured using C11-BODIPY 581/591 and MDA (50, 124). It should be noted that lipid peroxidation is not the only pathological change brought about by ferroptosis. Oxidative stress, apoptosis, necrosis, and other forms of RCD share characteristics of lipid peroxidation. So, if we want to prove that ferroptosis is DIC's mechanism, we must measure lipid peroxidation directly (56).

PREVENTION AND TREATMENT OF ANTHRACYCLINE-INDUCED CARDIOTOXICITY BASED ON FERROPTOSIS

Evaluations performed before the initiation of anticancer therapy in patients without significant CVDs should be regarded as the primary prevention strategy (128). An appropriate cancer treatment and anti-cardiotoxicity prevention and treatment strategies should be selected after a comprehensive discussion by a multidisciplinary team of cardiovascular, oncology and hematology experts, especially to balance the effects of drugs after cancer treatment regimens and the risk of specific CVDs in all aspects (129). Commonly used drugs and their mechanism of action are described as follows (**Figure 3** and **Table 2**):

Dexrazoxane

Dexrazoxane is the only formally preventive drug approved by the FDA. For patients planning to receive high-dose of ANT therapy, DXZ is recommended (2). DXZ is traditionally known as an iron chelator. Under the action of the iron-ANT complex, the ring of DXZ was opened and hydrolyzed to ADR-925. This presumably exerted its cardioprotective effects by either binding freely or loosely to iron or iron complexed with DOX, thus preventing or reducing site-specific oxygen radical production that damages cellular components (130). In addition, DOX-induced cardiac ferroptosis in rats was observed to be mediated by the upregulation of HMGB1, and correspondingly ferroptosis was inhibited when shHMGB1 was used. DXZ reversed DOX-induced elevation of HMGB1. DXZ also modulated iron metabolism-related protein levels in cardiac myocytes and reversed the upregulation of HO-1 induced by daunorubicin. It therefore inhibited the conversion of heme iron to non-heme iron, which reduced the Fe^{2+} content in the LIP in cardiac myocytes (131). In addition, this study also showed that DXZ reversed the DOX-induced decrease of FTH1 protein in the H9C2 cells (73). However, this view has been greatly challenged. An increasing number of studies believe that DXZ plays a protective role in the heart mainly because it inhibited DOX-mediated damage of cardiomyocyte topoisomerase IIβ (132, 133). Also, in one study, the chelating metabolite ADR-925 therapy on neonatal ventricular myocytes receiving was neither able to mitigate AIC, nor did it significantly impact daunorubicin-induced mortality, blood congestion, and biochemical and functional markers of cardiac dysfunction in a chronic rabbit model *in vivo* (132). Although the mechanism of DXZ chelating iron in mitochondria does not depend on the topoisomerase IIβ pathway (109), the contribution of DXZ in regulating the cardioprotective effect against ferroptosis is not apparent. Besides, DXZ has been shown to inhibit DOX-induced cardiomyocyte necrosis and apoptosis through several alternate mechanisms (134–136). Therefore, the cardiomyocyte protective effect exerted by DXZ is not achieved only through the inhibition of ferroptosis as it is not a simple, specific ferroptosis inhibitor.

Although DXZ is generally considered to reduce AIC and is recommended as the only approved cardioprotective agent (137, 138), the clinical use of DXZ is encountering various challenges. Firstly, there are concerns that DXZ may increase the risk of acute myeloid leukemia and secondary solid tumors in children (139–141). Secondly, the effectiveness of DXZ is also being questioned. One study found that the preventive use of DXZ before high-dose DOX in eight sarcoma patients did not reduce their cardiotoxicity satisfactorily. There were six patients with high-sensitivity troponin T levels exceeding 10 ng/ml, four patients with LVEF that decreased by more than 5%, and three patients with global longitudinal peak systolic strain changed by more than 15% (142). Finally, the European Society of Cardiology only recommends DXZ for patients with advanced or metastatic breast cancer receiving cumulative doses of DOX over 300 mg/m^2 (2). In spite of this, DIC development is not a dose-dependent response, and in our clinical work, we have found that some patients develop DIC when treated with small doses of anthracyclines.

Deferoxamine

Deferoxamine is a widely used iron chelator that can chelate excess intracellular iron, thereby reducing DOX-induced ferroptosis in cardiomyocytes. In addition, DFO can also be regarded as a protective agent for mitochondrial permeability transition pore, as it weakens the opening of calcium-dependent pores induced by iron and iron-DOX complexes and reduces the uptake of Fe^{2+} in mitochondria, thus protecting mitochondrial

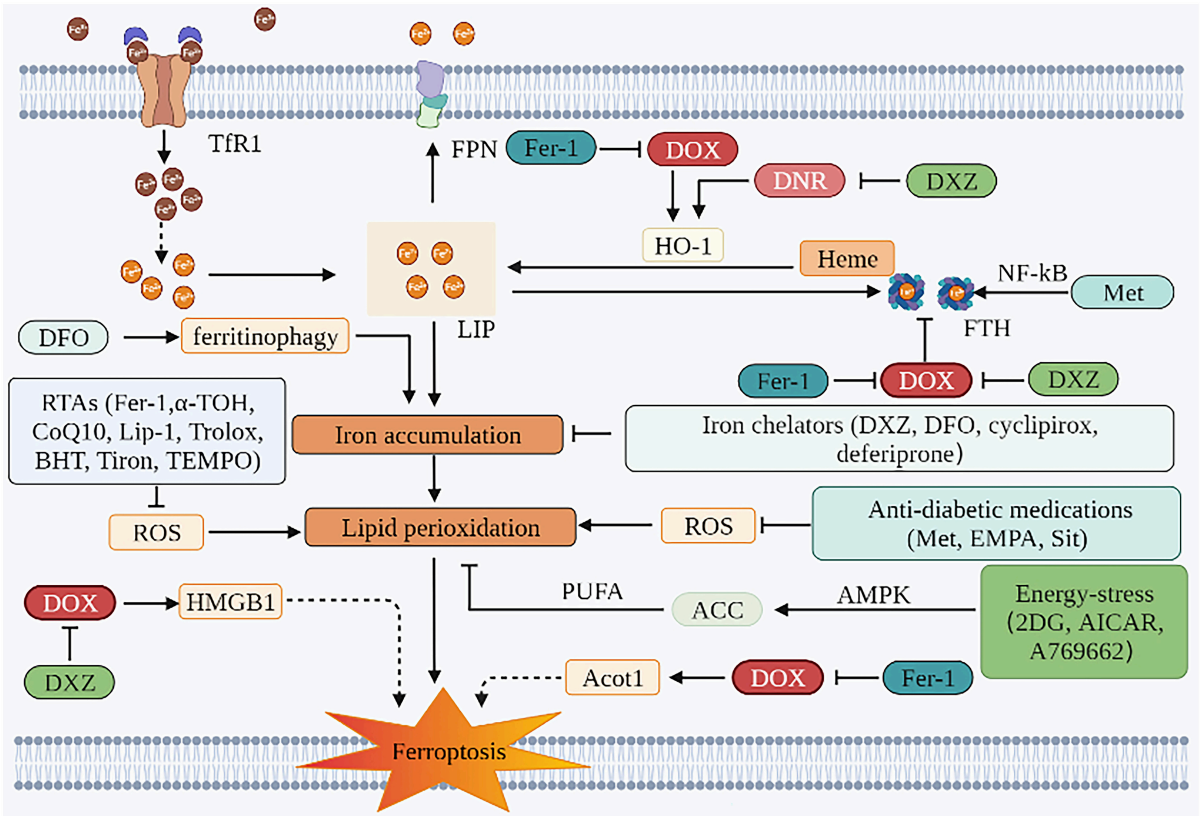

FIGURE 3 | Prevention and SOD treatment of AIC based on ferroptosis. The mechanism of preventing ferroptosis of cardiomyocytes is mainly in two aspects. One is to inhibit iron accumulation, and the other is to inhibit lipid peroxidation. Iron chelators can play a role through the former. The effects of RTAs, anti-diabetic medications, and energy-stress inducers are mainly attributed to the latter. TfR1, transferrin receptor 1; FPN, ferroportin; DOX, doxorubicin; DNR, daunorubicin; LIP, labile iron pool; HO-1, heme oxygenase 1; ROS, reactive oxygen species; Fer-1, ferrostatin-1; DFO, deferoxamine; DXZ, dexrazoxane; PUFA, polyunsaturated fatty acids; ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; EMPA, empagliflozin; Sit, sitagliptin; 2DG, 2-deoxy-d-glucose; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; HMGB1, high mobility group box 1; HO-1, heme oxygenase 1; FTH, ferritin heavy chain; NF-κB, nuclear factor-kappa B; α-TOH, α-tocopherol; CoQ10, coenzyme Q10; Lip-1, liproxstatin-1; BHT, butylated hydroxytoluene; Acot1, acyl-CoA thioesterase 1; TEMPO, 2,2,6,6-tetramethylpiperidin-N-oxyl; Met, metformin.

function (113). Intriguingly, DFO was demonstrated to aggravate ferroptosis by inducing ferritinophagy, leading to the accumulation of iron and ROS (10, 143, 144). The effect of DFO in protecting cardiomyocytes from DIC is still contentious. In this study, the cardioprotective benefit of DFO requires a strict dose, and a slight deviation from it would diminish this effect (145). Besides, DFO failed to reverse myocardial damage in a well-established spontaneously hypertensive rat models of chronic ANT cardiomyopathy (146). Moreover, DFO's side effects, such as hypotension and renal insufficiency, limit its clinical application (147).

Ferrostatin-1

As a radical-trapping antioxidant (RTA), Fer-1 attenuates lipid peroxidation by decreasing erastin-induced accumulation of cytosolic and lipid ROS, consequently inhibiting ferroptosis (11). This is primarily due to its powerful chain-carrying peroxyl trapping ability (148). The experimental results have shown that Fer-1 could reduce the lipid peroxides labeled with MDA and MitoPeDPP (50). In addition, the mechanisms by which Fer-1 protects the heart from DOX-induced ferroptosis could be describes as follows: Fer-1 inhibited DOX-induced elevation of non-heme iron in cardiomyocytes by downregulating Nrf2/HO-1, thereby inhibiting ferroptosis (74). In an alternate way, DOX downregulated the Acyl-CoA thioesterase 1 gene, causing alterations in the composition of free fatty acids in mitochondrial membranes, particularly in the proportion of C22:6N3, leading to ferroptosis. Fer-1 inhibited DOX downregulation of this gene which reduced the sensitivity of cardiomyocytes to ferroptosis induced by DOX (69). Besides, Fer-1 increased the expression of FTH1 protein to increase iron in storage, resulting in the reduction of Fe^{2+} in LIP (73).

Other Iron Chelators and Radical-Trapping Antioxidants

In addition to Fer-1, parallel experiments in GPX4 gene-deficient mouse embryonic fibroblast ferroptosis model and extracellular high concentration glutamate-induced ferroptosis

TABLE 2 | The therapeutic strategies against ferroptosis in DIC.

Agent	Study design	DOX administration	Agent dose	Mechanism	Parameters	References
DXZ	*In vivo* (mice)	3 mg/kg, IP; once per week for 6 weeks	30 mg/kg, IP; once per week for 6 weeks	Chelating iron	ECV ↓, GCS ↑, GLS ↑, LVEF ↑, T2 Avg ↓	(130)
DXZ	*In vivo* (mice)	6 mg/kg, tail vein injection; on days 0, 2, and 4	1,000 μM	Mitochondrial GPX4 ↑	Mitochondrial lipid peroxidation ↓, MDA ↓, cell survival rate ↑, mitochondrial iron ↓	(50)
DXZ	*In vivo* (rats)	20 mg/kg, IP; a single dose	NA	HMGB1 ↓	PTGS2 ↓, MDA ↓, Anp ↓, Bnp ↓, Myh7 ↓, LVEF ↑, LVFS ↑, cardiac heme ↑, serum heme ↑, non-heme iron ↓, Tfrc ↑, FTH1 ↓	(73)
DXZ	*In vitro* (H9C2 cells)	2 μM	100 μM/L	HMGB1 ↓	Cell viability ↑, PTGS2 ↓, MDA ↓, LDH ↓, Fe^{2+} ↓, GPX4 ↑, FTH1 ↑	(73)
DFO	*In vivo* (mitochondria from rat hepatocytes and cardiomyocytes)	50 μM	250 μM	Protect MPTP	Ca^{2+}-induced MPTP activation ↓, MMP ↑, SDH ↑	(113)
Fer-1	*In vitro* (H9C2 cells)	2 μM	50 μM	Mitochondrial GPX4 ↑	Mitochondrial lipid peroxidation ↓, MDA ↓, cell survival rate ↑, mitochondrial iron ↓	(50)
Fer-1	*In vivo* (mice)	10 mg/kg, IP; a single dose	1 mg/kg, IP; a single dose before DOX treatment	HO-1 ↓	Collagen ↓, Anp ↓, Bnp ↓, Myh7 ↓, EF ↑, FS ↑, heart rate ↑, PEox ↓, dioxide PEox ↓, trioxide PEox ↓	(74)
Fer-1	*In vivo* (mice)	15 mg/kg, IP at day 1 and 10 mg/kg IP at day 8	1 mg/kg, IP; every other day for 8 times	Acot1 ↑	Survival rate ↑, EF ↑, FS ↑, LVIDd ↓, LVIDs ↓, collagen area ↓, PTGS2 ↓, MDA ↓, mitochondrial morphological changes ↓	(69)
Fer-1	*In vitro* (HL-1 cells)	2 μM	10 μM	Acot1 ↑	Cell viability ↑, GSH ↓, PTGS2 ↓, lipid ROS ↓	(69)
Fer-1	*In vivo* (rats)	20 mg/kg, IP; a single dose	1 mg/kg, IP; a single dose before DOX treatment	HMGB1 ↓	PTGS2 ↓, MDA ↓, Anp ↓, Bnp ↓, Myh7 ↓, LVEF ↑, LVFS ↑	(73)
Fer-1	*In vitro* (H9C2 cells)	2 μM	10 μmol/L	HMGB1 ↓	Cell viability ↑, PTGS2 ↓, MDA ↓, LDH ↓, Fe^{2+} ↓, GPX4 ↑, FTH1 ↑	(73)
EMPA	*In vitro* (HL-1 cells)	100 nM	10, 50, and 500 nM	NLRP3/MyD88-related pathway ↓	Cell viability ↑, ROS ↓, MDA ↓, 4-HNA ↓, IL-1β ↓, IL-6 ↓, IL-8 ↓, leukotrienes B4 ↓	(156)
EMPA	*In vivo* (mice)	2.17 mg/kg/day, IP; for 7 days	10 mg/kg/day, oral gavage; for 10 days	NLRP3/MyD88-related pathway ↓	MitoPeDPP ↓, MDA ↓, xanthine oxidase ↓, IL-1β ↓, IL-6 ↓, IL-8 ↓, MyD88 ↓, NLRP3 ↓, EF ↑, FS ↑, fibrosis ↓	(156)
Met	*In vitro* (HL-1 cells)	5 μM	4 mM	FHC ↑, NF-κB ↑	Cell viability ↑, ROS ↓, CAT ↑, Gpx3 ↑, SOD ↑, free iron ↓ Complex I activity ↑, ATP ↑, loss of $\Delta\Psi m$ ↓	(92, 160)

model have shown that other RTAs (liproxstatin-1 and α-TOH) can also improve cell survival rate. In HEK-293 cells, the mechanism by which Fer-1, liproxstatin-1, and α-TOH inhibit lipid hydroperoxides and bring about ferroptosis was demonstrated to be achieved by capturing chain-carrying peroxyl radicals, rather than inhibiting LOXs or restoring GSH levels (148–150). However, in a striatal cell model, the mechanism of action of α-TOH against ferroptosis was proved to be associated with LOX. Its endogenous metabolite, α-tocopherol hydroquinone, inhibited 15-LOX activity by reducing its non-heme Fe^{3+} center to the inactive Fe^{2+}, thereby inhibiting lipid peroxidation (151). Other antioxidants (Trolox, butylated hydroxytoluene, Tiron, and TEMPO) have less inhibitory effects on erastin-induced ferroptosis than Fer-1 and this could be attributed to Fer-1 containing an aromatic amine (11). Another RTA, CoQ10, was shown to inhibit ferroptosis by inhibiting the propagation of lipid peroxides. Inhibition of CoQ10 synthesis with 4-chlorobenzoic acid or knockout of COQ2, an enzyme required for CoQ10 synthesis, can increase the sensitivity of cells to RSL3-induced ferroptosis *in vitro* (152, 153). Other iron chelators such as ciclopirox and deferiprone also alleviate iron-dependent lipid peroxidation by depriving iron (12).

Although many experiments have demonstrated that ferroptosis could be attenuated by inhibiting lipid peroxidation and iron accumulation, studies using DOX-induced

cardiomyopathy models are scarce, and the efficiency and safety of these drugs against ferroptosis are still questionable (56).

Anti-diabetic Medications

According to recent research, DIC was also associated with insulin signaling imbalance and cardiac insulin resistance (154, 155). Metformin, empagliflozin, and sitagliptin have been shown to reduce ferroptosis triggering lipid peroxidation in the mitochondria and cytoplasm of cardiomyocytes (156–158). Metformin and sitagliptin have been shown to attenuate myocardial lipid peroxidation in rats by reversing the DOX-induced decrease in GSH (159). In cardiomyocytes (HL-1 cell line) exposed to DOX and C57Bl/6 mice treated with DOX, empagliflozin reduced lipid peroxidation levels, decreased cardiomyocyte fibrosis, inhibited cardiomyocyte inflammation, increased ejection fraction percentage (% EF) and fractional shortening percentage (% FS), improved cardiac function, and protected cardiomyocytes from ferroptosis (156). Metformin also activated nuclear factor-kappa B, thereby increasing FTH and reducing iron accumulation in LIP, thus protecting adult mouse cardiomyocytes from DIC (92, 160).

Energy-Stress Inducers

In immortalized mouse embryonic fibroblasts (MEFs), the energy-stress inducers (2-deoxy-D-glucose, 5-aminoimidazole-4-carboxamide ribonucleotide, and A769662) were demonstrated to activate AMPK. This further inhibited acetyl-CoA carboxylase, and in turn palmitic acid (C16:0), thereby reducing the synthesis of PUFA, all of which suppressed ferroptosis (107).

In conclusion, anti-DIC therapy based on these ferroptosis triggering mechanisms mainly include two aspects: iron chelation and antioxidant treatment. Notably, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have a great potential in predicting patient susceptibility to DIC. Furthermore, the human-derived DOX cardiomyocyte injury model established by hiPSC-CMs overcomes the species differences of current research models and can be accurately used to understand the mechanism of ferroptosis in DIC (161).

CONCLUSION AND PERSPECTIVES

In summary, ferroptosis has been demonstrated by several studies to mediate the occurrence of AIC. In fact, DOX can increase the ROS content and affect iron metabolism in cardiomyocytes by acting on iron homeostasis regulatory proteins, such as, IREs/IRPs, and Nrf2/HO-1, resulting in the accumulation of lipid peroxides, thereby inducing ferroptosis. Mitochondria are the main organelle for inducing ferroptosis in cardiomyocytes. With further research, inhibition of ferroptosis could act as an effective strategy in both prevention and treatment of AIC. This would require screening for potential drugs that inhibit ferroptosis forcefully in cardiomyocytes or developing novel ferroptosis inhibitors and will surely benefit cancer patients with heart diseases as well as patients with high cardiovascular risk stratification.

AUTHOR CONTRIBUTIONS

YX and SS designed this study. GZ wrote the first draft of this manuscript and created the figures. CY and XS searched the literature. JZ and XY participated in discussions and improved pictures related to the manuscript. PG, GV, GL, NA, CL, WS, HC, and MW critically revised and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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Research on the Mechanism and Prevention of Hypertension Caused by Apatinib Through the RhoA/ROCK Signaling Pathway in a Mouse Model of Gastric Cancer

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Hypertension is one of the main adverse effects of antiangiogenic tumor drugs and thus limits their application. The mechanism of hypertension caused by tyrosine kinase inhibitors (TKIs) targeting vascular endothelial growth factors is mainly related to inhibition of the nitric oxide (NO) pathway and activation of the endothelin pathway, as well as vascular rarefaction and increased salt sensitivity; consequently, prevention and treatment differ for this type of hypertension compared with primary hypertension. Apatinib is a highly selective TKI approved in China for the treatment of advanced or metastatic gastric cancer. The RhoA/ROCK pathway is involved in the pathogenesis of hypertension and mediates smooth muscle contraction, eNOS inhibition, endothelial dysfunction and vascular remodeling. In this study, *in vivo* experiments were performed to explore whether the RhoA/ROCK signaling pathway is part of a possible mechanism of apatinib in the treatment of gastric cancer-induced hypertension and the impairment of vascular remodeling and left ventricular function. Y27632, a selective small inhibitor of both ROCK1 and ROCK2, was combined with apatinib, and its efficacy was evaluated, wherein it can reduce hypertension induced by apatinib treatment in gastric cancer mice and weaken the activation of the RhoA/ROCK pathway by apatinib and a high-salt diet (HSD). Furthermore, Y-27632 improved aortic remodeling, fibrosis, endothelial dysfunction, superior mesenteric artery endothelial injury, left ventricular dysfunction and cardiac fibrosis in mice by weakening the activation of the RhoA/ROCK pathway. The expression of RhoA/ROCK pathway-related proteins and relative mRNA levels in mice after apatinib intervention were analyzed by various methods, and blood pressure and cardiac function indexes were compared. Endothelial and cardiac function and collagen levels in the aorta were also measured to assess vascular and cardiac fibrosis and to provide a basis for the prevention and treatment of this type of hypertension.

Keywords: hypertension, apatinib, gastric cancer, RhoA/ROCK signaling pathway, Y27632

INTRODUCTION

Continued progress in cancer treatment technology has prolonged the survival of cancer patients, and cardiovascular disease has gradually emerged as an important cause of death in cancer patients. This phenomenon is partly due to the cardiovascular toxicity of antitumor therapy, which leads to cardiovascular problems in patients with cancer (1, 2). Hypertension is one of the most common adverse events caused by antiangiogenic antitumor drugs. Increased blood pressure (BP) not only causes discontinuation of antitumor therapy but also exacerbates the occurrence of cardiovascular events (3). A phase III clinical trial has proven that apatinib is safe and effective in patients with advanced gastric cancer (4).

Apatinib is a highly selective tyrosine kinase inhibitor (TKI) of vascular endothelial growth factor receptor-2 (VEGFR2) (5–7). Little is known about the mechanism of TKI-induced hypertension. The proposed mechanisms include activation of the endothelin (ET) system and inhibition of the nitric oxide (NO) pathway, vascular rarefaction, renal function impairment caused by glomerular injury, and increased salt sensitivity (8, 9). Endothelin (ET)-1 is a potent vasoconstrictor peptide produced and released primarily by endothelial cells (10) that inhibits endothelial nitric oxide synthase (eNOS) and, in turn, NO production (11, 12). Rho-kinase was first identified as a serine/threonine kinase that specifically binds to GTP-RhoA (an activated form of RhoA) (13).

The RhoA-associated kinase (RhoA/ROCK) signaling pathway plays an important role in the pathogenesis of salt-sensitive hypertension and hypertension-induced cardiac hypertrophy and enhances the vasoconstriction effect of ET-1. Moreover, activation of this pathway reduces endothelial NO production and vasodilation function, ultimately promoting the occurrence of hypertension (14–16). A high salt intake can significantly increase the elevation of BP induced by sunitinib (17). Therefore, it is speculated that the RhoA/ROCK pathway is involved in the occurrence of hypertension induced by TKIs and that ROCK pathway inhibitors may have therapeutic effects on this hypertension. However, no related research has been reported.

The RhoA/ROCK signaling pathway is deeply involved in arterial hypertension, cardiovascular–renal remodeling, hypertensive nephropathy and posttransplant hypertension (18). RhoA mainly interacts with two isoforms of Rho-associated coiled-coil domain-containing protein kinases (ROCK1 and ROCK2) to induce the phosphorylation and inhibition of myosin light chain phosphatase (MLCP) (19). ET-1, as an upstream effector, increases the phosphorylation level of MLC through the RhoA/ROCK pathway, enhances vascular endothelial oxidative stress, increases peroxide production, reduces NO production through this pathway, and enhances vasoconstriction, leading to hypertension (20–22). Moreover, RhoA/ROCK-mediated phosphorylation of MYPT-1 (p-MYPT-1) at specific residues is associated with inhibition of MLCP, leading to an increase in smooth muscle contraction (23–25).

The incidence of hypertension caused by apatinib is approximately 35.2%, of which approximately 4.5% is grade 3-4 hypertension (4, 26). However, the pathogenesis of this type of hypertension is not the same as that of essential hypertension, and the specific mechanism is unclear. Therefore, it is very important to explore the pathogenesis of apatinib-induced hypertension and to identify new specific therapeutic drugs that can promote the application of apatinib, prevent vascular events and improve patient prognosis. The goals of this study were to apply gastric cancer modeling technology to simulate the real *in vivo* environment to explore whether the RhoA/ROCK signaling pathway is involved in the occurrence of this type of hypertension and whether ROCK inhibitors have therapeutic effects.

MATERIALS AND METHODS

Cell Lines and Culture

The human gastric adenocarcinoma cell line MKN-45 was purchased from and validated by American Type Culture Collection (ATCC; Manassas, VA, United States). Cells were cultured following instructions from ATCC in Roswell Park Memorial Institute medium (RPMI 1640, Invitrogen, Shanghai, China). All cells were supplemented with 10% fetal bovine serum (FBS, Invitrogen, Shanghai, China), 100 U/ml penicillin and 100 U/ml streptomycin (Invitrogen, Shanghai, China) at 37°C in a humidified environment of 5% CO_2. All cells were used within 6 months after resuscitation and were free of mycoplasma.

Animals

All experiments were approved by the Ethics Committee of Lanzhou University Second Hospital (license number: D2019-101), and all manipulations were performed in accordance with the Guide for the Care and Use of Laboratory Animals. Female BALB/C nude mice (4–6 weeks old, 20 ± 5 g) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China; SCXK2012-0001).

Animal Experiments

The procedure used for establishing mouse orthotopic mammary xenograft tumors was described recently (27). Nude mice and MKN-45 were used for gastric cancer modeling.

Mice were fed in SPF-class barrier facilities that met the requirements of GB14925 (2010 Experimental Animal Environment and Facilities). All mice were reared on sterilized food and water and were housed eight mice per cage in standard polycarbonate cages with controlled temperature and humidity and a 12-h light and dark cycle (28). Adaptive feeding was performed for 1 week before the experiment, and all efforts were made to minimize suffering.

Nude Mouse Xenograft Tumor Assays

Mice were randomly placed into normal (NR) (n = 6 mice) or tumour-bearing (TB) groups. MKN-45 cells (1×10^7 in 100 μL of medium) and 50% matrix glue were inoculated subcutaneously into the right front armpit of the mice. Mice in the intervention groups were given apatinib 50 mg/kg/day. When the tumor volumes reached approximately 100–200 mm^3, the TB group was

randomly divided into four groups, with each group (n = 6 mice) having similar average tumor volumes: (i) control, 2 ml of normal saline gavage; (ii) high-salt diet (a custom 8% high-salt feed);

(iii) high-salt diet (a custom 8% high-salt feed) + 10 mg/kg Y27632 administered *via* intraperitoneal injection; (iv) high-salt diet (a custom 8% high-salt feed) + 50 mg/kg apatinib dissolved in 2 ml of normal saline by gavage; (v) 50 mg/kg apatinib dissolved in 2 ml of normal saline by gavage; and (vi) 50 mg/kg apatinib dissolved in 2 ml of normal saline by gavage + 10 mg/kg Y27632 administered *via* intraperitoneal injection. The mice were anesthetized by inhalation of 1.0–2.5% isoflurane on day 28 after drug intervention to collect their blood, and tissues were immediately frozen in liquid nitrogen. The anesthetized mice were sacrificed by carbon dioxide asphyxiation. The tumors were isolated and weighed. The tumor-free body weight at the last time point was calculated using the following formula: tumor-free body weight = (body weight with tumor)–(tumor weight) (29).

Blood Pressure Analysis

The tail pressure of the mice was measured every 3 days and before sacrifice. An IITCMRBP tail cuff blood pressure system (Yuyan Instruments, Shanghai, China) was used for measurements; the mice were placed within the instrument while the temperature was set to 36°C. After 15–20 min of relative quiet, the systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP) were collected when the mice were awake. Measurements were performed three times every 15 s, and the average value was recorded.

Echocardiography and Heart Weight Analysis

Mouse cardiac function was measured using a high-resolution echocardiography imaging system (Vinno 6 Lab, Vinno Technology, Beijing, China) following a sedation protocol (inhaled isoflurane 2.5%). Two trained, blinded operators (W.J.W. and Q.Y.W.) performed echocardiography to evaluate cardiac function. The ejection fraction (EF) and fractional shortening (FS) were calculated and considered systolic function. The left ventricular internal dimension-diastole (LVIDd) and left ventricular internal dimension-systole (LVIDs) were measured for left ventricular dilation. The mice were weighed throughout the experiment to properly administer the correct treatment dose. Heart weight (HW), tibia length (TL) and left ventricular mass (LVM) were measured, including the septum. Then, the heart weight-to-tibia length ratio (HW/TL) and left ventricular mass to-tibia length ratio (LVM/TL) were calculated to evaluate cardiac hypertrophy *in vivo*.

Histopathology and Immunohistochemistry

Tissues were fixed in 4% paraformaldehyde in PBS at room temperature for 24–48 h, embedded in paraffin (Solarbio, Beijing, China), sectioned into 3- to 5-μm pieces and placed in neutral resin to seal the film. Haematoxylin–eosin (HE) staining was performed to observe cell structure and organizational hierarchy. The experimental steps were carried out in strict accordance with the kit instructions (Solarbio, Beijing, China).

Masson's trichrome staining was used to evaluate the area of fibrosis. Staining was performed on each heart and aorta section according to the standard procedure of the Masson's trichrome staining kit (Solarbio, Beijing, China). Immunohistochemistry was performed by applying the UltraSensitive^{TM} SP (mouse/rabbit) IHC KIT-9710 protocol method (Manxin, Fuzhou, China) with anti-COL I (Proteintech, Wuhan, China) and anti-COL III (Proteintech, Wuhan, China) antibodies to each aorta section. Images were collected using a Nikon Eclipse 80i (Nikon, Japan), and subsequent analysis was performed using ImageJ software.

Western Blotting

Western blotting was performed as previously described (29). Total protein was extracted in radioimmunoprecipitation assay (RIPA) buffer and phenylmethanesulfonyl fluoride (PMSF) (Beyotime Biotechnology, Shanghai, China) at a ratio of 100:1. The protein concentration was measured by a BCA assay kit (Biosharp, AnHui, China), and the protein was loaded onto a 5 × sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gel; a loading buffer (Biosharp, Anhui, China) at a volumetric ratio of 5:1 was used.

After denaturation and SDS–PAGE electrophoresis, the proteins were transferred to polyvinylidene fluoride (PVDF) membranes, which were blocked with 5% non-fat dried milk (Sigma, Shanghai, China) and incubated with primary antibodies including anti-ROCK1, anti-RhoA, anti-ROCK2, anti-α-SMA, anti-eNOS, anti-iNOS anti-ET-1, anti-CD31 phospho-MYPT-1, MYPT-1 (Abcam, Shanghai, China), phospho-MLC, MLC (Cell Signaling Technology), and GAPDH (Proteintech, WuHan, China) overnight at −20°C. The PVDF membranes were washed with TBST and then with secondary antibodies. Finally, the membranes were washed again and incubated in chemiluminescent ECL substrate (Fisher). The images were analyzed by ImageJ software, and the results were normalized to those of GAPDH. Analysis was performed using ImageJ software.

qRT–PCR Analysis

Total RNA was extracted using TRIzol (Takara, Japan). For mRNA quantification, a PrimeScript^{TM} RT reagent Kit (Takara) was used to prepare cDNA. Real-time PCR was performed with SYBR Green (Bio-Rad, United States) on a Bio-Rad CFX-96 real-time system (Bio-Rad, Hercules, California, United States). Relative RNA expression was determined by the relative standard curve method ($2^{-\Delta\ \Delta\ CT}$). PCR primers were synthesized by Sangon Biotech (Shanghai, China). The following primers were used in this study: RhoA (forward: 5′- GAG TTG GAC TAG GCA AGA AAC TC-3′; reverse: 5′-ACC CAA ACC CTC ACT GTC TTC -3′); ROCK1 (forward: 5′-TGG GAG AGT GAG CCT GTT CT -3′; reverse: 5′-TAG AGG TGG TCT AGC CCT GCA T -3′); ROCK2 (forward: 5′-TGT TAG GGT TCC CAG GGT GA -3′; reverse: 5′-AGA AGC TCG GAA GCT ACG TG -3′); eNOS (forward: 5′-TCT GCG ATG TCA CTA TG -3′; reverse: 5′-ATG ACG TCA CCG GCT TCA TC -3′); iNOS (forward: 5′- TCT AGT GAA GCA AAG CCC AAC -3′; reverse: 5′- GGA ACA TTC TGT

GCT GTC CCA -3′); ET-1 (forward: 5′-CTA CGA AGG TTG GAG GCC AT -3′; reverse: 5′-TCG GTT GTG CGT CAA CTT CT -3′); COLI (forward: 5′- TCG GAC TAG ACA TTG GCC CT -3′; reverse: 5′- TCG TCT GTT AGG GTT GG -3′); COLIII (forward: 5′- CCA AGG CTG CAA GAT GGA TG-3′; reverse: 5′-TGT CCA GTG CTT ACT TG-3′); and CX43 (forward: 5′- TCT GAG AGC CCG AAC TCT CCT T -3′; reverse: 5′-CTG GGC ACC TCT CTT TCA CTT –3′).

Statistical Analysis

All experiments were performed at least three times. SPSS version 23 (IBM, Armonk, New York, United States) and GraphPad Prism version 8.0 (San Diego, CA, United States) were used for statistical analysis. Data are presented as the mean ± standard error of the mean (SEM) or geometric means with 95% confidence intervals (CIs). Differences between two independent groups were determined using an unpaired Student's *t*-test, while one-way or two-way ANOVA followed by either Dunnett's or Tukey's *post-hoc* test was used to compare means across multiple groups. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Apatinib Treatment Increases Mouse Weight and Decreases Tumor Volume, While Y27632 Treatment Does Not Affect Body Weight or Tumor Volume

Weight changes in each group, including weight at baseline, after tumor modeling, after treatment, and before death, were recorded weekly (**Figure 1A**).

Over the course of the experiment, body weight increased gradually in the NR group and reached 23.7 ± 1 g, while body weight in the control group began to show a downward trend after tumor modeling (**Figure 1A**) and reached 17.1 ± 0.5 g before death. Body weight in the HSD group tended to decrease continually and reached 13.7 ± 0.21 g before death, while body weight in the HSD + Y27632 and HSD + apatinib groups increased for 1 week after treatment and reached 16.5 ± 0.8 g before death. However, body weight in the apatinib group and the apatinib + Y27632 group tended to decrease after tumor modeling but gradually increased in the week after treatment (**Figure 1A**). Compared with that in the NR group, body weight tended to decrease in the control group ($p < 0.001$). Compared with that in the control group, body weight decreased in the HSD group ($p < 0.001$) but increased slightly in the HSD + Y27632, HSD + apatinib, and apatinib + Y27632 groups ($p < 0.001$). There was no significant difference in weight between the apatinib and apatinib + Y27632 groups or between the HSD + apatinib and HSD + Y27632 groups.

The changes in tumor volume in each group were evaluated and recorded after tumor modeling. The tumor volumes in the control and HSD groups increased over time and reached 779.4 ± 144.1 mm^3 by death, while those in the HSD + apatinib and HSD + Y27632 groups and the apatinib and apatinib + Y27632 groups decreased in the week after treatment (**Figure 1B**). Compared with the control group, tumor volume decreased in the apatinib and apatinib + Y27632 groups ($p < 0.001$), but tumor volume did not differ significantly between the apatinib and apatinib + Y27632 groups (**Figure 1B**). Compared with that in the HSD group, tumor volume decreased in the HSD + apatinib and HSD + Y27632 groups ($p < 0.001$), but tumor volume did not differ significantly between the latter two groups (**Figure 1B**).

Apatinib Induces Elevated Blood Pressure and Can Enhance High-Salt Diet-Induced Elevated Blood Pressure, and Y27632 Mitigates These Effects

BP was recorded weekly in each group. The baseline BP was 114.50/67.17 ± 1.78/6.51 mmHg, and the baseline MBP was 83.50 ± 4.60 mmHg in all groups. After tumor modeling, there was a slight increase in BP in all groups, which gradually decreased to baseline at week 3. There were no significant differences in SBP, DBP or MBP in the control group compared with the NR group, while BP significantly increased in the apatinib group from the fifth week and the HSD and HSD + apatinib groups from the third week, regardless of whether it was SBP, DBP or MBP, reaching a plateau after 7 weeks ($p < 0.001$). The increase in BP was most significant in the HSD + apatinib group ($p < 0.001$) (**Figure 2**). Before the mice were killed, SBP/DBP was 158.83/101.83 ± 2.71/1.54 mmHg, 188.17/133.67 ± 2.07/1.17 mmHg, and 167.33/105.33 ± 2.11/5.78 mmHg in the HSD, HSD + apatinib and apatinib groups, respectively. Notably, Y27632 significantly mitigated the apatinib-induced and HSD-induced elevation in BP. SBP/DBP was 101/57 ± 1.67/1.84 mmHg in the HSD + Y27632 group, and the BP was lower than that in the HSD group ($p < 0.001$). The BP of the apatinib + Y27632 group was 98.17/66.17 ± 1.25/3.51 mmHg, which was lower than that of the apatinib group ($p < 0.001$).

Apatinib Activates the RhoA/ROCK Signaling Pathway in Mouse Models of Gastric Cancer

RhoA/ROCK signaling pathway-related mRNA levels and protein expression were detected and measured by western blotting and qRT–PCR. The relative mRNA levels and protein expression of RhoA, ROCK1, and ROCK2 in mouse aortas increased after HSD, and this effect of HSD was enhanced by apatinib ($p < 0.001$) (**Figures 3A–D**. Y27632 treatment decreased the mRNA and protein expression levels of RhoA, ROCK1 and ROCK2 in mouse aortas compared with apatinib treatment alone ($p < 0.001$). The same effect was observed in the HSD + Y27632 group ($p < 0.001$). This effect was more obvious for ROCK2 than for the mRNA and protein levels of ROCK1, and ROCK1 expression was still higher than that in the control group (**Figures 3A–D**).

Subsequently, the phosphorylation of MLC and MYPT-1 in this pathway was assessed. p-MLC/P-MLC/and P-MYPT/MYPT-1 protein levels were increased in the aortas of mice treated with

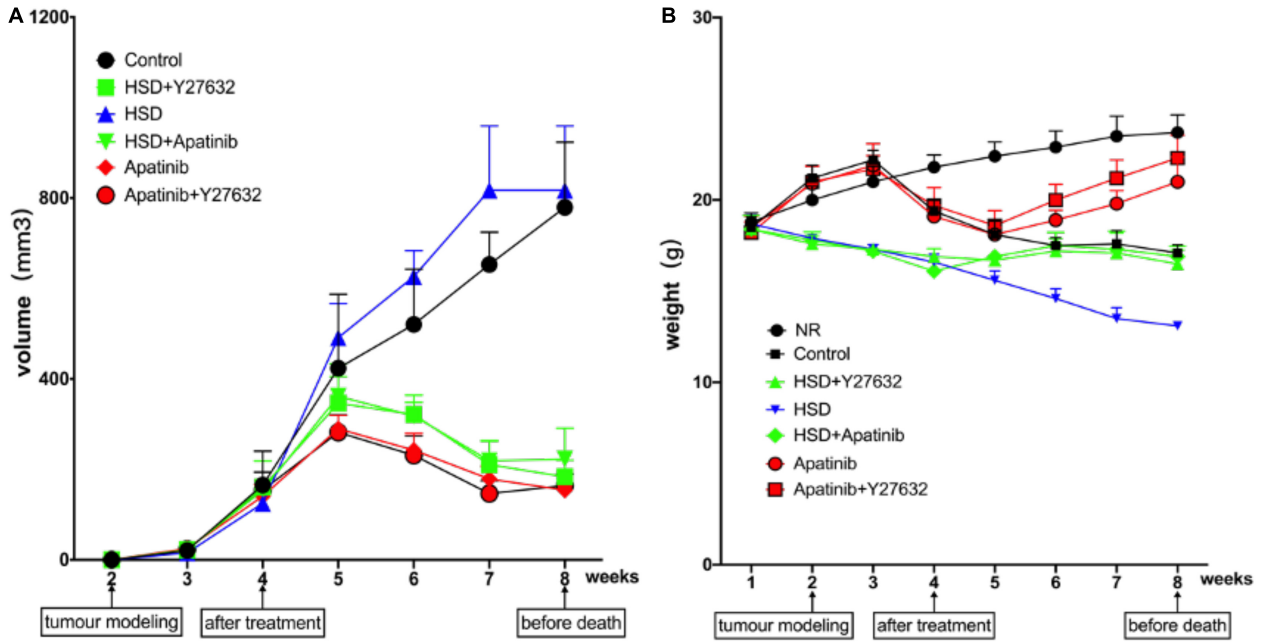

FIGURE 1 | Changes in body weight and tumor volume in mice. **(A)** The trend of tumor volume after modeling (mm^3). Tumor volume showed a decreasing trend in the fifth week after apatinib intervention for 1 week and showed a decreasing trend after Y27632 treatment. However, there was no statistical significance for the influence of the two drugs on tumor volume ($P > 0.05$). Similar trends were observed when apatinib and Y27632 were given with a high-salt diet. **(B)** Change trend of body weight at baseline, after modeling, after drug intervention and at death (g). Body weight showed an increasing trend in the sixth week after apatinib intervention for 2 weeks and showed an increasing trend after Y27632 treatment. However, there was no statistical significance in the influence of the two drugs on body weight ($P > 0.05$). Similar trends were observed when apatinib and Y27632 were given with a high-salt diet. Data are representative of three independent measurements. Mean ± SEM.

apatinib, and the HSD + apatinib group showed a more obvious trend ($p < 0.001$) (**Figure 3E**). However, this effect was mitigated when Y27632 was administered. Similarly, administration of Y27632 after HSD reduced the protein expression of p-MLC and P-MYPT-1 ($p < 0.001$) (**Figure 3E**). That is, apatinib induced the activation of the RhoA/ROCK pathway by stimulating the phosphorylation of MLC and MYPT-1, while treatment with Y27632 inhibited the expression of phosphorylated proteins.

Apatinib Upregulates ET-1 and Downregulates the NO System in Mouse Models of Gastric Cancer

ET-1 and NO levels in mouse aortas were assessed by qRT–PCR and western blotting (**Figures 4A–D**). The mRNA and protein expression of ET-1 increased after HSD + apatinib treatment and was higher than the levels in the apatinib and HSD groups ($p < 0.001$). Moreover, Y27632 attenuated the increase in ET-1 induced by apatinib, and ET-1 levels were lower in the HSD + Y27632 group than in the HSD group ($p < 0.001$) (**Figures 4A–D**). The mRNA and protein expression of iNOS increased in the HSD + apatinib group compared with the apatinib and HSD groups ($p < 0.001$) and decreased after Y27632 treatment ($p < 0.001$) (**Figures 4A–D**). In contrast, eNOS protein expression and relative mRNA levels in mouse aortas decreased after apatinib treatment but were higher than those in the aortas of mice treated with HSD + apatinib ($p < 0.001$). However, after Y27632 treatment, eNOS protein expression and relative mRNA levels were increased compared with apatinib ($p < 0.001$) and were also higher in the HSD + Y27632 group than in the HSD group ($p < 0.001$) (**Figures 4A–D**).

Apatinib Induces Vascular Remodeling in Mouse Models of Gastric Cancer, and Y27632 Ameliorates This Effect

Haematoxylin–eosin staining showed that the aortic lumen was narrowed and the vascular wall was thickened in mice treated with HSD and apatinib, and these effects were enhanced in the HSD + apatinib group ($p < 0.001$). The lumen area was increased in mice treated with Y27632, and the wall was thinner than that in mice treated with apatinib ($p < 0.001$) (**Figures 5A,E**). The vascular wall was also thinner in the HSD + Y27632 group than in the HSD group ($p < 0.001$) (**Figures 5A,E**). Masson staining showed that the degree of aortic blue staining increased after HSD and apatinib treatment, and this effect was enhanced in the HSD + apatinib group ($p < 0.001$). The area of blue staining decreased after Y27632 treatment compared with apatinib treatment ($p < 0.001$) and was also decreased in the HSD + Y27632 group ($p < 0.001$) (**Figures 5B,F**). The observed patterns of collagen overexpression inspired us to investigate the mechanical metrics of the mid-aorta. Immunohistochemistry showed that the positive signals of COLI and COLIII increased significantly after apatinib treatment

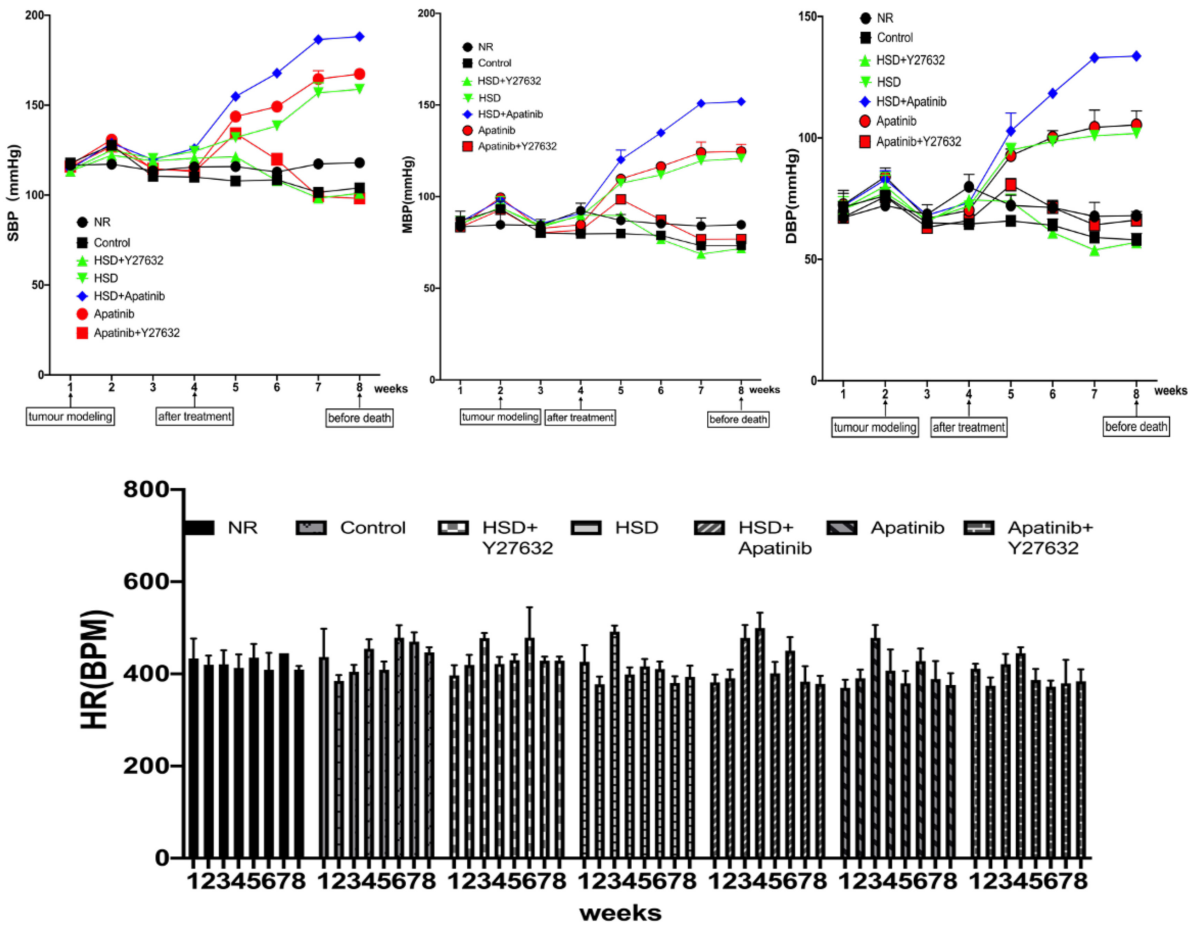

FIGURE 2 | Changes in the BP of mice in each group. After 1 week of gastric cancer modeling, BP showed a temporary upward trend and returned to the baseline BP level in the third week. Apatinib and the high-salt diet increased BP from the fourth to the eighth week of treatment, and the increase in BP after the high-salt diet combined with apatinib treatment was most obvious and reached a plateau between weeks 7 and 8. Y27632 mitigated the apatinib-induced elevation in BP in the apatinib ($P < 0.001$) and high-salt diet ($P < 0.001$) groups. Data are representative of three independent measurements. Mean ± SEM. BP, blood pressure; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure.

and HSD, and the HSD + apatinib group showed the highest positive signal ($p < 0.001$). After treatment with Y27632, the positive signal was lower, and the fibrosis was relieved compared with apatinib treatment ($p < 0.001$). Fibrosis was also lower in the HSD + Y27632 group than in the HSD group ($p < 0.001$) (**Figures 5C,D,G,H**). From the mRNA levels, the results were similar to those of immunohistochemistry ($p < 0.001$) (**Figures 5I,J**).

Apatinib Reduced the Vascular Density of the Superior Mesenteric Artery, and This Effect Was Attenuated by Y27632 in Mouse Models of Gastric Cancer

To determine whether the vascular remodeling in the superior mesenteric artery of mice was due to a reduction in vascular density, we performed CD31 staining. The results of immunohistochemistry showed that the positive signals of CD31 and eNOS decreased significantly after HSD and apatinib treatment, and the downward trend of the positive signal in the HSD + apatinib group was the most obvious ($p < 0.001$). The positive signals of CD31 and eNOS were stronger in the apatinib + Y27632 group than in the apatinib group ($p < 0.001$) (**Figures 6A–D**) but lower in the HSD + Y27632 group than in the HSD group ($p < 0.001$). The stronger positive signals of CD31 and eNOS in the apatinib + Y27632 and HSD + Y27632 groups suggest that administration of Y27632 can improve vascular injury to the superior mesenteric artery (**Figures 6A–D**).

Y27632 Improves Hypertension-Induced Cardiac Hypertrophy Caused by Apatinib in Mouse Models of Gastric Cancer

Left ventricular function in each experimental group was evaluated using the long-axis view of the mouse sternum and M-mode echocardiography (**Figures 7A,B**). There were no significant differences in functional indexes between the NR and control groups, suggesting that tumor modeling may not

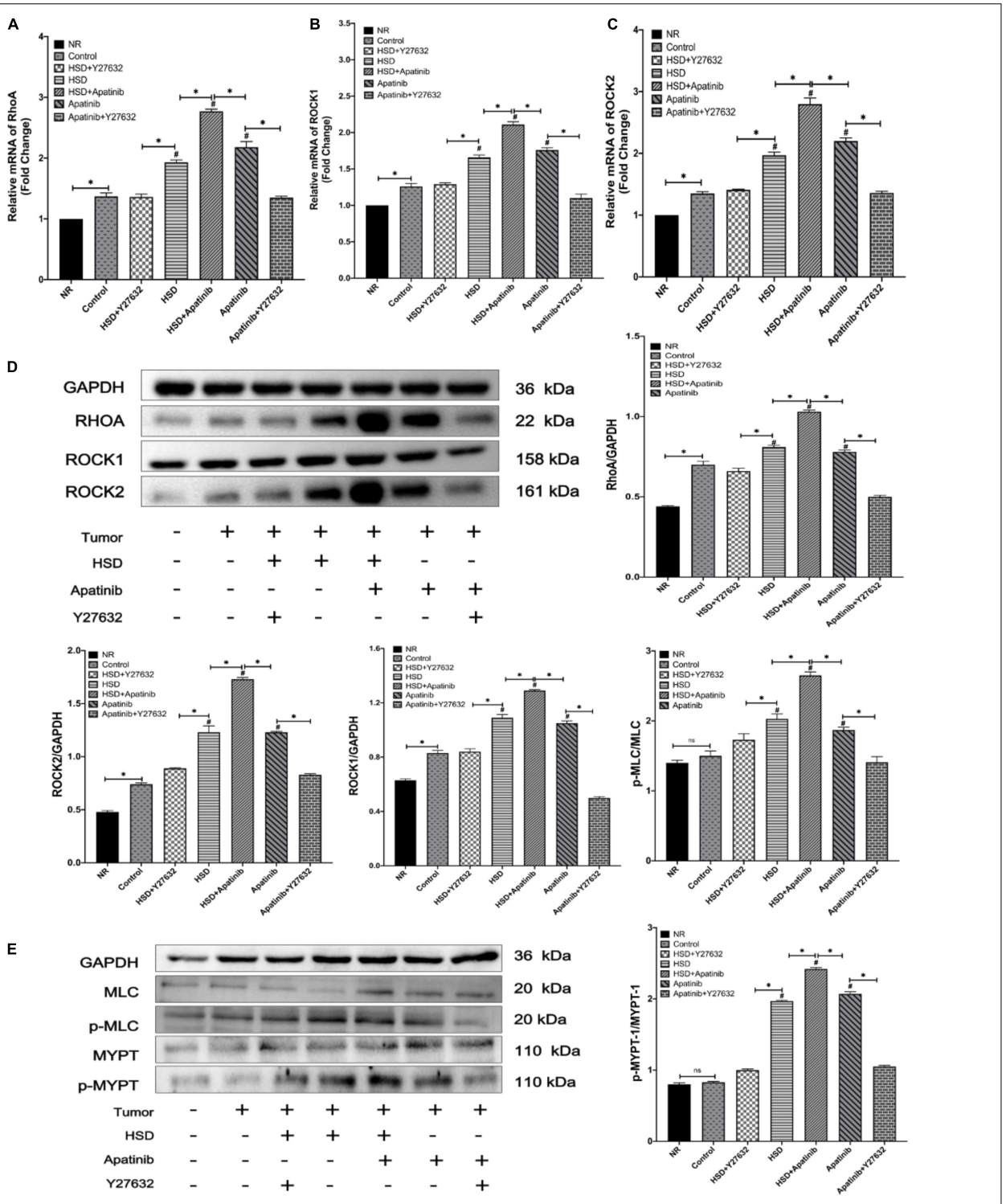

FIGURE 3 | Expression of the RhoA/ROCK pathway was detected by qRT–PCR and western blotting. **(A–C)** The levels of RhoA and Rho-associated coiled-coil domain-containing protein kinase mRNAs. **(E)** The protein expression levels of RhoA/Rho-associated coiled-coil domain-containing protein kinase signaling pathway members. **(E)** Changes in the phosphorylation levels of MLC and MYPT1 after apatinib and high-salt diet intervention. Apatinib and HSD upregulated the expression of RhoA ROCK1 and ROCK2 proteins and mRNAs. Administration of Y27632 reduced the phosphorylation levels of MLC and MYTP downstream of the RhoA/ROCK signaling pathway induced by apatinib and a high-salt diet (n = 6 in each group). Data are representative of three independent measurements. Mean ± SEM. *P less than 0.001. $^{\#}P$ less than 0.001, comparison between each group and the control group. ROCK, Rho-associated coiled-coil domain-containing protein kinase. MLC, myosin light chain phosphatase. MYPT, myosin phosphatase target subunit.

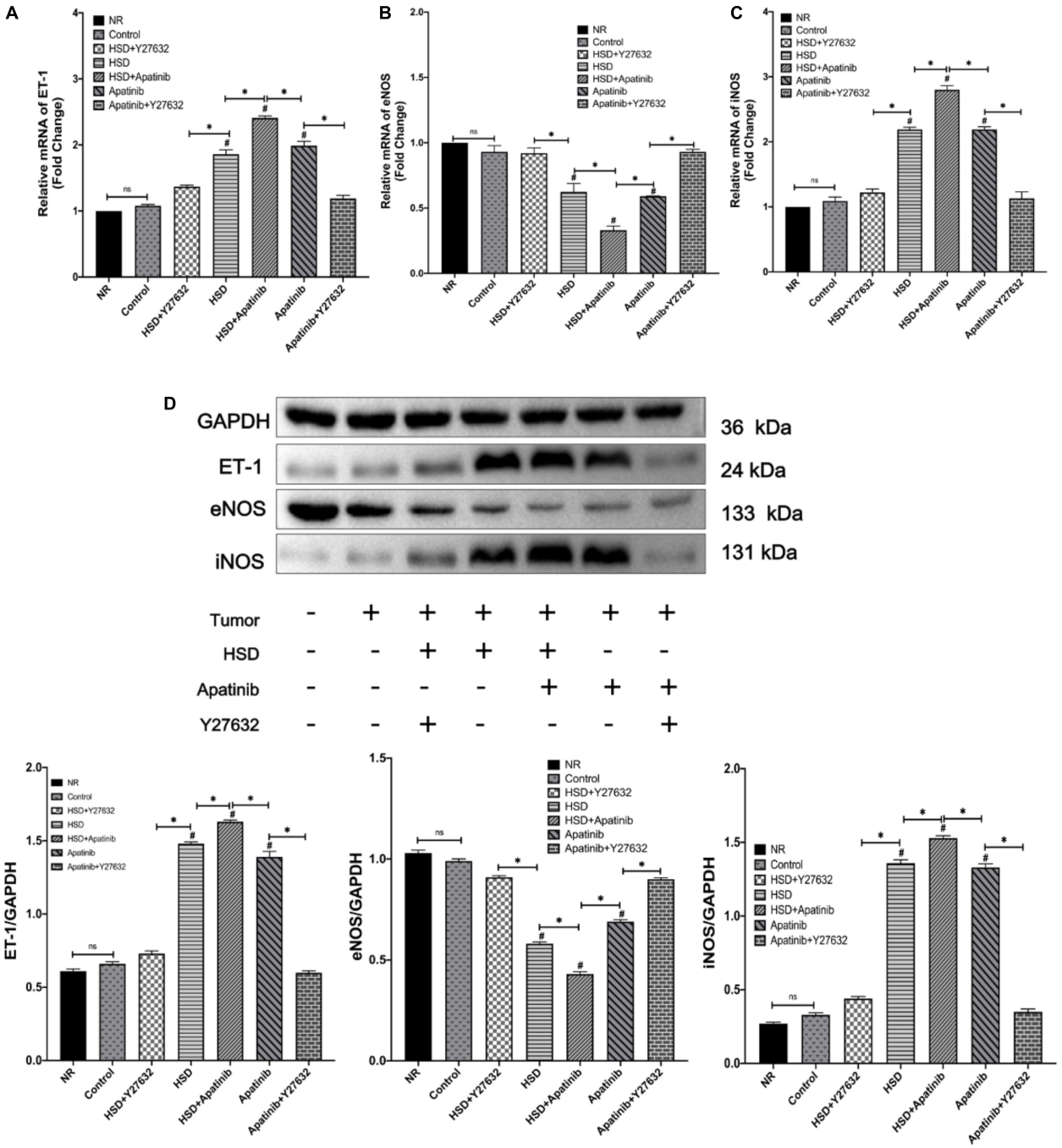

FIGURE 4 | Expression of proteins related to vascular contraction and vasodilation systems was detected by qRT–PCR and western blotting. **(A–C)** The levels of vascular contraction and vasodilation mRNAs. **(D)** The protein expression levels of vascular contraction and vasodilation systems. The protein levels and mRNAs of ET-1 were upregulated with the administration of apatinib and a high-salt diet. Y27632 mitigated both. The protein levels and mRNAs of nitric oxide synthase showing that apatinib and a high-salt diet downregulated eNOS and upregulated iNOS, while the effect was mitigated by Y27632 (n = 6 in each group). Data are representative of three independent measurements. Mean ± SEM. $^{*}P$ less than 0.001. $^{\#}P$ less than 0.001, comparison between each group and the control group. ET-1, endothelin-1, eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase.

cause damage to cardiac function in the short term. In the HSD and apatinib groups, LVPW, LVIDd, LVIDs, HM, and LVM, which were measured for left ventricular dilation, increased; these elevations were most pronounced in the HSD + apatinib group ($p < 0.001$) (**Figure 7**). FS and EF decreased, indicating left ventricular systolic dysfunction, and these decreases were significant in the HSD + apatinib group compared with the HSD and apatinib groups ($p < 0.001$) (**Figure 7**). The ratio of HW/TL

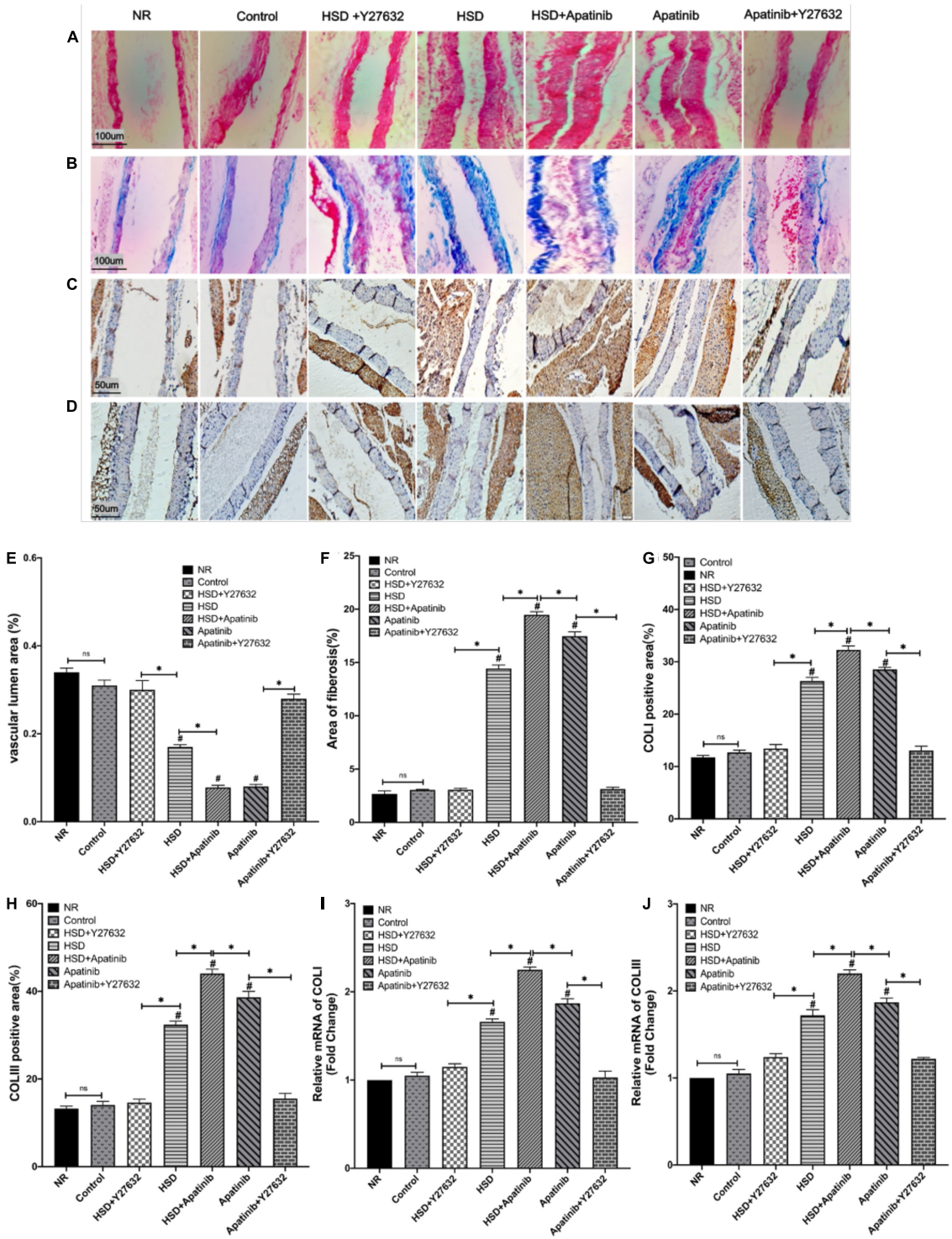

FIGURE 5 | Histological sections of aortic fibrosis *in vivo*. **(A)** Sections stained with H&E. **(B)** Sections stained with Masson staining. **(C,D)** COLI and COLIII immunohistochemical staining. **(E)** The subluminal area of the vessels (%) was decreased in the HSD, HSD + apatinib, and apatinib groups. However, after Y27632 treatment, the area of the vessels was increased. **(F)** The area of Masson staining for fibrosis (%) was increased in the HSD, HSD + apatinib and apatinib groups; after Y27632 treatment, the area of the vessels was decreased. **(G,H)** Positive signal areas of COLI and COLIII (%) were increased in the HSD, HSD + apatinib and apatinib groups. After Y27632 treatment, the vessel diameter was decreased, which is consistent with the fibrosis biomarkers, including COLI and COLIII, detected at the mRNA level **(I,J)** (n = 6 in each group). Data are representative of three independent measurements. Mean ± SEM.*P less than 0.001. #P less than 0.001, comparison between each group and the control group. COLI, collagen I; COLIII, collagen III.

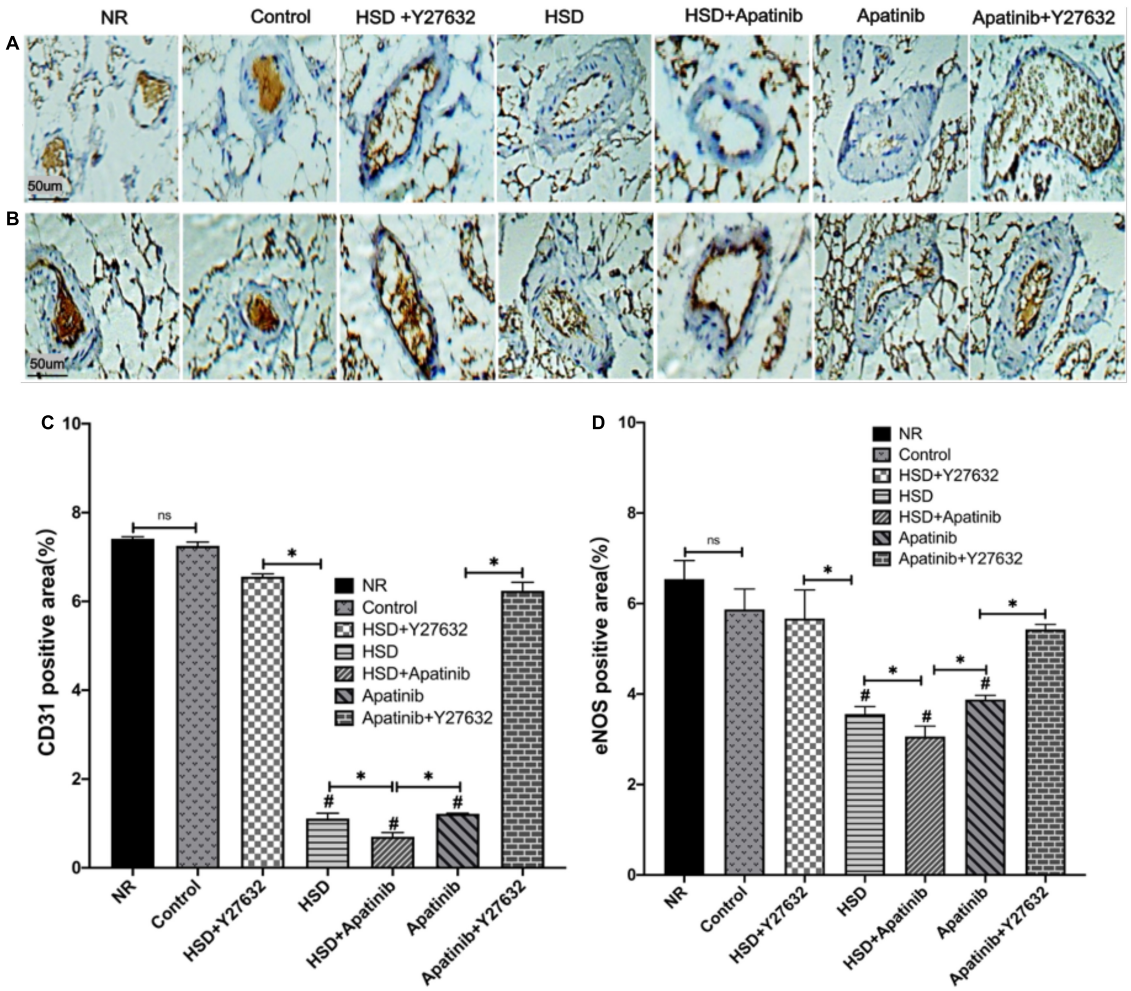

FIGURE 6 | Histological sections of the superior mesenteric artery *in vivo*. **(A,B)** CD31 and eNOS immunohistochemical staining. **(C,D)** Positive signal areas of CD31 and eNOS assessed by immunohistochemistry (%). Positive signal areas of CD31 and eNOS (%) were decreased in the HSD, HSD + apatinib and apatinib groups, and after Y27632 treatment, the area of the vessels was increased (n = 6 in each group). Data are representative of three independent measurements. Mean ± SEM. $^{*}P$ less than 0.001. $^{\#}P$ less than 0.001, comparison between each group and the control group. eNOS, endothelial nitric oxide synthase. CD31, Platelet endothelial cell adhesion molecule-1 (PECAM-1).

and LVW/TL used to evaluate cardiac hypertrophy increased in the apatinib group and the HSD group ($p < 0.001$), and the increase was most significant in the HSD + apatinib group ($p < 0.001$). After treatment with Y27632, LVPW, LVIDd, LVIDs, HW, LVM and the ratios of HW/TL and LVW/TL decreased ($p < 0.001$), and EF and FS increased ($p < 0.001$) compared with those in the apatinib and HSD groups, which suggests that Y27632 can improve left ventricular dysfunction induced by apatinib and HSD (**Figure 7**).

Y27632 Improves Cardiac Fibrosis Caused by Apatinib in Mouse Models of Gastric Cancer

HE staining showed that after treatment with HSD and apatinib, the myocardial fibers were disordered, and the gap increased ($p < 0.001$). These changes were most significant in the HSD + apatinib group compared with the HSD and apatinib groups ($p < 0.001$). After treatment with Y27632, the myocardial fibers were orderly, and the gap size was similar to those of the NR group and control group (**Figure 8A**).

Masson staining showed that the degree of cardiac tissue blue staining increased most significantly in the HSD + apatinib group compared with the HSD and apatinib treatment groups ($p < 0.001$), while the area of blue staining decreased in the apatinib + Y27632 and HSD + Y27632 groups, suggesting that cardiac fibrosis induced by apatinib and HSD was decreased ($p < 0.001$) (**Figures 8B,D**).

Positive signals for α-SMA in the immunohistochemical assay and the relative mRNA content of COLI and COLIII increased significantly after HSD and apatinib treatment, and these effects were enhanced in the HSD + apatinib group ($p < 0.001$). After treatment with Y27632, the positive signal was lower,

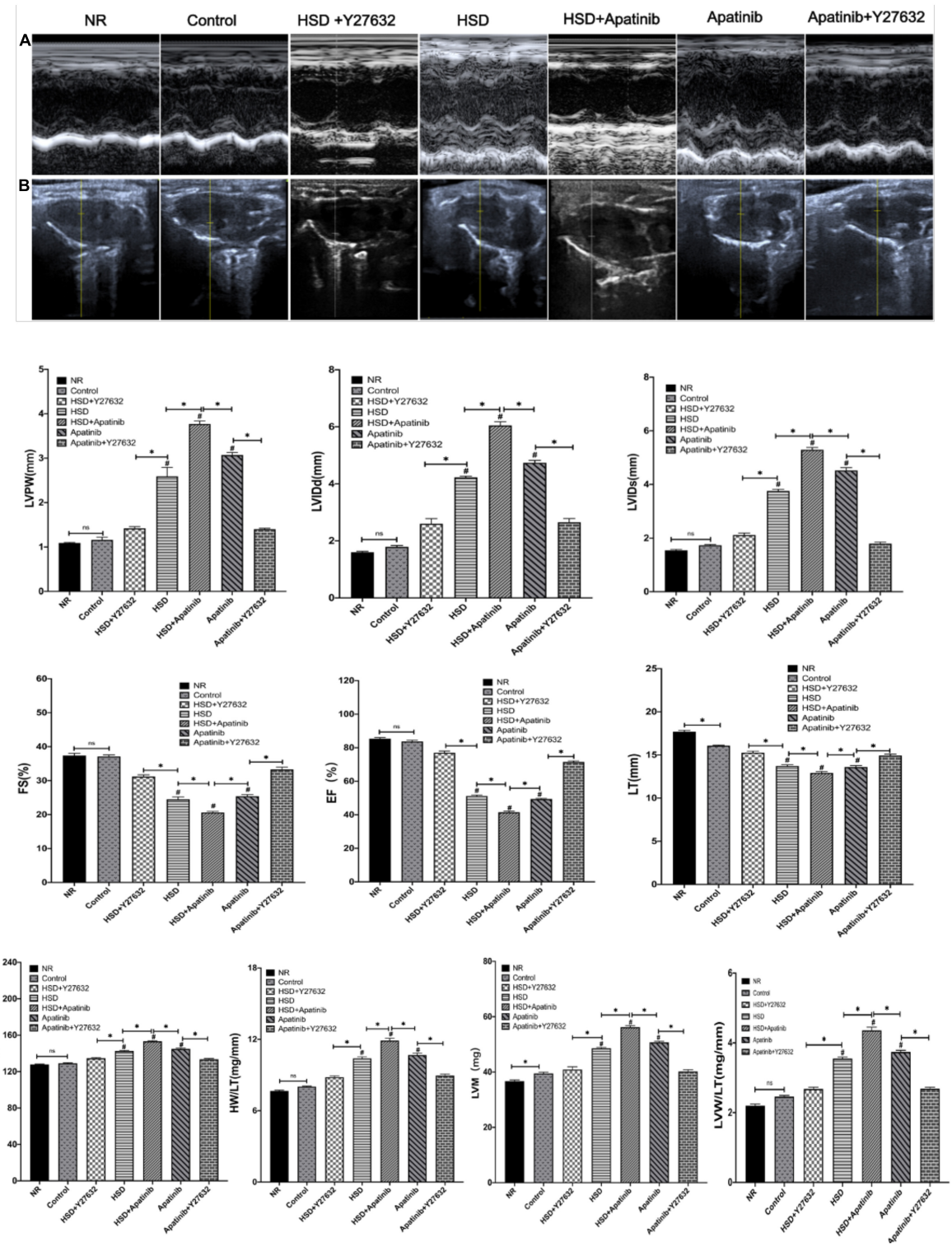

FIGURE 7 | The effects of apatinib and Y27632 on left ventricular function *in vivo*. **(A)** M-mode echocardiography of the left ventricular chamber, including the FS (%), EF (%), IVPW, LVIDd (mm) and LVIDs (mm). **(B)** Long axis section of the parasternal left ventricle. In the HSD, HSD + apatinib and apatinib groups, LVPW, LVIDd, LVIDs, HW (mg), and LVM (mg) increased, while after Y27632 treatment, these indicators were decreased. The FS and EF have the opposite trends. Then, the ratios of HW/TL and LVM/TL were calculated to evaluate cardiac hypertrophy, which increased in the HSD, HSD + apatinib and apatinib groups, and after Y27632 treatment, the ratios were decreased (n = 6 in each group). Data are representative of three independent measurements. Mean ± SEM. $^{*}P$ less than 0.001. $^{\#}P$ less than 0.001, comparison between each group and the control group. LVPW, left ventricular posterior wall thickness; LVIDd, left ventricular internal dimension-diastole; LVIDs, left ventricular internal dimension-systole; FS, fraction shortening; EF, ejection fraction; HW, heart weight; LVM, left ventricular mass; TL, tibial length.

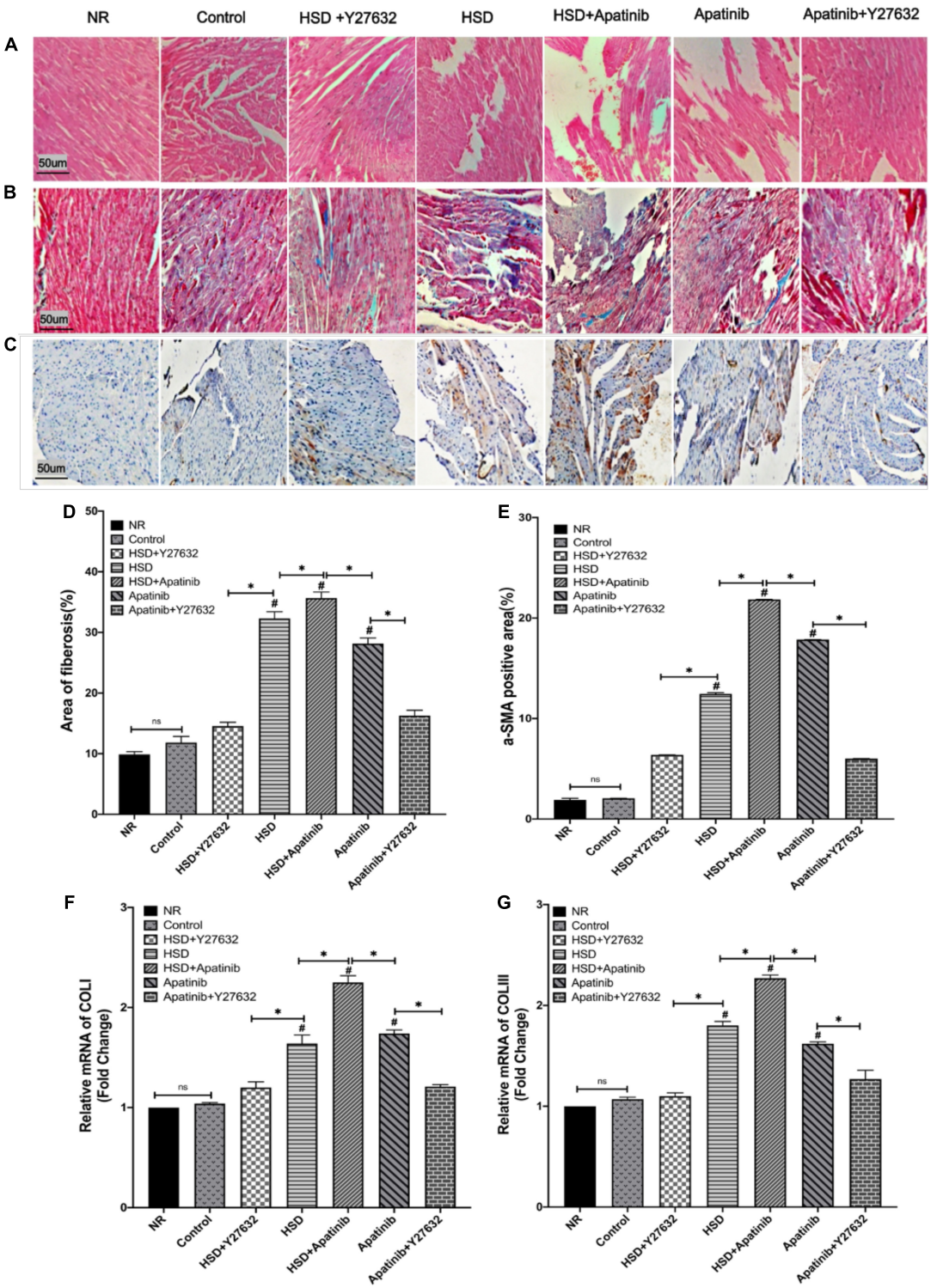

FIGURE 8 | Histological sections of myocardial fibrosis *in vivo*. **(A)** Sections stained with H&E. **(B)** Sections stained with Masson staining. **(C)** α-SMA immunohistochemical staining. **(D)** The area of Masson staining for fibrosis (%) was increased in the HSD, HSD + apatinib and apatinib groups. After Y27632 treatment, the area of fibrosis was decreased. **(E)** Positive signal areas of α-SMA (%) have the same result as Masson staining. **(F,G)** Fibrosis indicators, including COLI and COLIII, were detected at the mRNA level, which was consistent with the Masson staining and α-SMA immunohistochemical staining (n = 6 in each group). Data are representative of three independent measurements. Mean ± SEM. $^{*}P$ less than 0.001. $^{\#}P$ less than 0.001, comparison between each group and the control group. COLI, collagen I; COLIII, collagen III; α-SMA, alpha-smooth muscle actin.

and the mRNA level decreased in the apatinib + Y27632 and HSD + Y27632 groups, indicating that fibrosis induced by apatinib and HSD was relieved (**Figures 8C,E–G**).

DISCUSSION

Hypertension is one of the major adverse reactions in the treatment of gastric cancer with apatinib, which limits its application and may affect patient prognosis. A previous study showed that grade ≥3 treatment-related adverse events (TRAEs) were reported in 147 (77.4%) of 190 patients treated with apatinib, with the most common being hypertension (34.2%) (30). However, the pathogenesis of this type of hypertension is different from that of essential hypertension; the specific mechanism is not clear at present, and there is no specific drug for prevention and treatment. Therefore, we conducted *in vivo* experiments to explore the mechanism by which apatinib causes hypertension.

Previous studies have explored apatinib doses of 30–150 mg/kg per day in mice (31–35) and Y27632 doses of 10–30 mg/kg (36–38). Therefore, we used apatinib 50 mg/kg per day to explore the effects of apatinib on BP over a 28-day period and Y27632 10 mg/kg per day to observe the effect on apatinib-induced hypertension. These drug doses are much closer to those used in clinical settings.

In our *in vivo* experiments, we first confirmed that tumor volume was reduced after apatinib treatment, confirming previous studies of the obvious therapeutic effect of apatinib on gastric cancer in mice (39–42). Another problem with ROCK inhibitors is their impact on cancer therapy. ROCK blockade promotes cancer cell phagocytosis and induces antitumor immunity by enhancing T-cell priming *via* dendritic cells, thereby suppressing tumor growth in syngeneic tumor models (43). The ROCK pathway inhibitors include Y27632, fasudil (44), GSK429286 (45) and RKI1447 (46).

One of the most widely investigated ROCK inhibitors is Y-27632 (47), which is a selective small inhibitor of both ROCK1 and ROCK2 that suppresses the invasiveness and metastasis of rat and human hepatoma cells, bladder cancer cells, colorectal cancer cells, lung cancer cells, and cancer cell types (48–53). Accumulating evidence strongly suggests that ROCK is dominantly involved in the regulation of vascular contraction, and Y27632 most likely targets ROCK and normalizes contractile dysfunction (54). Therefore, Y27632 may counteract the BP-increasing effects of VEGF antagonists and promote their antineoplastic effects (3). We confirmed that the tumor volume decreased after Y27632 intervention, which means that Y27632 does not affect the antitumor efficacy of apatinib in the treatment of gastric cancer and is the best choice for the treatment of TKI-induced hypertension.

Increased ARHGEF11 expression in the Dahl salt-sensitive (SS) rats leads to actin cytoskeleton-mediated changes in cell morphology and cell function that promote hypertension and a decline in kidney function (15). As ARHGEF11 is known to act through RhoA, it was expected to be involved in the canonical (RhoA-ROCK) pathway (15). In Dahl SS rats, salt influences blood pressure, proteinuria, and renal hemodynamics through the Rho-ROCK pathway (55, 56). Previous studies have shown that TKI-induced hypertension and salt-sensitive hypertension have similar effects under high salt levels (14, 17, 57). On the other hand, Lankhorst et al. (58) demonstrated that sunitinib-induced hypertension has a similar mechanism to salt-sensitive hypertension and could be aggravated by a high salt intake. Therefore, we hypothesized that the RhoA/ROCK pathway may be involved in the occurrence of hypertension induced by TKIs and that ROCK pathway inhibitors may have a therapeutic effect on this hypertension. Therefore, mice fed an HSD were included in this experiment to explore the activation of the RhoA/ROCK signaling pathway by apatinib. The HSD activated the RhoA/ROCK signaling pathway, leading to increased BP. Moreover, the increase in BP was significantly greater in mice fed the HSD and treated with apatinib, confirming our hypothesis that apatinib can also affect the elevation of BP by activating the RhoA/ROCK signaling pathway. Additionally, after Y27632 was administered, BP decreased in the HSD + apatinib group, which means that the ROCK inhibitor Y27632 may be a potential therapeutic target for salt sensitivity-induced hypertension. Finally, there was no significant difference in heart rate between the groups ($p > 0.05$) (**Figure 2**), indicating that apatinib and the HSD had no significant effect on heart rate in mice with gastric cancer.

ROCK, a serine/threonine kinase and an important downstream effector of the small G protein RhoA, is an important factor in the development of smooth muscle tone (59). We previously demonstrated that apatinib increases BP in a rat model by activating the RhoA/ROCK signaling pathway and that this increase is reversed by Y27632 (3). On this basis, we carried out experiment to further simulate the real tumor environment. MKN-45 cells were used to build a tumor model. The tumor activated the RhoA/ROCK signaling pathway but not downstream phosphorylation, ET or the NO system. Only apatinib treatment caused further changes in the signaling pathway. This means that apatinib enhances the activation of the RhoA/ROCK signaling pathway in the tumor environment, causing hypertension. Chemokines and cytokines regulate the activation of RhoA to initiate the cytoskeletal changes required for the physical movement of the cells into the tumor stroma (60, 61). ROCK activity increases due to extracellular matrix rigidity in the tumor microenvironment and promotes a malignant phenotype *via* actomyosin contractility. The mechanical rigidity of the tumor microenvironment may drive ROCK signaling through distinct pathways to enhance the invasive migration required for cancer progression and metastasis (62). In this experiment, the tumor environment only caused increases in RhoA, ROCK1 and ROCK2, and other components of this signaling pathway were not elevated. However, after apatinib and HSD intervention, there was a superimposed elevation of these proteins that caused changes in other factors of the signaling pathway. The inactive form of RhoA (RhoA.GDP) is present in the cytosol bound to a guanine dissociation inhibitor (GDI). The activation of RhoA,

such as *via* apatinib induction, is mediated by various Rho-specific guanine nucleotide exchange factors (RhoGEFs), which in turn promote the exchange of GDP for GTP. Upon GTP binding and activation, RhoA interacts with and stimulates the activity of downstream effectors, including ROCK. Active RhoA (GTP-bound) is in turn inactivated by GTPase-activating proteins (GAPs), which hydrolyze GTP to GDP (63). Y27632 is a structural analog of ATP, which has been shown to have potent inhibitory activity against both ROCK1 and ROCK2 (64). In this experiment, we further found that Y27632 inhibited ROCK and RhoA activity caused by activation of the RhoA/ROCK pathway induced by apatinib and HSD, which may be a potential therapeutic target for the treatment of hypertension caused by apatinib and HSD.

The results of qRT–PCR and western blot analyses revealed that apatinib treatment mediated the significant upregulation of RhoA, ROCK1 and ROCK2 expression. Activation of the RhoA/ROCK pathway maintains the level of MLC20 phosphorylation, an essential step in smooth muscle contraction, *via* the inhibition of MLC phosphatase activity by phosphorylation of the MLC phosphatase target subunit (MYPT-1) (59). Activating ROCK2 inhibits MLCP activity in a dose-dependent manner, thereby increasing p-MLC and increasing contraction (65). Therefore, we verified the enhancement of ROCK activity by apatinib and HSD in **mouse models of gastric cancer** by measuring p-MYPT-1 and p-MLC protein expression. Our results also revealed that apatinib treatment caused significant upregulation of p-MLC and p-MYPT-1 in mouse models of gastric cancer. These changes can lead to vascular contraction and were reversed by concomitant treatment with the non-specific ROCK inhibitor Y27632, confirming our hypothesis.

Previous studies have demonstrated that increased ET-1 secretion and disordered NOS are the key mechanisms through which TKIs induce hypertension (66, 67). *In vivo* and *in vitro* studies have shown that vatalanib, a VEGFi, decreases the activation of eNOS and NO in mice, resulting in endothelial dysfunction and vascular hypercontractility (68, 69). In the present study, apatinib treatment increased ET-1 and iNOS and decreased eNOS in the aorta. Furthermore, Y27632 significantly downregulated ET-1 and iNOS and increased eNOS expression in the aorta, indicating that the RhoA/ROCK signaling pathway could be involved in the regulation of the ET system and NOS.

The CD31 antigen, which is expressed on all continuous endothelia, is a single-chain type-1 transmembrane protein belonging to the immunoglobulin superfamily. The constitutive expression of the CD31 antigen on endothelial cells defines the morphology of these cells (70). eNOS uncoupling and the resulting oxidative stress are major drivers of endothelial dysfunction and atherogenesis (71, 72). During endothelial injury, however, a reduction in NO release may lead to vasoconstriction and arterial obliteration (70, 73). Therefore, the expression of the endothelial markers CD31 and NOS can be used to evaluate endothelial integrity (74). The integrity of the small vascular endothelium was disrupted, which may promote fibroblast activation, leading to progressive vascular fibrosis.

In the superior mesenteric artery, the effect of apatinib on the endothelial integrity of small vessels was observed by immunohistochemistry. Apatinib and the HSD impaired the endothelial integrity of small vessels. In addition, histological methods and qRT–PCR showed that apatinib and HSD resulted in vascular lumen narrowing and increased COLI and COLIII expression, indicating vascular hypertrophy and increased vascular stiffness. Therefore, apatinib and HSD promoted aortic remodeling. Y27632 can improve the integrity of the small vessel endothelium and the fibrosis of aortic vessels.

Increased hemodynamic stress imposed by hypertension on the LV leads to major alterations in the myocardium, including hypertrophy and structural remodeling (75, 76). The RhoA/ROCK pathway is associated with myocardial hypertrophy (77). In our study, echocardiography revealed that apatinib induced hypertension through the RhoA/ROCK pathway and eventually led to left ventricular hypertrophy, consistent with previous studies. Myocardial fibrosis is a common pathophysiological manifestation of hypertensive heart diseases (78). Masson staining and immunohistochemistry analysis of α-SMA were performed to detect myocardial fibrosis. In addition to α-SMA, we also examined the expression of COLI and COLIII in myocardial tissues by qRT–PCR. Furthermore, left ventricular function was measured by echocardiography. The results suggested that apatinib and the HSD led to myocardial fibrosis and left ventricular hypertrophy after the increase in BP. ROCK inhibitors may improve ventricular hypertrophy and heart function in hypertensive rats (79, 80). In this study, we found that Y27632 reversed myocardial fibrosis and left ventricular hypertrophy induced by apatinib and the HSD.

In conclusion, our experiment found that both apatinib and an HSD not only elevated blood pressure in a gastric cancer mouse model through the RhoA/ROCK signaling pathway but also promoted vascular remodeling and left ventricular hypertrophy. Y27632, which improved aortic remodeling, fibrosis, endothelial dysfunction, and superior mesenteric artery endothelial injury, as well as left ventricular dysfunction and cardiac fibrosis in mice, is a potential drug for the treatment of hypertension caused by apatinib and an HSD.

Of course, there are limitations in the study. First, a comparison of ROCK inhibitors is lacking. Fasudil, the best known ROCK inhibitor, is clinically relevant and clinically approved in Japan and China. In the current experiment, fasudil was not used in a comparative mode to Y-27632, which weakens our findings. Additionally, as ARHGEF11 is known to act through RhoA, it was expected that it is involved in the RhoA/ROCK pathway, and ARHGEF11 gene knockout may have attenuated activation of the ROCK pathway. Therefore, ARHGEF11 gene knockout mouse models should be established to further elucidate the mechanism of the RhoA/ROCK signaling pathway. Moreover, *in vitro* experiments were not performed, and future studies applying ARHGEF11 gene knockout technology will provide a more detailed view of the molecular changes, combined with cellular level changes, in hypertension induced by activating the RhoA/ROCK signaling pathway.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics Committee Board of Lanzhou University Second Hospital (license number: D2019-101).

AUTHOR CONTRIBUTIONS

WW performed the experiments and wrote the initial draft of the manuscript. JY, QW, CL, and WW designed the study. HZ, CZ, and XF analyzed the data. All authors have read and approved the final manuscript.

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REVIEW
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Bidirectional Relationship Between Cancer and Heart Failure: Insights on Circulating Biomarkers

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Cancer and heart failure are the two leading causes of death in developed countries. These two apparently distinct clinical entities share similar risk factors, symptoms, and pathophysiological mechanisms (inflammation, metabolic disturbances, neuro-hormonal and immune system activation, and endothelial dysfunction). Beyond the well-known cardiotoxic effects of oncological therapies, cancer and heart failure are thought to be tied by a bidirectional relationship, where one disease favors the other and vice versa. In this context, biomarkers represent a simple, reproducible, sensitive and cost-effective method to explore such relationship. In this review, we recapitulate the evidence on cardiovascular and oncological biomarkers in the field of cardioncology, focusing on their role in treatment-naïve cancer patients. Cardioncological biomarkers are useful tools in risk stratification, early detection of cardiotoxicity, follow-up, and prognostic assessment. Intriguingly, these biomarkers might contribute to better understand the common pathophysiology of cancer and heart failure, thus allowing the implementation of preventive and treatment strategies in cardioncological patients

Keywords: cancer, inflammation, cardiovascular disease, cardio-oncology, circulating biomarkers, neuro-hormonal activation

INTRODUCTION

Cardiovascular disease, namely heart failure (HF), is the leading cause of death in industrialized countries, and its incidence is increasing. Recently, several studies have drawn the scientific community attention to the overlap between these two diseases, which were previously considered as distinct from each other (1). In particular, the bidirectional relationship between cancer and HF has been highlighted, demonstrating the presence of common risk factors (such as aging, male sex, obesity, diabetes mellitus, sedentariness and smoking) as well as shared etiopathogenetic pathways in both diseases (2). Oxidative stress and inflammation have been implicated in the pathogenesis of cancer and cardiovascular diseases, promoting the tumor microenvironment and cancer invasiveness on the one hand, and inducing endothelial dysfunction, fibrotic processes, and the formation of atherosclerotic plaques on the other (3). The renin-angiotensin-aldosterone system (RAAS) has also been shown to play a role in certain steps of cancer development, along with typical biomarkers of cardiac stress (3).

In contrast, some preclinical studies have suggested a possible role for some oncometabolites (e.g., D-2-hydroxyglutarate) in promoting HF (4). As a result of this evidence, in recent years there has been a growing interest in the new field of cardioncology, aimed at understanding and analyzing the precise biological mechanisms underlying the overlap between these two diseases.

In this narrative review, we provide an overview of the main biomarkers of cardiovascular disease and cancer, their pathophysiological role and their overlap in the two diseases (**Figure 1**). The proper use of these biomarkers for diagnostic and therapeutic purposes is still unclear, and future studies will be needed to eventually introduce them into clinical practice.

THE ROLE OF CARDIOVASCULAR BIOMARKERS IN CANCER

Cardiac biomarkers have been investigated as inexpensive and easily accessible tools for risk assessment, prediction of response to treatment, early diagnosis of cardiotoxicity, as well as monitoring disease progression and evaluating the prognosis of cancer-related cardiac involvement (5). However, while there is extensive literature on the role of cardiac biomarkers for the early detection of cardiotoxicity from cancer therapies (6, 7), much less is known about cardiac biomarkers role before the start of chemotherapy (8). One of the best settings to explore the bidirectional relationship between cancer and HF is, indeed, the one considering treatment-naïve cancer patients (8) [**Table 1**, (9–14)]. In such case, an elevation of cardiovascular biomarkers may result from cardiovascular comorbidities, systemic perturbances (e.g., inflammation, oxidative stress, sepsis) or, more intriguingly, as a direct effect of cancer itself.

Cardiac Troponins

Cardiac troponin T and I (cTnT and cTnI, respectively) are cardiac regulatory proteins that control the calcium-mediated interaction between actin and myosin in cardiomyocytes (15). The troponin complex consists of three subunits: TnT, which binds to tropomyosin and facilitates contraction; TnI, which binds to actin and inhibits actin-myosin interactions; and troponin C, which binds to calcium ions (16). The majority of cTns is bound to myofilaments, and the remainder is free in the cytosol, which accounts for 3%−8% of the total amount (17). After disruption of the sarcolemmal membrane of the cardiomyocyte, troponin from the cytoplasmic pool is initially released, followed by a more protracted release from myofibril-bound cytosolic complexes (18, 19). However, an increase in serum troponin levels may rely not only on direct myocardial damage, but also demand ischemia, myocardial ischemia, myocardial strain due to volume and pressure overload, and chronic kidney disease (CKD) (16). Methods for determining cTnT and cTnI, first developed in the late 90s, have been continuously improved, increasing analytical sensitivity and specificity (20). Nowadays, highly sensitive (hs) immunoassays are available to determine hs-cTnT and hs-cTnI concentrations; they allow detecting very low but diagnostically significant concentrations of cardiac troponins in blood serum (17).

Cardiac troponins are the gold standard biomarkers for the detection of myocardial injury, namely in the setting of acute coronary syndromes (ACS) (21). In peripheral blood, troponins begin to rise within 3–4 h after the onset of myocardial injury and remains increased for 10–14 days (22). However, increased levels may be found in several different conditions, both physiological (e.g., physical exertion or psycho-emotional stress) and pathological, including chronic HF, diabetes, arterial hypertension, inflammatory heart disease, pulmonary embolism, chronic renal failure and sepsis (20, 21, 23).

Cardiac troponins are also the most widely used biomarkers to detect cardiotoxicity in cardioncology (24). With the advent of hs assays, it is possible to detect early subclinical cardiomyocyte damage and help provide treatments to prevent cardiotoxicity prior to the development of irreversible left ventricular (LV) dysfunction (24). In addition to this, cardiac troponins have been increasingly studied in cancer patients before receiving oncological treatment: cTnI was found to be significantly higher in 25 anthracycline-naïve cancer patients (36.5 pg/ml; 95% confidence interval [CI], 25.1–47.9 pg/ml), as compared to 60 healthy controls ($p < 0.01$) (25), and in 25 patients with ovarian cancer prior to treatment, as compared to women with endometriosis or benign ovary masses (26). A study on 452 treatment-naïve women with breast cancer explored the prognostic value of troponins I, T, and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) to predict baseline susceptibility to trastuzumab-related cardiac dysfunction (11). Elevated baseline troponin I (>40 ng/L) and T (>14 ng/L), occurring in 56 of 412 (13.6%) and 101 of 407 (24.8%) patients, respectively, were associated with an increased significant LV ejection fraction (EF) drop risk (hazard ratio [HR] 4.52, $p < 0.001$ and HR 3.57, $p < 0.001$, respectively). A similar conclusion for NT-proBNP could not be drawn because of the lack of a well-established elevation threshold; however, higher increases from baseline were seen in patients with cardiotoxicity compared with patients without (11).

The prognostic role of hs-TnT before immune checkpoint inhibitors (ICIs) treatment start has been investigated for the first time in 30 patients with different lung cancer types (12). The

Abbreviations: AML, acute myeloid leukemia; ANP, atrial natriuretic peptide; BNP, brain-type natriuretic peptide; CA125, carbohydrate antigen 125; CAD, coronary artery disease; CANTOS, canakinumab anti-inflammatory thrombosis Outcome Study; CEA, carcinoembryonic antigen; CHIP, clonal hematopoiesis of undetermined potential; CI, confidence interval; CKD, chronic kidney disease; CRC, colorectal cancer; CRD, carbohydrate recognition domain; CRP, C-reactive protein; cTnI, cardiac troponin I; cTnT, cardiac troponin T; CXCL, C-X-C motif chemokine ligand; ECM, extracellular matrix; EF, ejection fraction; ESMO, european society for medical oncology; Gal-3, galectin-3; GDF-15, growth differentiation factor-15; HF, heart failure; HR, hazard ratio; hs, highly sensitive; ICI, immune checkpoint inhibitor; IL, interleukin; LV, left ventricular; MACE, major adverse cardiovascular events; MI, myocardial infarction; MIC-1, macrophage inhibitory cytokine-1; MPO, myeloperoxidase; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-atrial natriuretic peptide; NO, nitric oxide; NPs, natriuretic peptides; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PlGF, placental growth factor; RAAS, renin-angiotensin-aldosterone system; RR, relative risk; sFlt-1, soluble fms-like tyrosine kinase-1; ST2, suppression of tumorigenicity 2; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

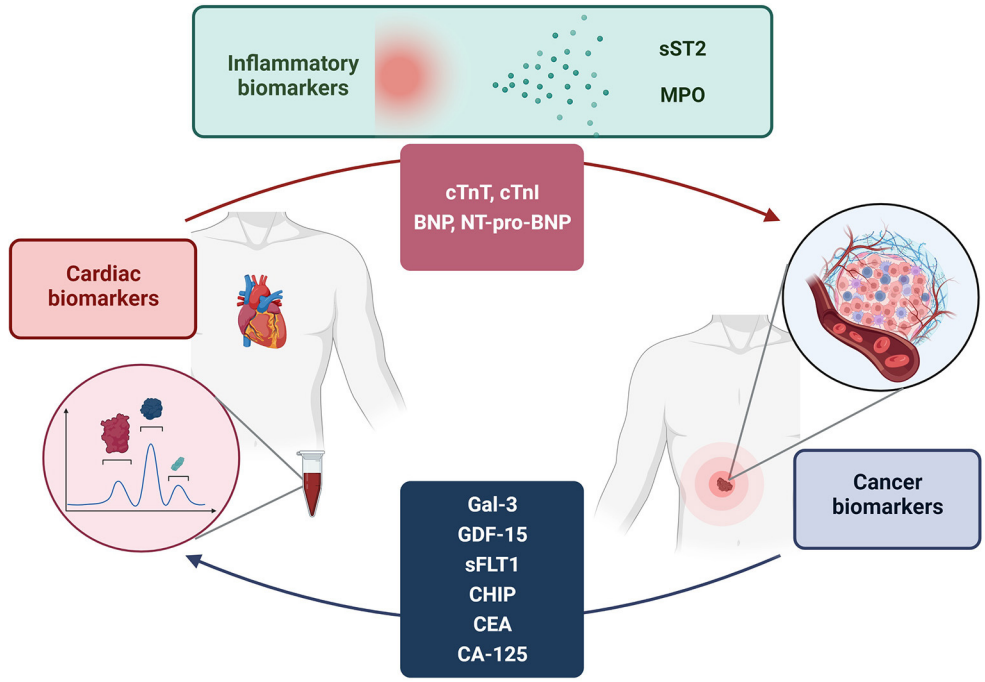

FIGURE 1 | Bi-directional relationship between cancer and cardiovascular disease. Analysis of the elevation of disease-specific biomarkers in both conditions suggests a possible influence of cancer on the pathogenesis of cardiovascular disease and vice versa. The elevation of biomarkers is also justified by several pathogenetic pathways shared by both diseases.

TABLE 1 | Prognostic value of cardiovascular biomarkers in treatment-naïve cancer patients.

Biomarker	References	Cut-off/ range of value found	Cancer type	Association with cancer outcome
cTnT	Pavo et al. (9)	≥0,005 ng/mL	Several types	Increased mortality risk (HR 1.21, $p < 0.001$)
cTnT	Kitayama et al. (10)	Not defined	Breast	No predictive value of cardiotoxicity
cTnT	Zardavas et al. (11)	>14 ng/L	Breast	Increased cardiotoxicity risk (HR 3.57, $p < 0.001$)
cTnI	Zardavas et al. (11)	>40 ng/L	Breast	Increased cardiotoxicity risk (HR 4.52, $p < 0.001$)
cTnT	Petricciuolo et al. (12)	≥14 ng/L	Lung	TnT predicted CV death, stroke or TIA, pulmonary embolism and new-onset HF
cTnT	Rini et al. (13)	Not defined	Advanced renal cell carcinoma	Increased risk of MACE (RR 3.31)
NT-proBNP	Pavo et al. (9)	≥125 pg/mL	Several types	Increased mortality risk (HR 1.54, $p < 0.001$)
NT-proBNP	Rini et al. (13)	Not defined	Advanced renal cell carcinoma	No increased risk of MACE
BNP	Rini et al. (13)	Not defined	Advanced renal cell carcinoma	No increased risk of MACE
Neprilysin	Pavo et al. (14)	Median values 276 pg/ml	Several types	Lack of association with mortality but for myelodysplastic disease (HR 1.27, $p = 0.044$)

primary endpoints considered in the study were cardiovascular death, stroke or transient ischemic attack, pulmonary embolism and new-onset HF, while the secondary endpoint was progression of cardiac involvement. After 3 months of follow-up from the ICIs treatment start, 14 ng/L proved to be the best cutoff for both primary (100% sensitivity, 73% specificity) and secondary endpoints (sensitivity 75%, specificity 77%). The primary endpoint occurred only in patients with hs-TnT ≥14 ng/L at baseline (12). Data from the phase III JAVELIN Renal 101 trial have also recently shown that patients with advanced renal cell carcinoma and higher baseline TnT are at increased risk of developing major adverse cardiovascular events (MACE) after receiving a combined treatment with ICIs and vascular endothelial growth factor receptor (VEGFR) inhibitors (relative risk [RR] 3.31; 95% CI, 1.19–9.22) (13). Other cardiac biomarkers (TnI, natriuretic peptides [NPs], and creatine kinase MB) measured at baseline were not significantly predictive for MACE (13).

Natriuretic Peptides

NPs are a family of structurally related peptide hormones mainly produced by cardiovascular, brain and renal tissues (27, 28). Atrial natriuretic peptide (ANP) is a 28-amino acid peptide that is synthesized, stored, and released by atrial myocytes mainly in response to cardiomyocytes mechanical stretch due to volume overload (29). Other factors leading to ANP secretion include exercise, hypoxia and cold, as well as angiotensin, endothelin, vasopressin, catecholamine and glucocorticoid stimulation (30). Brain-type natriuretic peptide (BNP) is a 32-amino acid peptide that is largely synthesized by the ventricles and the brain, where it was first identified (31). BNP is first synthesized as pre-pro-BNP, which is then cleaved to pro-BNP, which proteolysis by furin (or corin) results in the active BNP and the inactive NT-pro-BNP (76 amino acids) (32). Various causes induce BNP synthesis in cardiomyocytes, such as tissue hypoxia, transmural pressure or volume overload, and pro-inflammatory cell factors (e.g., interleukin-1β [IL-1β], interleukin-6 [IL-6] and tumor necrosis factor-α [TNF-α] (30, 33).

NPs mediate a wide range of physiologic effects achieved by interaction with specific guanylyl cyclase receptors (30), including direct vasodilation of veins and arteries, respectively lowering central venous pressure (i.e., preload) and systemic vascular resistance and arterial pressure (i.e., afterload) (34). In the kidney, NPs induce natriuresis and diuresis, suppress renin secretion and aldosterone synthesis, and increase glomerular filtration rate by vasodilating afferent arterioles (35). In addition, NPs provide antiproliferative, antihypertrophic, and antifibrotic effects, thus hindering adverse cardiac remodeling (36, 37).

Both BNP and NT-proBNP are useful biomarkers routinely used in the diagnosis, risk stratification, therapy management and prognosis assessment in patients with acute or chronic HF (38). Increased levels of NPs are also found in the setting of pulmonary diseases, cardiac inflammatory or infectious diseases, endocrine disorders and high output status, such as sepsis, kidney failure, liver cirrhosis, and intracranial pathologies (39).

NPs have been reported to be markedly and constantly increased in the cancer population. NPs typically increase after treatment with various oncological treatments, namely anthracyclines (40). However, NPs elevation may also depend on causes other than cancer therapy, such as release from cancer cells themselves (41), volume overload (42) or cancer-related systemic inflammation (43). Burjonroppa et al. first demonstrated that there is lack of association between markedly elevated BNP levels (>1,000 pg/mL) and clinical evidence of volume overload or LV dysfunction in cancer patients with multiple comorbidities (44). On the contrary, Popat et al. found that very high NT-proBNP (>3,000 pg/mL) in cancer patients is usually encountered in the context of fluid overload and most often in hematologic malignancies (42). In a study by Sachiko et al., both plasma BNP and serum C-reactive protein (CRP) levels were significantly higher in cancer patients before treatment than non-cancer patients (43). There was also a significant positive correlation between plasma BNP and serum CRP levels in cancer patients ($R = 0.360$, $p < 0.01$) but not in those without. In cancer patients, CRP correlated with BNP independent of the age, creatinine level, hypertension, and body mass index (43).

In a prospective cohort study, Pavo et al. enrolled 555 patients with a primary diagnosis of cancer and no prior oncological therapies. NT-proBNP, mid-regional pro-atrial natriuretic peptide (MR-proANP), mid-regional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1, copeptin, hsTnT, proinflammatory markers IL-6 and CRP, cytokines serum amyloid A, haptoglobin and fibronectin have been measured (9). All cardiovascular hormones and hsTnT levels rose with tumor stage progression. All markers were significant predictors of mortality with HRs of 1.54 (95% CI; 1.24–1.90, $p < 0.001$) for NT-proBNP, and 1.21 (95% CI; 1.13–1.32, $p < 0.001$) for hsTnT, independent of age, gender, tumor entity and stage, and presence of cardiac comorbidities. NT-proBNP, MR-proANP, MR-proADM and hsTnT displayed a significant correlation with the inflammatory markers IL-6 and CRP. This study showed for the first time that cardiovascular hormones are related to cancer disease progression and severity, suggesting the presence of subclinical functional and morphological myocardial damage independent of cancer treatment (9). Thereafter, the authors tried to assess the prognostic role of neprilysin, an enzyme degrading NPs, in the same population. Although neprilysin seems to be involved in tumor biology as well as in cardiovascular diseases, no association was observed between neprilysin levels and overall survival ($p = 0.887$) except for myelodysplastic malignancies (HR 1.27; 95% CI, 1.01–1.61; $p = 0.044$) (14).

To summarize, cardiovascular biomarkers (cardiac troponins and NPs) are found to be remarkably increased in the cancer population. This may depend on either a direct or indirect cancer-related systemic perturbance, which is also fostered by aging-linked phenomena of inflammation and oxidative stress. Further studies are therefore needed to define the precise role of these biomarkers in clinical practice in terms of risk prediction, screening, and therapeutic monitoring of both HF and cancer.

THE ROLE OF ONCOLOGICAL BIOMARKERS IN CARDIOVASCULAR DISEASE

Cancer is a complex disease marked by the uncontrollable proliferation of genetically abnormal cells, and it has long been the main cause of mortality in several countries (45). Early diagnosis, achieved through the use of specific biomarkers, is critical in terms of successful and timely therapy and patient survival. According to the National Institutes of Health, a biomarker is a feature that is objectively tested and assessed as a sign of normal biologic processes, pathogenic processes, or pharmaceutical reactions to a therapeutic intervention (46).

A reliable and appropriate biomarker must have several characteristics: it must be diagnostic while also allowing for early diagnosis, provide prognostic information, and have predictive potential to confirm therapy effectiveness (47). Enzymes, metabolites, DNA and RNA, as well as surface receptors, are all examples of cancer biomarkers (48). Several cancer biomarkers, such as the prostate-specific antigen and

carcinoembryonic antigen (CEA), are now widely used in clinical practice and are universally regarded as useful diagnostic and prognostic tools (45). In recent years, studies investigating novel biomarkers in cancer have focused on the junction of malignancy and cardiovascular disease at several levels (49) [**Table 2**, (6, 51–77)]; these findings could be linked to the already well-known cardiotoxicity of neoplastic agents and radiation therapy, as well as to the multiple common biological mechanisms in cancer and cardiovascular disease development (6, 77). Furthermore, multiple studies have found that patients with both cardiovascular disease and cancer had a greater mortality rate than patients with either condition alone, underscoring the importance of treating both diseases jointly (78). It is therefore essential to identify and study biomarkers shared by both diseases, which could be useful tools in diagnostic and prognostic terms, allowing to understand the complicated dialogue between these two conditions.

CA125 and CEA

Serum carbohydrate antigen 125 (CA125), a high-molecular-weight membrane glycoprotein, is a peptide repeat epitope of the mucin MUC16. It has a C-terminal portion with a short cytoplasmic tail and a transmembrane domain, as well as an extracellular N-terminal region with numerous partially conserved tandem repeats (79). CA125 is the most well-studied serologic tumor marker used to assess the clinical status of ovarian cancer patients and for the differential diagnosis of pelvic masses (80, 81).

CA125 antigen is not exclusively expressed on ovarian-cancer tumor cells. Acute leukemia, non-lymphoma Hodgkin's, melanoma, breast and lung cancers, and gastrointestinal carcinoma have all been linked to increased serum CA125 levels (82). Furthermore, CA125 expression has also been found under physiological conditions in tissues of mesothelial origin, such as the pleura, pericardium, and peritoneum (83). Moreover, in subsequent research, it has also been detected in the kidney, gallbladder, pancreas, lung, stomach, and colon, implying that CA125 lacks organ specificity due to its extensive diffusion across the body tissues (84, 85).

The clinical usefulness of the CA125 assay has been investigated in preclinical, translational, and clinical research since the 1980s (86–88). Firstly, in a cohort of 101 patients with ovarian cancer, Bast et al. found a positive CA125 antigen in 82% of patients, using a cutoff of 30–35 U/ml (89). Moreover, in subsequent research, the level of tumor biomarker elevation was found to be dependent on factors such as the type of tumor histology and the stage of the disease (84, 85).

In addition to its widespread usage as a predictive and diagnostic biomarker for ovarian cancer and other malignancies, CA125 has been shown to have a possible role in cardiovascular disease. Specifically, several clinical studies have found a correlation between elevation of tumor biomarker, pericardial effusion (73), and LV dysfunction (50).

In a cohort of patients with chronic HF, D'Aloia et al. found that elevated CA125 levels were associated to the severity of the New York Heart Association (NYHA) class. Furthermore, CA125 levels were also shown to be lower in patients with moderate to severe chronic HF who were getting aggressive pharmacological therapy, suggesting that the biomarker could be used to verify the treatment's therapeutic efficacy. Additionally, in short-term follow-up, CA125 has also been found to be a valuable prognostic factor (51).

The pathophysiological mechanism causing the simultaneous rise of CA125 and the development of cardiovascular disease is still unknown: according to some studies, an increase in the tumor marker is linked to the inflammatory process that underpins cardiovascular disease, which is exacerbated by a change in the hydrostatic balance, resulting in the development of HF (90).

Similar to CA125, CEA, a glycoprotein overexpressed on the cell surface in the majority of colorectal cancer [CRC] patients, has shown to be altered in cardiovascular disease as well. CEA is an oncofetal antigen that was first discovered as a CRC marker in 1965 (91). It is produced physiologically in the fetus but it is found only in small amounts in normal adult cells (92). CEA is one of the most commonly overexpressed cell surface molecules in CRC, and it is responsible for activating the cytokine cascade via direct interaction with monocytes, which is essential for cell adhesion and metastatic spread of intestinal cancer (93). However, CEA appears to be an insufficiently sensitive tool for primary CRC screening, especially in the early stages of the disease (sensitivity and specificity for Dukes' A and B 36 and 87%, respectively) (94).

Although most patients do not show elevated CEA at the time of diagnosis, CEA levels and disease stage have been found to correlate, making CEA a significant prognostic predictor independent of histologic grade and Dukes' stage (95).

An association between CEA and chronic inflammation, one of the key promoters of the development of various cardiovascular diseases including atherosclerosis, myocardial infarction, and HF (96, 97), has been proposed due to the strong correlation between CEA and elevated leukocyte counts (98).

In a study involving 2,079 patients from the BIOSTAT-CHF cohort, Shi et al. (55) explored the relationship between several tumor biomarkers (including CEA) and the outcome of HF. CEA levels increased in lockstep with NT-proBNP across a 21-month follow-up period, and were associated with all-cause death (HR 1.45, 95% CI, 1.30–1.61; $p = 0.0001$) and cardiovascular mortality (HR 1.18, 95% CI, 1.06–1.32; $p = 0.003$). Similarly, Bracun et al. (56) discovered a substantial connection between CEA levels and cardiovascular mortality over an 11.5-year follow-up period. CEA was also found to be associated with all-cause mortality and to be an independent predictor of cardiovascular events (56).

Galectin-3

Human galectin-3 (Gal-3) is a 35-kDa protein belonging to the galectins family of galactoside-binding proteins. Its structure is characterized by the presence of a unique N-terminal domain followed by the carbohydrate recognition domain (CRD) (99). The first 12 amino acids of the protein are essential for its secretion or translocation from the cytoplasm to nucleus, whereas the CRD is crucial for binding glycoconjugates containing N-acetyllactosamine (99). Thus, although located mainly in the cytoplasm, Gal-3 can be found both inside and

TABLE 2 | Elevated cancer biomarkers in cardiovascular disease.

Biomarker	References	Cut-off/ range of value found	Population characteristics	Association with CV disease
CA-125	Toshihiko et al. (100)	>35 U/mL	HF Pericardial, metastasis, renal failure, HypothyroidismRF, hypothyroidism	Pericardial effusion
CA-125	Nägele et al. (50)	>35 U/mL	Patient with HF admitted for HTX, patients after HTX	Association with NPs, severity of HF, response to medical therapy
CA-125	D'aloia et al. (51)	>35 U/mL 68 ± 83 U/ml	CHF	CHF severity and short-term prognosis
CA-125	Turk et al. (52)	>35 U/mL 100.0 ± 129.4 U/ml	CHF	Pleural effusion
CA-125	Faggiano et al. (53)	NYHA classes III (60 ± 22 UI/ml) and IV (192 ± 115 UI/ml)	CHF	Severity of HF, response to medical therapy
CA-125	Durak-Nalbantic et al. (54)	71.05 [30.70–141.47] U/ml	CHF	Pleural effusion, pericardial effusion, decompensated HF
CEA	Faggiano et al. (53)	>5 ng/ml	CHF	No association with HF
CEA	Shi et al. (55)	>5 ng/ml	Patients of the BIOSTAT-CHF cohort	Association with NPs, prediction of all-cause mortality
CEA	Bracun et al. (56)	>5 ng/ml	UAE >10 mg/L	CV morbidity, CV mortality and all-cause mortality
Gal-3	Motiwala et al. (57)	>20 ng/ml	HF	Incidence **o**f CV events
Gal-3	Meijers et al. (75)	>17.8 ng/mL	HF	Risk of rehospitalization at 30, 60, 90, 120 days
Gal-3	Xi Zhang et.al (58)	>384,7 ng/mL* >9,76 ng/mL	HF	Diagnosis of HF
Gal-3	Veli Polat et al. (59)	>1,79 ng/mL	HFpEF	Diagnosis and severity of HFpEF
Gal-3	Medvedeva et al. (74)	>21 ng/mL	HF	Independent factor of death, correlation with oxidative stress and renal failure
GDF-15	Kempf et al. (60)	Δ 1,194–3,577 ng/L	HFrEF	All-cause mortality
GDF-15	Kempf et al. (61)	Δ 850–1,553 ng/L	Stable angina pectoris	Coronary heart disease mortality
GDF-15	Wang et al. (62)	Δ 306–14,493 ng/L	3,428 individuals from the Framingham Offspring Study	Death, HF, MACE
GDF-15	Schopfer et al. (63)	Δ 1,589–3,057 ng/L	CAD	All-cause mortality, CV events, MI, HF, hospitalization
GDF-15	Chan et al. (64)	Δ 1,555–4,030 ng/L Δ 1,812–4,176 ng/L	HFrEF; HFpEF	Death or HF hospitalization
GDF-15	Skau et al. (65)	None	AMI	Long-term predictor of all causes of mortality
PlGF and sFlt-1	Lenderink et al. (66)	> 27 ng/l	ACS	Adverse long-term outcomes
PlGF and sFlt-1	Hochholzer (2010)	>20 ng/L; >84 ng/L (sFlt-1)	Suspected MI	Mortality
PlGF and sFlt-1	Marković et al. (67)	>13.2 ng/L	NSTEMI	Short term death, decrease in renal function
PlGF and sFlt-1	Glaser et al. (68)	>19.5 ng/L	Suspected ACS	Risk of MACE
PlGF and sFlt-1	Matsui et al. (69)	>19.6 pg/mL	CKD	CV events, all-cause mortality
CHIP	Genovese et al. (70)	/	12,380 persons, unselected for cancer or hematologic phenotypes	Risk of CV disease
CHIP	Jaiswal et al. (71)	/	17,182 persons who were unselected for hematologic phenotypes	All-cause mortality, risks of incident coronary heart disease
CHIP	Calvillo et al. (72)	/	AML	Increased prevalence of CV diseases

ACS, acute coronary syndrome; AMI, acute myocardial infarction; AML, acute myeloid leukemia; CA125, carbohydrate antigen 125; CAD, coronary artery disease; CEA, carcinoembryonic antigen; CHF, chronic heart failure; CHIP, clonal hematopoiesis of undetermined potential; CKD, chronic kidney disease; CV, cardiovascular; Gal-3, galectin-3; GDF-15, growth differentiation factor-15; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HTX, heart Transplantation; MACE, major adverse cardiovascular events; MI, myocardial infarction; NP, natriuretic peptide; NSTEMI, non ST-segment elevation myocardial infarction; NYHA, new york heart association; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; UAE, urinary album excretion.

outside the cell, in the nucleus and on cell surface, as well as in biological fluids. Gal-3 is expressed in a variety of human tissues, including endothelium, epithelial cells, sensory neurons, and immune cells (101).

Gal-3 is involved in a variety of biological processes, and its roles vary depending on whether it is found inside or outside the cell; in the cytoplasm, it plays a key role in cell survival by inhibiting apoptosis through binding to Bcl-2 and influencing Ras-mediated AKT signaling (102). Nuclear-localized Gal-3, on the other hand, contributes to the formation of the spliceosome structure for pre-mRNA splicing and regulates gene transcription by enhancing the binding of specific transcription factors to the gene promoter. Finally, it appears to participate in cell-cell interactions in the extracellular environment, regulating cell adhesion and migration. Thus, Gal-3 plays a key role in several physiological processes including cell adhesion, angiogenesis, proliferation, inflammation and fibrosis (102).

Gal-3 has been linked to cancer in several studies, with evidence that it promotes neoplastic transformation, cell cycle progression, and apoptosis (103). According to Song et al., increased expression of Gal-3 was found in a K-ras mutant mouse model and in pancreatic cancer patients, and greater expression of Gal-3 was linked to increased tumor proliferation and infiltration (103). Furthermore, short hairpin RNA-induced downregulation of Gal-3 was demonstrated to reduce *in vitro* and *in vivo* tumor proliferation, invasion, and growth (103). Increased Gal-3 production improves cancer cell adherence to the extracellular matrix (ECM) which enhances, coupled with immune surveillance evasion, malignant cell movement and metastasis (104). According to preclinical research, increased serum levels of Gal-3 have been linked to a higher frequency of metastases (104). Furthermore, increased nuclear Gal-3 concentration promotes the expression of cyclin D1, thyroid transcription factor-1, and mucin 2, all of which are directly associated to cancer pathogenesis and progression (105). Gal-3 has also been discovered to have an intricate prognostic role, which changes according on the type of tumor: higher Gal-3 expression has been linked to a worse prognosis in numerous malignancies, including lymphoma, thyroid cancer, and leukemia, whereas lower Gal-3 expression has been linked to a worse outcome in prostate cancer and chronic lymphoblastic leukemia (106). The inconsistent results regarding Gal-3 expression based on tumor type could be related to differences in Gal-3 localization inside tumor cells, since the molecule's function differs depending on whether it is found in the nucleus, cytoplasm, or extracellular regions. Califice et al. have shown that nuclear Gal-3 has a pro-apoptotic effect in prostate cancer cells, whereas cytoplasmic Gal-3 has an anti-apoptotic effect (107). Although possible between Gal-3 and apoptosis-associated protein Nucling have been suggested, the exact pro-apoptotic pathway remains unknown (107).

Due to its proliferative action, Gal-3 has been intensively examined for a possible pathogenic role in cardiovascular disease and has been identified as a marker of fibrosis and inflammation. Specifically, Gal-3 appears to be involved in the development of HF and may act as a diagnostic and prognostic biomarker, suggesting higher rates of mortality and morbidity (58, 59). In a prospective cohort study, Medvedeva et al. found an increase in Gal-3 levels in patients with chronic HF of all NYHA classes. Gal-3 levels >21 ng/mL were also found to be an independent predictor of death across a 26-month follow-up, and were correlated to markers of oxidative stress, renal failure, and inflammation (74). Furthermore, Meijers et al. reported that plasma Gal-3 levels >17.8 ng/mL predict HF re-hospitalization and mortality, and offer a more accurate risk stratification, regardless of age, gender, LV EF, NYHA class, or serum BNP levels (75). Moreover, the predictive value of Gal-3 in patients with HF has been found to be unaffected by the therapeutic strategies used to treat HF or by age (57, 108). However, data comparing Gal-3 predictive value to established biomarkers for HF are conflicting, suggesting that Gal-3 has major prognostic efficacy when used in combination with other HF biomarkers than alone (101).

GDF-15

Growth differentiation factor-15 (GDF-15), also known as macrophage inhibitory cytokine-1 (MIC-1), is a divergent member of the transforming growth factor (TGF)-β superfamily. In healthy individuals, with the exception of the placenta and prostate, GDF-15 shows low to absent constitutive expression (109). Increased blood levels of GDF-15 are related to stressogenic events, anoxia and acute injury, and are found to be increased in several diseases including inflammation, obesity, cardiovascular disease and cancer (110, 111). In addition, several cell types express GDF-15 under stress conditions, including cardiomyocytes, adipocytes, macrophages, endothelial cells and vascular smooth muscle cells (112). Being an inflammatory and stress-induced cytokine, GDF-15 is also significantly expressed in response to various growth factors and inflammatory proteins, including IL-1ß, TNF-α, IL-2, and macrophage colony-stimulating factor-1, which implies a complex and multidimensional regulation (113). Specifically, GDF-15 appears to play a role in limiting the inflammatory response in the aftermath of tissue damage, reducing leukocyte infiltration and fibrosis (114). Chung et al. found increased expression of GDF-15 in the liver after administration of carbon tetrachloride or alcohol (114). In GDF-15 knockout mice, they also observed an increased degree of liver infiltration by monocytes, CD4+ and CD8+ lymphocytes and macrophages, as well as a lower degree of fibrosis (114). Moreover, the transcription factor p53 binds to the *GDF15* gene promoter region via two distinct binding sites. As a result, GDF-15 expression appears to be linked to the synthesis of p53, which is activated by conditions such as hypoxia, telomere erosion, and oxidative stress (115). This explains why GDF-15 expression rises with aging, a condition known to be linked to several markers of stress and damage, including ROS production, protein glycation, inflammation and hormonal changes.

Elevated serum levels of GDF-15 have been found in several types of cancer (116), also confirmed by biopsy analysis of various tumor tissues (117). Several studies have correlated high levels of GDF-15 with the development of cancer-related anorexia and cachexia (118, 119), as well as worse survival (120). Furthermore, Wallentin et al. in the Uppsala Longitudinal Study of Adult Men (ULSAM) study, identified GDF-15 as an independent predictor of all-cause mortality, cardiovascular and cancer mortality (121).

However, the exact role of GDF-15 within tumorigenesis is still unclear, with some evidence supporting its action in promoting malignancy, while others showing its inhibitory effects on cancer. Boyle et al. found that subcutaneous injection of GDF-15-producing metastatic melanoma cells into nude mice resulted in faster tumor development than controls (122). In contrast, some preclinical investigations on transgenic mice have shown that GDF-15 has tumor-suppressing activity: for example, Husaini et al. found that overexpression of this protein reduced tumor mass growth and enhanced survival (123). The conflicting results regarding GDF-15 role in tumorigenesis may be due to the timing of the protein's tumor-promoting or tumor-suppressing effects. It has been hypothesized that GDF-15 primarily performs a tumor-suppressing function in the early stages of tumor development, and then a tumor-promoting function in the later stages (124).

Similarly to cancer, great attention has been paid to the association between elevated serum concentration of GDF-5 and outcomes of various cardiovascular diseases, including atherosclerosis, HF, coronary artery disease (CAD) and ischemic reperfusion injury. By enrolling 3,428 participants in the Framingham Heart Study, Wang et al. demonstrated a strong correlation between high GDF-15 levels, mortality and the development of HF (62). Cotter et al., using data from the RELAX-AHF study, assessed GDF-15 values at admission and at several subsequent time points (125). GDF-15 levels were found to be an independent predictor of short-term cardiovascular mortality and rehospitalization (125). Similarly, in a study on 847 patients with myocardial infarction (MI), using The Proximity Extension Assay proteomics chip (capable to analyze 92 different cardiovascular biomarkers), GDF-15 and TRAIL receptor 2 were identified as the best biomarkers in predicting long-term all-cause mortality (65). Furthermore, synthesis of GDF-15 in the infarcted area of a mouse model of MI was shown to be responsible for reduced leukocyte infiltration, lowering the probability of fatal heart rupture, thus demonstrating the local anti-inflammatory role of GDF-15 (126). GDF-15 has also been identified as a potential biomarker for the risk of cardiovascular events and mortality in patients with ACS and CAD (127). Some preclinical studies have investigated the function of GDF-15 in the pathophysiology of atherosclerosis; however, results have been conflicting, with both a protective and a disease-promoting effect reported in different studies (128). Despite multiple studies strongly linking GDF-15 serum levels with an increased risk of cardiovascular events and death, the specific involvement of the protein in the development of cardiovascular disease is still unknown.

PlGF and sFlt-1

Vascular endothelial growth factor (VEGF)-A, B, C, D, and E, and placental growth factor (PlGF) are all members of the *VEGF* gene family. The pro-angiogenic action of this glycoproteins is characterized by increased endothelial cell proliferation and survival, as well as improved vascular permeability (129). Furthermore, by serving as a chemoattractant for monocytes and stimulating the production of adhesion molecules on endothelial cells, VEGF has a function in inflammatory responses and ischemic events (130). The pro-angiogenic actions of VEGF are mediated by the VEGFR-2, while the VEGFR-1 (also known as fms-like tyrosine kinase-1, Flt-1) is responsible for sequestering VEGF and functioning as a negative regulator of the neoangiogenic process (131). PlGF, on the other hand, can only bind Flt-1 and its soluble form, sFlt-1, which is the second version of the receptor produced by alternative pre-mRNA splicing. Hypoxia, a stress condition found in both cardiovascular disease and cancer, regulates *FLT1* gene expression and is responsible for the preferential synthesis of sFlt-1. VEGF and PlGF bind to sFlt-1 with a high affinity, allowing it to block their pro-angiogenic activity (131).

PlGF beneficial effects on angiogenesis and cardiac function preservation in post-ischemic myocardium have been investigated in various preclinical studies (132). Furthermore, PlGF appears to be involved in the inflammatory mechanisms supporting atherosclerosis, although data from preclinical studies are conflicting (132). PlGF also seems to play a key role in the cardiorenal connection, possibly as a result of the increased degree of atherosclerosis seen in patients with chronic kidney disease CKD (69). Matsui et al. it have shown that higher serum levels of PlGF are independent predictors of all-cause mortality and cardiovascular events in patients with CKD, with greater strength than traditional risk factors (69); Specifically, patients with plasma PlGF levels of 19.6 pg/mL showed an 8.42-fold increase in cardiovascular mortality and a 3.87-fold increase in all-cause mortality when compared to patients with lower PlGF levels (10.1 pg/mL) (69). A prospective trial of patients with suspected ACS, showed that low serum PlGF and BNP levels were efficient predictors of the risk of MACE, with an incidence at 1 year of <1% (68). Furthermore, an induction of PlGF production within 12 h of MI was observed in patients with ACS without ST-segment elevation, with blood values remaining stable for up to 30 days after the event, implying that PlGF is involved in the healing process of the infarcted area. Interestingly, PlGF serum levels >13.2 ng/L were associated with a greater risk of short-term death (HR 2.28, 95% CI, 1.21–4.7;, $p = 0.0125$), as well as decreased renal function (67). Finally, it was found that PIGF values at baseline were effective prognostic indicators of adverse long-term outcomes in patients with ACS, regardless of platelet activation and myocardial necrosis (doi: 10.1016/j.jacc.2005.08.063). As PlGF and sFlt-1 may show independent plasma alterations during ACS, the PlGF/Flt-1 ratio was also investigated (132). Hochholzer et al. found in a sample of patients with symptoms suggestive of acute MI that both biomarkers gave additional prognostic information when compared to established blood biomarker, such as TnT and NT-proBNP (76). Matsumoto et al. also examined the efficacy of the PlGF/sFlt-1 ratio in predicting death from all causes, cardiovascular death, and total cardiovascular events in patients with ACS at baseline (133). The ratio was predictive of an increased risk of adverse events and death from all causes, when compared to the individual biomarkers studied separately. It was also found that a greater PIGF/sFlt-1 ratio value was associated with a higher number of coronary arteries with stenosis at baseline (133).

Through the correct supply of oxygen and nutrients, neovascularization plays a critical role in the growth dynamics

of the tumor mass as well as metastases (134). sFlt-1 is expressed in several tumors, including breast cancer, colorectal cancer, and acute myeloid leukemia (AML), and the PIGF/sFlt-1 ratio, as well as sFlt-1, have been linked to the prognosis of a variety of malignancies (135). Furthermore, tumor PIGF synthesis is crucial for keeping the inflammatory response in the TME, and it also appears to induce an immunosuppressive state favorable to tumor growth via NFAT-mediated binding to sFlt-1 (136).

Despite their efficacy in the treatment of a variety of tumor types, the introduction of drugs targeting members of the VEGF family into clinical practice has been linked to cardiotoxicity and the development of a variety of cardiovascular diseases, including hypertension, cardiomyopathy, and deep vein thrombosis (137). These findings emphasize the impact of VEGF in the pathophysiology of cardiovascular disease and cancer, and future studies are needed to clarify the appropriate clinical use of VEGF family biomarkers.

CHIP

Hematopoiesis is a polyclonal process in which equipotential hematopoietic stem cells differentiate into erythroid, lymphoid, myeloid, or megakaryocytic cells (138). This stem population may develop mutations providing it a proliferative advantage, leading to the formation of clonally expanded stem populations (clonal haematopoiesis), which produce mutant progenies that can be sampled in peripheral blood (139). At the age of 70, more than 10% of people have these clones, which account for about 20% of their peripheral white blood cells on average (71). Most people who are affected by these mutated clones will never develop a hematological malignancy, which is why this condition is called clonal hematopoiesis of undetermined potential (CHIP). It is a pre-malignant state, with rates of progression to hematological malignancy of about 0.5 % per year (71). CHIP is diagnosed when the number of mutant clones in peripheral leukocytes count exceeds 2% (140).

In recent studies, CHIP has been linked to a 2- to 4-fold higher risk of cardiovascular disease (70, 71). Some preclinical studies have also reported an overlap between CHIP and the development of cardiovascular disease: mouse models with mutations in Tet2 (one of the key genes in the pathogenesis of CHIP) showed accelerated formation of atherosclerotic lesions (141). Furthermore, mice knockout for Tet2 showed higher serum levels of inflammatory markers such as C-X-C motif chemokine ligand (CXCL)1, CXCL2, CXCL3, IL-1, and IL-6, implying that Tet2 may have a role in atherogenesis pathogenesis (142).

In a recent retrospective cohort study of 623 patients with AML, 63% of whom carried CHIP-related mutations, Calvillo et al. found an increased prevalence of cardiovascular diseases at baseline (72). Of note, the presence of 1 or more CHIP-related mutations was an independent risk factor for the development of cardiovascular adverse events in patients treated with anthracycline chemotherapy, but not in other patients (72). Furthermore, the timing of the onset of cardiovascular adverse events after a diagnosis of AML was discovered to be a poor prognostic factor, independently related with all-cause mortality (72).

Several concerns remain unanswered in understanding the processes underlying the vascular risk posed by CHIP. More research is needed to understand the genetic and molecular mechanisms behind CHIP, as well as the investigation of environmental risk factors that regulate CHIP, to identify a suitable application for this strong new risk factor. In addition, appropriate screening and treatment strategies for CHIP patients, as well as adjustments in cancer treatment regimens, must be examined.

MARKERS OF PRO-INFLAMMATORY STATUS

Pro-inflammatory Cytokines

Aging is associated with the development of a pro-inflammatory status that is characterized by high levels of pro-inflammatory markers in cells and tissues (143). These pro-inflammatory markers include IL-1, IL-6, IL-8, CRP, TGF-β, and TNF-α, among others (143). A systemic inflammatory state may originate from genetic susceptibility, visceral obesity, cellular senescence, impaired recycling and elimination of degraded cellular material, as well intrinsic defects in immune cells and chronic infections (143). It is noteworthy that senescent cells acquire a senescence-associated secretory phenotype that involves the secretion of a wide range of soluble mediators, including IL-1, IL-6, chemokines, growth factors, and metalloproteinases (MMPs) (143, 144).

Despite its fundamental physiological role as a defense mechanism against infections or extraneous agents, when inflammation becomes sustained and prolonged it becomes pathologically detrimental (143). Several studies have indeed shown that inflammation is a risk factor for cardiovascular disease, cancer, CKD, dementia, depression, osteoporosis, sarcopenia, and anemia (143, 145, 146). There is strong evidence suggesting that chronic inflammation is both a risk factor and a pathogenic mechanism in cardiovascular disease; for instance, vascular endothelial cell inflammation participates in the pathogenesis of atherosclerotic plaques, whereas atherosclerosis itself produces antigens that trigger and sustain an inflammatory response (143, 147, 148). In HF, concentrations of several interleukins are increased, including IL-1β, IL-6, IL-8, IL-13, and IL-18, whereas the levels of anti-inflammatory interleukins IL-5, IL-7, or IL-33 are down-regulated (149).

During the last couple of decades, the contribution of inflammation to cancerogenesis has been increasingly recognized. At present, cancer cells are investigated within a network of stromal and inflammatory immune cells that all together form the tumor microenvironment (150). Inflammation drives all stages of cancerogenesis, namely tumor initiation, growth, progression, metastasis, and therapy resistance (150). Furthermore, around 15–20% of all cancers are preceded by infection, chronic inflammation, or autoimmunity; examples of inflammatory pre-cancerous disorders are inflammatory bowel disease, chronic hepatitis, and Helicobacter-induced gastritis (150). Increased IL-6 serum levels seem to be closely associated

with cancer patients' clinical condition and to correlate with survival independent of the cancer type (151).

Myeloperoxidase

Myeloperoxidase (MPO) is a heme-containing peroxidase produced by polymorphonuclear leukocytes (152), which plays an important role in inflammation and microbial killing by neutrophils. MPO has also shown to have pro-atherogenic and pro-oxidant properties, being responsible for lipid peroxidation, nitric oxide (NO) scavenging and NO synthase inhibition (153). MPO may serve as both a marker and mediator of vascular inflammation. In patients with ACS and acute HF, elevated MPO levels importantly predict adverse outcomes and worse prognosis (154, 155).

MPO has also proven to have a promising role in cardioncology (24). In a study by Ky et al., 78 patients with breast cancer undergoing doxorubicin and trastuzumab therapy received a baseline evaluation with 8 different biomarkers: hsTnI, hsCRP, NT-proBNP, GDF-15, MPO, PlGF, sFlt-1, and Gal-3 (156). Among these markers, MPO baseline levels were the only to be significantly associated with cardiotoxicity development ($p = 0.052$). However, interval changes in hsTnI, GDF-15, MPO, sFlt1, and Gal-3 from baseline to 3 months were also associated with subsequent cardiotoxicity (156).

sST2

Suppression of tumorigenicity 2 (ST2) is a member of the IL-1 receptor superfamily that exists in two main isoforms: a soluble form (referred to as soluble ST2 or sST2) and a membrane-bound receptor form (referred to as ST2 receptor or ST2L) (157). The interaction between IL-33 and ST2L exerts cardioprotective effects in the myocardium by reducing fibrosis, hypertrophy and enhancing survival. The circulating isoform sST2, by sequestering IL-33, abrogates this favorable effect (158). Circulating sST2 is released in response to vascular congestion and inflammatory and pro-fibrotic stimuli, and it serves as a marker of adverse remodeling and fibrosis, cardiac dysfunction, impaired hemodynamics and higher risk of progression. In patients with HF, sST2 is an independent predictor of mortality and HF hospitalization (158).

Serum levels of sST2 and IL-33 were reported to be significantly higher in several different cancer types as compared to healthy controls (159–161). However, their role has not been fully understood yet. Akimoto et al. have shown that sST2 negatively regulates tumor growth and the metastatic spread of CRC through modification of the tumor microenvironment; in particular, sST2 suppresses IL-33-induced angiogenesis, Th1- and Th2-responses, macrophage infiltration and macrophage M2a polarization (162). On the contrary, in other studies sST2 is associated with advanced and metastatic disease in gastric cancer and significantly correlates with the duration of the disease (163).

CURRENT RECOMMENDATIONS

The 2020 European Society for Medical Oncology (ESMO) consensus recommendations suggest performing a comprehensive baseline cardiovascular risk assessments before starting anticancer therapy (164). This includes baseline measurement of cardiac biomarkers (cardiac troponins and NPs), electrocardiogram, echocardiography (with LV EF and diastolic function evaluation). Biomarkers are useful to detect cardiotoxicity early before changes in LVEF, or clinical signs and symptoms of HF have developed (24). However, biomarkers should not be used in isolation, but rather placed in a comprehensive assessment including imaging, risk factors, clinical symptoms and the cancer specific characteristics (24).

THERAPEUTIC PERSPECTIVES

The assessment of biomarkers in patients with cancer and/or HF provides strong evidence of the physiopathogenetic overlap between these two conditions. The advantage of unveiling the bidirectional relationship between cancer and HF may also rely upon the development of a therapeutical strategy suitable for both (8). To this purpose, inflammation represents an extremely useful target, and anti-inflammatory drugs targeting the IL-1, IL-6, or CRP axis, may lead to improved cardiovascular and oncological outcomes (165). Apart from calorie restriction and physical activity, systemic inflammation can be hindered by small molecules or antibodies interfering with inflammatory mediators or their biological targets (143).

The CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study) was a randomized, double-blinded, placebo-controlled trial that investigated the use of canakinumab, a monoclonal antibody targeting IL-1β, in over 10.000 high-risk patients with established atherosclerotic disease who had already had a MI) (166). At a median follow-up of 3.7 years, canakinumab led to a significantly lower rate of recurrent cardiovascular events (nonfatal MI, stroke, or cardiovascular death) than placebo ($p = 0.02074$) (166). Strikingly, an exploratory analysis from the CANTOS group revealed that canakinumab can significantly reduce incident lung cancer (HR 0.33; 95% CI, $p < 0.0001$) and lung cancer mortality (HR 0.23; 95% CI, $p = 0.0002$) (167). The analysis also reported that baseline concentrations of CRP (6.0 mg/L vs. 4.2 mg/L; $p < 0.0001$) and IL-6 (3.2 vs. 2.6 ng/L; $p < 0.0001$) were significantly higher among participants subsequently diagnosed with lung cancer than among those not diagnosed with cancer (167).

A meta-analysis of two large prospective cohort studies has also shown that long-term use of aspirin, a nonsteroidal anti-inflammatory drug, was associated with a modest but significantly reduced risk for overall cancer (relative risk [RR], 0.97; 95% CI, 0.94–0.99), which was primarily owing to a lower incidence of gastrointestinal tract cancers (RR, 0.85; 95% CI, 0.80–0.91), especially CRC (RR, 0.81; 95% CI, 0.75–0.88) (168). Similarly, a meta-analysis of four randomized clinical trials, revealed that long-term aspirin intake of at least 75 mg daily reduced long-term incidence (HR 0.76, $p = 0.02$) and mortality (HR 0.65, $p = 0.005$) due to CRC (169).

CONCLUSIONS

Cancer and cardiovascular diseases, more specifically HF, represent some of the most commonly recognized causes of death and comorbidity world-wide. These two entities often share common risk factors and clinical symptoms. While recent findings have highlighted that HF is associated with an increased risk of cancer and cancer-related mortality, heightened in decompensated states (2, 3, 170), common pathophysiological systemic changes (inflammation, metabolism, activation of the neuro-hormonal and immune system, endothelial dysfunction) often subtend both these chronic conditions.

Circulating biomarkers represent a sensitive and specific diagnostic and prognostic tool (as a potential therapeutic target) for evaluation of pre-clinical as well as follow-up of disease condition, potentially aiding in identification of multiple (including cardiovascular) injuries and toxicities.

Although the potentially adverse effects of chemotherapy on the heart are well known (cardiotoxicity), there is limited evidence on the impact of cancer, per sé, on the heart of untreated oncologic patient as well as on the bidirectional relationship between cancer and HF. In pre-clinical and human models, the presence of active cancer has been associated with subclinical metabolic and myocardial cellular damage oncometabolites, (4) and patients with active neoplasms have been shown to have, independently of cardiological comorbidity, increased levels of multiple cardiac biomarkers before chemotherapy, with a demonstrated prognostic role of the same (8).

This milieu might represent, *per sé,* a condition at increased risk of cardiotoxicity, even before chemotherapy. Since oncologists refer patients to cardiological evaluation for risk stratification and monitoring, this evidence might provide further guidance on the management of patients who are candidates to specific treatments: thus far circulating biomarkers represent an easy, bedside and reproducible clinical tool for the entire course of the cardioncological patient, from initial risk stratification (increase of cardiac biomarkers = increased risk for chemotherapy), conventional early detection of cardiotoxicity, short/medium and long term follow-up (survivorship). Finally, circulating biomarkers might aid in the identification of a common pathophysiology between cancer and HF (increase of baseline circulating biomarkers = "cancer cardiomiopathy"), thus prompting the implementation of preventive and treatment strategies on multiple targets (164).

AUTHOR CONTRIBUTIONS

MC, GP, IF, SL'A, and DC contributed to the conception of the review, the bibliographic research, and the drafting of the manuscript. AG, AA, GV, CG, VC, CP, CC, AF, and ME contributed to critical revision and editing. MC, GP, and SL'A designated tables and figures. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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Shared Genetic Risk Factors Between Cancer and Cardiovascular Diseases

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Cancer and cardiovascular diseases (CVD) account for approximately 27.5 million deaths every year. While they share some common environmental risk factors, their shared genetic risk factors are not yet fully understood. The aim of the present study was to aggregate genetic risk factors associated with the comorbidity of cancer and CVDs. For this purpose, we: (1) created a catalog of genes associated with cancer and CVDs, (2) visualized retrieved data as a gene-disease network, and (3) performed a pathway enrichment analysis. We performed screening of PubMed database for literature reporting genetic risk factors in patients with both cancer and CVD. The gene-disease network was visualized using Cytoscape and the enrichment analysis was conducted using Enrichr software. We manually reviewed the 181 articles fitting the search criteria and included 13 articles in the study. Data visualization revealed a highly interconnected network containing a single subnetwork with 56 nodes and 146 edges. Genes in the network with the highest number of disease interactions were *JAK2*, *TTN, TET2*, and *ATM*. The pathway enrichment analysis revealed that genes included in the study were significantly enriched in DNA damage repair (DDR) pathways, such as homologous recombination. The role of DDR mechanisms in the development of CVDs has been studied in previously published research; however, additional functional studies are required to elucidate their contribution to the pathophysiology to CVDs.

Keywords: cancer, cardiovascular disease, risk factor, chemotherapy, comorbidity, genetic variant, interaction network, cardio-oncology

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INTRODUCTION

Cancer and cardiovascular diseases (CVD) are among the leading causes of death worldwide. An estimated 17.9 million people die every year as a result of CVDs and approximately 9.6 million deaths per year are caused by cancer (1, 2). While they are two distinct types of illness, they display a significant level of comorbidity (3). A portion of this comorbidity has been attributed to the cardiotoxic effects of chemotherapeutic cancer treatments (4). Cancer and CVD also share contributing risk factors, such as hypertension, obesity, smoking, diabetes mellitus and lifestyle choices, as well as pathophysiological mechanisms, such as oxidative stress, neuro-hormonal activation and inflammation (5). The identification of these common risk factors and mechanisms has led to increased focus on the various possible connections between the two diseases in the field of cardio-oncology. However, much of this research has been focused on the effects of medication

and common risk factors. As a result, potential underlying genetic contributors to this comorbidity are poorly understood.

Among the known genetic factors that contribute to the development of both cancer and CVDs is clonal hematopoiesis of intermediate potential (CHIP). CHIP refers to the presence of a sub-population of clonally expanded hematopoietic stem cells within an individual. This may occur as a result of age-related genetic drift or due to genetic variants. While the presence of CHIP is not inherently malignant, it does confer a higher risk for the development of blood malignancies, such as leukemia and CVDs. CHIP has also been associated with worsened heart failure outcomes (6). It has also been shown that patients with congenital heart disease (CHD) carry more damaging gene variants in cancer risk genes, suggesting shared biological pathways (7). Associations between cancer and CVDs on a genetic level have therefore already been identified. Further exploration into their shared genetic background could thus yield valuable results and contribute to our understanding of both cancer and CVDs.

The aim of the present study was to aggregate genetic risk factors associated with the comorbidity of cancer and CVDs. Our goal is to visualize gene-disease interactions in genes with variants associated with cancer and CVD comorbidity. Additionally, the goal is to perform a protein-protein interaction (PPI) analysis for genes included in the study and to perform pathway enrichment analysis to identify biological pathways in which disease-associated genes are involved. This would contribute to the field of cardio-oncology by compiling genetic risk factors and their roles for multiple disease types, which could be used for future development of risk assessment, more accurate prognoses and personalized treatment choices.

MATERIALS AND METHODS

The PubMed database was accessed in order to collect relevant articles. Four database searches were conducted, containing common keywords pertinent to the subject matter:

1. Cardiac, mutations, risk, prevalence, predispose*, cancer
2. Heart, cancer, mutation, risk, cardiooncology
3. Cardiovascular, propensity, mutation, risk factor, cancer
4. Heart, cancer, gene*, risk, cardiooncology

The articles within the search results were then manually reviewed and screened for studies that fit two criteria:

1. Both cancer and CVD phenotypes were observed in patients
2. The study is concerned with shared genetic risk factors for cancer and CVDs

Studies that fit both screening criteria were then included in the study. Cancer type, CVD type, chemotherapeutic methods, as well as names of genes with disease-associated variants, were retrieved from studies. STRING software was then used to identify protein-protein interactions (PPIs) between proteins encoded by genes included in the present study (8). Only PPIs categorized as "retrieved from curated databases" or "experimentally validated" were included. The PPIs, genes, and their associated cancer and cardiovascular conditions were then visualized as a network using Cytoscape software (9). Secondary literature review was then conducted for genes with the highest number of disease-associated interactions in the network.

A pathway enrichment analysis using Enrichr was performed on genes included in this study, drawing from the BioPlanet 2019, KEGG 2021 and WikiPathway 2021 databases (10). In order to include the most reliable enrichments, pathway enrichment analysis results were then sorted by lowest adjusted *P*-value. The 10 lowest *P*-value results of each database were included in the study. A workflow diagram of the study is presented in **Figure 1**.

RESULTS

In this study, we conducted manual literature review of genes with variants associated with cancer and CVD comorbidity and then visualized this data as a gene-disease network. Additionally, we expanded upon the network with PPIs obtained from the STRING database. An analysis revealed the obtained gene list was significantly enriched in pathways responsible for DDR. Finally, we conducted secondary literature review of genes identified with the study methodology.

The PubMed database was accessed with four queries containing common keywords for the field of cardio-oncology; the four queries combined yielded 181 articles as search results. After screening and manual review, 13 articles were included in the final data set (11–23). Out of 13 articles included in the study, 10 included chemotherapy as a study parameter. Data extracted from manually reviewed articles is available in **Supplementary Table 1**. A list of PPIs identified with STRING is available in **Supplementary Table 2**. Data visualization revealed a large interconnected network of associations between genes, cancer types and CVDs as well as PPIs (**Figure 2**). The network was composed of 29 loci (28 genes and 1 intergenic region), 13 cancer types and 14 CVDs; thus containing 56 nodes and 146 edges.

Genes in the network with the most disease-connected edges were *JAK2*, *TTN, TET2*, and *ATM*, with 16, 12, 8, and 6 edges, respectively. Diseases with the highest number of gene-connected edges were peripartum cardiomyopathy, breast cancer, clonal hematopoiesis of intermediate potential (CHIP) and coronary artery disease, with 24, 15, 10, and 5 edges, respectively.

Furthermore, we conducted a pathway enrichment analysis of the 28 disease-associated genes. Of the 30 pathway enrichments, 28 enrichments had an adjusted *P*-value ≤ 0.05 (**Table 1** and **Supplementary Table 3**). Among them, 3 were associated with CVDs, 4 were associated with cancer, and 16 were associated with DNA damage repair. Other enrichments include nucleotide methylation, meiosis or meiotic recombination, membrane transport proteins and other functions.

DISCUSSION

In this study we conducted a database search and literature review for genes associated with cancer and CVD comorbidity, including chemotherapy-induced cardiotoxicity. Of the 181

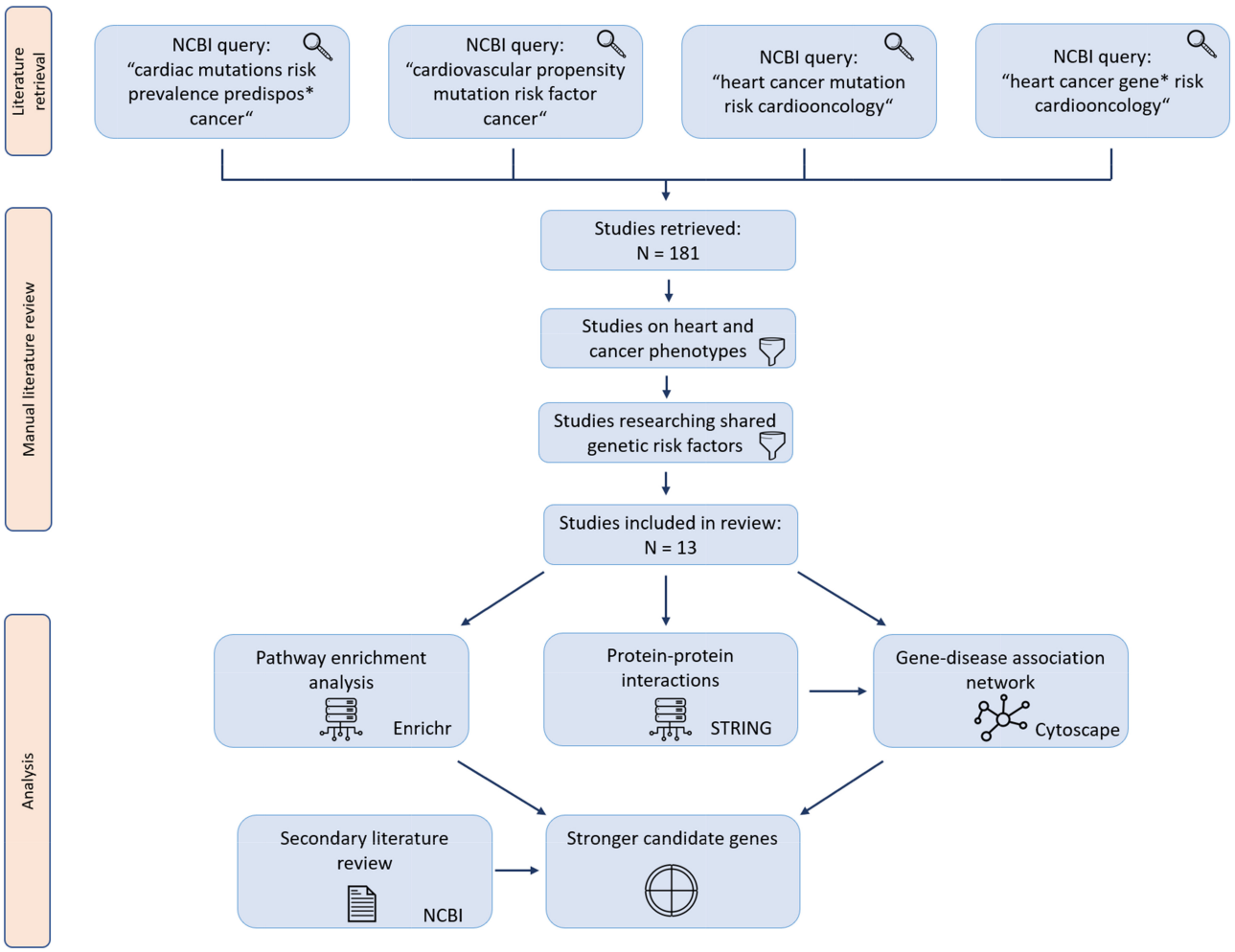

FIGURE 1 | Study workflow diagram. Retrieved studies were manually reviewed for studies matching the screening criteria.

manually reviewed articles, 13 were included in the analysis. We then formed a network of gene-disease associations, which was expanded upon with PPI data. Analysis revealed a large interconnected network containing a single subnetwork with 56 nodes and 146 edges. Enrichment analysis results also showed that genes were enriched in pathways associated with DNA damage repair, cancer and CVDs. Genes in the gene-disease association network with the highest number of edges were *JAK2*, *TTN, TET2*, and *ATM*.

Janus kinase 2 (JAK2) is encoded by the *JAK2* gene. It serves as a kinase for cytokine receptors and is part of the JAK-STAT signaling pathway, which allows the cell to respond to various stress factors, including endotoxins, hypoxia, UV radiation and hyperosmolarity (24). The pathway is also important for the regulation of the immune system through the polarization of T helper cells (25). JAK2 plays an important role in hematopoiesis, as *JAK2* gene disruptions in animal models are embryonically lethal due to a lack of definitive erythropoiesis (26). *JAK2* has been associated with the copresence of CHIP and myocardial infarction (16). A variant of *JAK2* has also been associated with an increased risk of developing myeloproliferative neoplasms such as polycythemia vera, essential thrombocythemia and primitive myelofibrosis (18, 27). As such JAK2 has been proposed as a potential target for the treatment or management of these diseases (28).

The *TTN* gene encodes titin (TTN), a large protein that plays a structural, scaffolding and signaling role in sarcomeres of striated muscle tissue (29). TTN spans from the Z-disc to the M-band, functioning as a molecular spring and giving muscles passive stiffness (30). The protein is also present in the heart's muscle tissue. While its mechanical and scaffolding properties are critical for sarcomere function, its signaling role is also important. TTN binds to telethonin via its NH_2 terminal domain, thus recruiting the muscle LIM protein (MLP) to the Z-line, where it interacts with other signaling molecules (31, 32). TTN could also be involved in hypertrophic signaling through its N2-A domain. This domain interacts with multiple signaling molecules, including muscle-ankyrin-repeat-proteins (MARPs), which are stress-response molecules and signal transducers (31). *TTN* variants have been associated with several CVDs, including

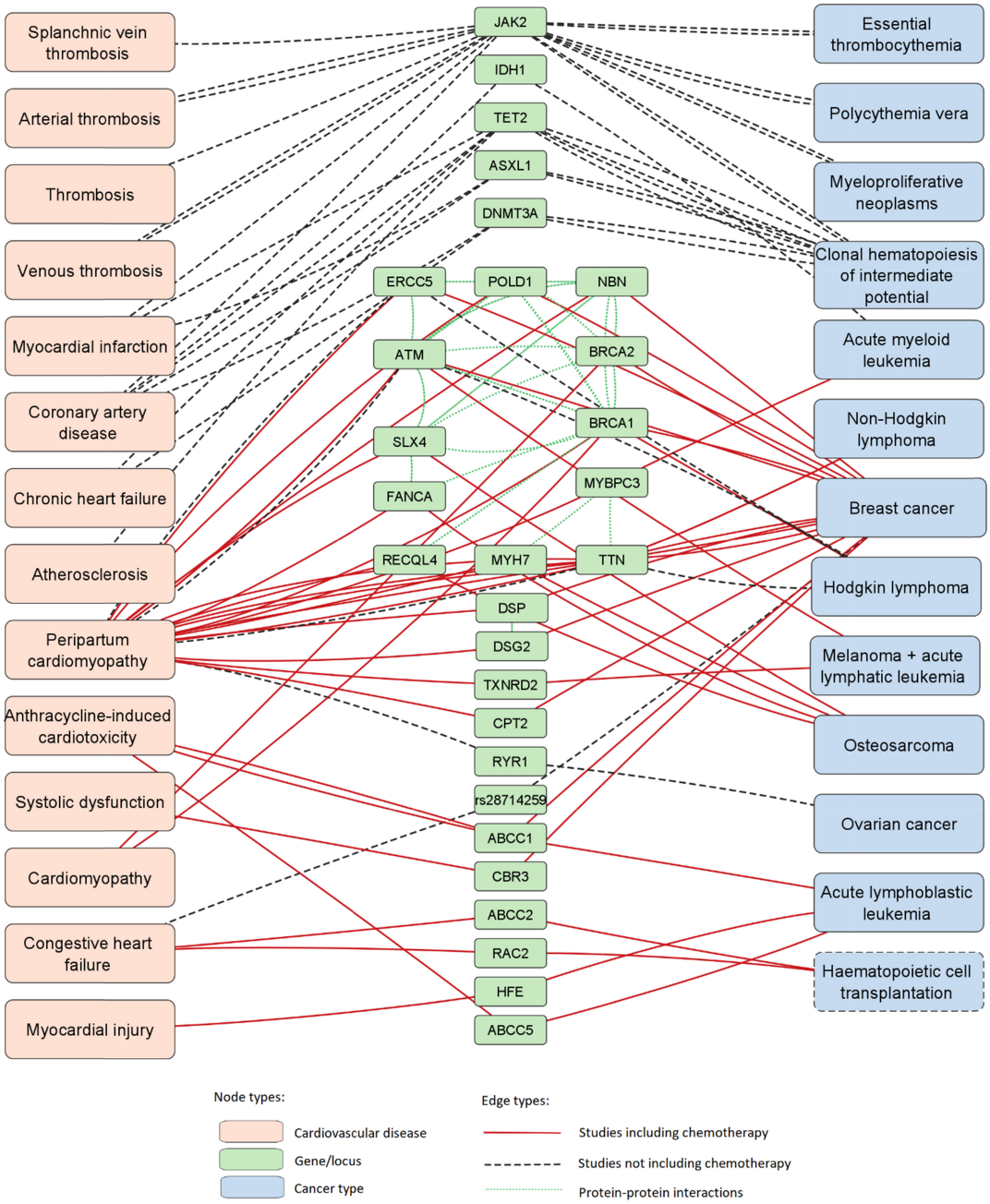

FIGURE 2 | Network of associations between 29 loci (28 genes and 1 SNP), cancer types and CVDs. This network was expanded with data of PPIs. Hematopoietic cell transplantation is marked with a dashed node outline as it is not a disease.

dilated cardiomyopathy, fatal cardiomyopathy and heart failure (29, 33, 34). *TTN* has also been associated with peripartum cardiomyopathy, Hodgkin lymphoma, non-Hodgkin lymphoma and breast cancer (12). Furthermore, it has been suggested that the mutation load of *TTN* is indicative of a high tumor mutation burden (35).

Tet methylcytosine dioxygenase 2 (TET2), encoded by *TET2*, plays a role in epigenetic DNA modification by converting 5-methylcytosine to 5-hydroxymethylcytosine, thus promoting DNA demethylation (36). Through its regulatory functions TET2 affects hematopoiesis by promoting the self-renewal of stem cells, lineage commitment and monocyte differentiation (37). Li et al. report that 8% of *TET2*-knockout mice developed lethal myeloid malignancies within their first year of life (38). *TET2* variants have also been associated with increased risk of myeloid malignancies. These malignancies include B- and T-cell lymphomas, myeloproliferative neoplasms, chronic myelomonocytic leukemia, acute myeloid leukemia,

myelodysplastic syndrome, CHIP and others (16, 19, 36, 38). Sano et al. have shown that *TET2* deficiency in mice is associated with greater cardiac dysfunction and heart failure, accelerated by *TET2*-induced clonal hematopoiesis. This is likely caused by the influence of *TET2* deficiency on the IL-1β/NLRP3 inflammatory pathway, as treatment with an NLRP3 inflammation inhibitor protected against the development of heart failure (39).

The *ATM* gene encodes the ATM serine/threonine kinase (ATM), which regulates multiple processes, including DNA damage response, oxidative stress levels and mitochondrial homeostasis (40). ATM plays a role in the cellular response to double-stranded breaks (DSB) (41). It phosphorylates TP53 – one of the most important tumor suppressor proteins (41, 42). Furthermore, ATM has also been shown to phosphorylate BRCA1, NBN and other tumor suppressor proteins (43, 44). *ATM* variants have been associated with a predisposition for developing ischemic heart disease (45). Beside CVDs, *ATM* variants have been associated with conveying an increased risk of developing some cancers, including chronic lymphocytic leukemia, breast cancer, pancreatic cancer and mantle cell

TABLE 1 | Pathway enrichment analysis for 28 genes associated with cancer and CVDs.

Pathway source	Pathway name	*P*-value	Adjusted *P*-value	Odds ratio	Combined score	Network genes included in pathway
Bioplanet 2019	BRCA1, BRCA2 and ATR roles in cancer susceptibility	1.15E-10	1.33E-08	244.53	5595.75	*FANCA, ATM, BRCA1, NBN, BRCA2*
Bioplanet 2019	DNA repair	1.27E-10	1.33E-08	64.52	1470.09	*POLD1, FANCA, ATM, ERCC5, BRCA1, NBN, BRCA2*
Bioplanet 2019	ATM-mediated phosphorylation of repair proteins	2.74E-08	0.000001138	1152.06	20062.49	*ATM, NBN, BRCA2*
Bioplanet 2019	Double-strand break repair	2.56E-08	0.000001138	177.36	3100.38	*ATM, BRCA1, NBN, BRCA2*
Bioplanet 2019	Fanconi anemia pathway	2.56E-08	0.000001138	177.36	3100.38	*FANCA, ATM, BRCA1, BRCA2*
Bioplanet 2019	Homologous recombination	8.25E-08	0.000002453	127.65	2082.04	*POLD1, ATM, NBN, BRCA2*
Bioplanet 2019	BARD1 signaling events	8.25E-08	0.000002453	127.65	2082.04	*POLD1, ATM, NBN, BRCA1*
Bioplanet 2019	Recruitment of repair and signaling proteins to double-strand breaks	1.53E-07	0.000003971	460.75	7231.36	*ATM, BRCA1, NBN*
Bioplanet 2019	Meiotic recombination	0.000001072	0.00002477	63.75	876.27	*NBN, ATM, BRCA1, BRCA2*
Bioplanet 2019	Meiosis	0.000006051	0.0001259	40.29	484.07	*NBN, ATM, BRCA1, BRCA2*
WikiPathways 2021	DNA Repair Pathways Full Network WP4946	3.28E-10	3.97E-08	55.92	1221.07	*POLD1, FANCA, ATM, ERCC5, BRCA1, NBN, BRCA2*
WikiPathways 2021	Homologous recombination WP186	2.53E-09	1.53E-07	354.88	7025.6	*POLD1, ATM, NBN, BRCA2*
WikiPathways 2021	DNA IR-damage and cellular response via ATR WP4016	9.94E-08	0.000004007	55.27	891.15	*FANCA, ATM, BRCA1, NBN, BRCA2*
WikiPathways 2021	DNA IR-double strand breaks and cellular response via ATM WP3959	0.000001155	0.00003493	62.49	854.39	*ATM, BRCA1, NBN, BRCA2*
WikiPathways 2021	ATM Signaling Pathway WP2516	0.00002612	0.0006321	62.16	656.01	*ATM, BRCA1, NBN*
WikiPathways 2021	DDX1 as a regulatory component of the Drosha microprocessor WP2942	0.00004244	0.0008558	295.79	2977.88	*ATM, NBN*
WikiPathways 2021	Cytosine methylation WP3585	0.00007262	0.001098	211.26	2013.35	*IDH1, TET2*
WikiPathways 2021	Breast cancer pathway WP4262	0.00006911	0.001098	21.14	202.54	*ATM, BRCA1, NBN, BRCA2*
WikiPathways 2021	Irinotecan pathway WP229	0.0001568	0.001725	134.41	1177.53	*ABCC1, ABCC2*
WikiPathways 2021	DNA damage response WP707	0.0001289	0.001725	35.34	316.48	*ATM, BRCA1, NBN*
KEGG 2021	Homologous recombination	3.22E-09	3.16E-07	115.36	2255.82	*POLD1, ATM, BRCA1, NBN, BRCA2*
KEGG 2021	Fanconi anemia pathway	0.000001072	0.00005252	63.75	876.27	*SLX4, FANCA, BRCA1, BRCA2*
KEGG 2021	ABC transporters	0.00003733	0.001219	54.75	558.21	*ABCC1, ABCC2, ABCC5*
KEGG 2021	Hypertrophic cardiomyopathy	0.0002958	0.007011	26.37	214.29	*MYBPC3, TTN, MYH7*
KEGG 2021	Dilated cardiomyopathy	0.0003577	0.007011	24.66	195.72	*MYBPC3, TTN, MYH7*
KEGG 2021	MicroRNAs in cancer	0.0009902	0.01617	10.28	71.13	*ABCC1, DNMT3A, ATM, BRCA1*
KEGG 2021	Nucleotide excision repair	0.002107	0.0295	32.8	202.12	*POLD1, ERCC5*
KEGG 2021	Viral myocarditis	0.003411	0.04178	25.43	144.47	*RAC2, MYH7*

lymphoma (12, 46, 47). While ATM has several tumor suppressing functions, ATM-induced signaling can cause tumor progression via the αvβ3 integrin pathway in some cancers (48). ATM-dependent signaling in tumor cells can also increase resistance to radiotherapy and chemoresistance (49). Due to its role in signaling pathways and DNA damage repair, ATM has been proposed as a potential target for the development of future chemotherapies (50).

In the present study, a pathway analysis revealed that the genes included in the analysis were enriched in pathways associated with DDR. Among them were pathways such as Nucleotide excision repair, DNA damage response WP707 and Double-strand break repair. While variants of DDR genes are known to contribute to cancer development, they have also been associated with some CVDs, such as heart failure and atherosclerosis (51). Results of a study of DDR in experimental heart failure in rats conducted by Yndestad et al. suggest that DDR mechanisms could play an important part in counteracting CVD-related genotoxic stress and damage to tissue (52). DDR mechanisms are important for counteracting the effects of oxidative stress on the cell (51). Among other shared risk factors, increased oxidative stress contributes to the development of both cancer and CVDs (53). Variants of DDR genes could thus be related to an increased susceptibility to damage from endogenous oxidative stress. Additional functional research is necessary to determine the role of DDR mechanisms in CVDs.

Several genes included in this study were identified in research concerned with the effects of chemotherapies on CVD development. Chemotherapeutics are a diverse group of molecules that remains a widely used approach in cancer treatment. These molecules often target rapidly replicating cancer cells by inducing DNA damage (54). However, this treatment also affects other cells, leading to various side effects, including cardiotoxicity. A common class of chemotherapeutics are anthracyclines and are known to have dose-dependent cardiotoxic side effects (54). The main mechanism of anthracycline-induced cardiotoxicity (ACT) is thought to affect heart cells by topoisomerase 2β inhibition, leading to cardiomyocyte apoptosis and mitochondrial biogenesis inhibition (55). The mechanisms of cardiotoxicity are not equally understood in all chemotherapeutics, however. The pathophysiology of HER-2-targeting agents, used for the treatment of breast cancer, is currently unclear (56). Further studies into the mechanisms of chemotherapy-induced cardiotoxicity for various treatments could serve to improve future therapeutic developments.

Genes included in this study could serve as potential variant screening targets for chemotherapy-induced cardiotoxicity risk assessment. Aminkeng et al. have identified variants of several genes associated with ACT, which could also serve as potential screening targets (57). Gene panels may be more cost effective than whole genome sequencing (WGS) for this purpose, but lack the option of re-analysis as knowledge of genetic risk factors advances (58). Pharmacogenomic testing is recommended for childhood cancer patients with indication for the anthracyclines daunorubicin and doxorubicin for specific variants of *RARG*, *SLC28A3* and *UGT1A6*4*. However, testing is not currently recommended for adult patients or children with indications for other anthracycline treatment (57). As the cost of WGS and gene panels fall and risk assessment strategies improve, screening a larger number of patients may become viable.

Accurately predicting cardiotoxic effects in patients and elucidating their pathophysiological mechanisms are of vital importance in cardio-oncology. This requires processing large data quantities, which is a task well-suited for machine learning. In cardio-oncology studies, machine learning has been used to perform retrospective cardiotoxicity risk assessment (59) and to estimate the safety of hERG blockers (60). Computational approaches could also have potential to improve cardiovascular imaging (61). Current research also suggests that these methods could be useful for predicting the cardiotoxic effects of drugs (62, 63). The use of machine learning may, in the future, assist clinicians in identifying probable pathogenic pathways and the treatment option with the highest likelihood of success.

CONCLUSION

In this study we conducted manual literature review and data synthesis of genetic factors associated with cancer and CVD comorbidity. We visualized a network of gene-disease associations based on data extracted from 13 articles. Additionally, we added information on PPIs of proteins encoded by genes in this study and included the PPIs in the network. The results revealed a large, highly interconnected network of known genetic risk factors shared between cancer and CVDs. Pathway enrichment analysis indicate that the genes included in this study are enriched in various DDR pathways, such as homologous recombination. While prior literature has associated some DDR gene variants with cancer and CVD comorbidity, the mechanism behind this has not yet been sufficiently studied. Additional functional studies are required to elucidate the role of DDR gene variants in the development of both cancer and CVDs and chemotherapy-induced cardiotoxicity. In the future, gene variants screening before cancer treatment could improve outcomes for patients at risk of cardiotoxicity.

STUDY LIMITATIONS

Patient chemotherapy information was extracted from articles during manual literature review. Some articles analyzed in the present study did not include chemotherapeutics as a study parameter, as it was not the intended focus of the studies. While treatment was not a study parameter, patients may have undergone chemotherapeutic treatment, which could contribute to CVD development. Therefore, it is possible that chemotherapies contributed to disease development in these

studies as well. Moreover, in this study we focused on changes at the DNA level. In the future, taking into account other omics levels, such as transcriptomics, proteomics and epigenomics may yield a more holistic view of the study field. It is also possible that some relevant research articles may not have been included based on the present study's queries. Searches for specific associations between CVD and cancer types may yield more publications as a database search output. We highlighted a few genes with the highest number of edges in the gene-disease association network. It should be noted, however, that an increased number of connections may be due to research interest and it is possible that other genes in the network could also be of great significance.

FUTURE DEVELOPMENTS

Variants of genes involved in DDR pathways appear to contribute to cancer and CVD comorbidity in cancer patients undergoing chemotherapy. Further functional studies would contribute to the understanding of the underlying disease mechanisms and allow for the development of precision medicine approaches to disease treatment.

The present study could serve as a basis for the development of a cardio-oncology database. Such a database could prove invaluable for the understanding of the molecular relationship between diseases and identifying potential novel molecular targets for treatment. Additionally, it could serve to identify biomarkers, which could be used for risk assessment and optimal treatment choices.

While cardio-oncology is a rapidly expanding field, many genetic risk factors shared by cancers and CVDs are currently not yet known. Thus, the network formed in this study is incomplete and will require follow-up studies as more research in the field is conducted.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

AT conducted literature review, screening and analysis, wrote the manuscript, and created graphics. AT and TK designed study methodology and conducted manuscript review. Both authors contributed to the article and approved the submitted version.

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Evidence for reciprocal network interactions between injured hearts and cancer

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Heart failure (HF) and cancer are responsible for 50% of all deaths in middle-aged people. These diseases are tightly linked, which is supported by recent epidemiological studies and case control studies, demonstrating that HF patients have a higher risk to develop cancer such as lung and breast cancer. For HF patients, a one-size-fits-all clinical management strategy is not effective and patient management represents a major economical and clinical burden. Anti-cancer treatments-mediated cardiotoxicity, leading to HF have been extensively studied. However, recent studies showed that even before the initiation of cancer therapy, cancer patients presented impairments in the cardiovascular functions and exercise capacity. Thus, the optimal cardioprotective and surveillance strategies should be applied to cancer patients with pre-existing HF. Recently, preclinical studies addressed the hypothesis that there is bilateral interaction between cardiac injury and cancer development. Understanding of molecular mechanisms of HF-cancer interaction can define the profiles of bilateral signaling networks, and identify the disease-specific biomarkers and possibly therapeutic targets. Here we discuss the shared pathological events, and some treatments of cancer- and HF-mediated risk incidence. Finally, we address the evidences on bilateral connection between cardiac injury (HF and early cardiac remodeling) and cancer through secreted factors (secretoms).

KEYWORDS

cardiotoxicity, cancer, heart failure, risk factors, mechanism, bilateral interaction, secretoms, inflammation

Introduction

Patients with cardiovascular disease have a higher risk of developing cancer

Heart failure (HF) and cancer are tightly linked (1, 2) which is supported by recent studies on epidemiological cohort and case-control, research synopsis and meta-analyses. An increased cancer risk in HF patients was shown by the international cohorts such as America (3–5), Denmark (6), Japan (7), and Korea (8). These studies

demonstrate that HF patients have a higher risk to develop cancer, independently of age (9–11). Furthermore, women with HF are at higher risk than men, indicating that gender is an important factor (3). The most common types of cancer in HF patients below age 55 are colorectal (21%), lung (18%), gastrointestinal (20%); prostate (16%) (6).

Patient with cancers have higher risk of dying from heart disease and stroke

Cancer patients can develop cardiovascular diseases (CVD) mainly for the three reasons: (1) the anticancer drugs can have direct adverse effect on cardiovascular system (2), soluble factors (secretoms) such as chemokines, hormones, and vesicles released from tumor cells can damage the cardiac cells as a paracrine manner (3), cancer itself or anticancer drugs induce cachexia that leads to cardiac dysfunction (12). As a consequence, approximately 20–30% of cancer patients die from cardiovascular dysfunctions, regardless of the time passed after cancer diagnosis (13). Indeed, 50% of patients with breast, prostate, endometrial, and thyroid cancer die because of CVD (14, 15). In the most aggressive cancer cases such as cancers of the lung, liver, brain, stomach, gallbladder, pancreas, esophagus, ovary, and multiple myeloma, patients die primarily due to cancer (16). However, the CVD -related mortality were higher among the survivals with cancer of bladder (19% of patients), larynx (17%), prostate (17%), uterus (16%), bowel (14%), and breast (12%).

Sturgeon et al. using databases of the Surveillance, Epidemiology and End Results (SEER) found that among the 3,234,256 cancer patients with 28 different types of cancer, 38% of mortality was due to cancer, whereas 11% mortality was from CVDs including hypertension, cerebrovascular disease, arterial diseases, and cardiac ischemia (17). More interestingly, the highest mortality among the younger cancer patients (< 35 years old) 1 year after the cancer diagnosis was due to CVD.

Co-occurrence of both diseases causes a major clinical burden and has a strong outcome on the quality of life and survival rates (10, 18). Early diagnosis and better understanding of bilateral interaction between HF and cancer is critical for an optimal treatment and management strategies, because a one-size-fits-all treatment approach is ineffective for these patients (7, 8).

Here we outline the known common mechanisms and preclinical studies emphasizing the interactions to help mechanistic understanding that may impact on identifying biomarkers and innovative therapeutic strategies targeting both diseases simultaneously.

Common mechanisms involved in tumor growth and heart failure

CVD and cancer share common risk factors such as smoking, aging, genetic predisposition, obesity, and diabetes mellitus (9). Indeed, cardiac regeneration and diseases are reminiscent of processes of tumor development (19). A growing body of studies have suggested that several mechanisms can be involved in development of both HF and cancer such as inflammation, metabolic remodeling, clonal hematopoiesis, angiogenesis, the extracellular matrix (ECM), and stromal cells activations (20, 21) (**Figure 1**).

Inflammation

Any changes in homeostasis by stresses, tissue damage, infection, metabolic alterations induces low-grade inflammation to achieve wound healing and tissue regeneration, and to prevent loss of tissue function (22). However, maladaptive chronic inflammation leads to progressive myocardial injury, development of vascular dysfunction, and reduces cardiac tissue survival. The cytokines chemokines, and lipid mediators are involved in inflammatory signaling. Accordingly, high circulating levels of pro-inflammatory cytokines [e.g., interleukin (IL)-1β, and IL-6] have been found in acute and chronic decompensated HF (23). Many cohort studies (> 50) have showed that both high-sensitive CRP (C-reactive protein) and IL-6 can predict development of myocardial infarction (MI) and stroke (24, 25). However, chronic inflammation predisposes to the development of cancer and affects the tumorigenesis and tumor-permissive state by promoting proinflammatory cytokines and chemokines (26). For example, IL-1β and IL-6 have also been reported as key players in development of cancer (27). Specifically, in the solid malignancies, the infiltration of immune cells and the production of pro-inflammatory mediators play key roles for malignant transformation, via epithelial to mesenchymal transition, and metastasis (28).

In the Whitehall II study cohort, Ridker demonstrated that low level of systemic inflammation detected by increased levels of CRP and IL-6 was associated with prediction of cardiovascular and cancer-related mortality in midlife patients (24). Anti-Inflammatory Thrombosis Outcome Study (CANTOS) has also confirmed this study showing that canakinumab an IL-1β-targeting antibody has beneficial effects on cardiovascular events (29). Moreover, canakinumab significantly decreased incident of mortality in patients with lung cancer.

The role of other inflammatory mediators eicosanoids, such as prostanoids (prostaglandins and prostacyclines) in cancer and CVD has not been extensively investigated yet.

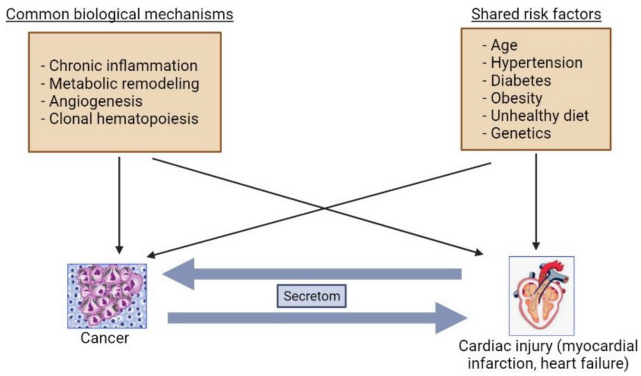

FIGURE 1
Bilateral interactions between cancer and heart failure. The common mechanisms and risk factors are involved in cancer and heart failure development. The secretoms can be involved in communications between cancer and heart failure.

For example, prostacyclin has been used to treat pulmonary arterial hypertension (30). Unfortunately, in this condition, the pulmonary cancer incidence has not been studied. In contrary, in mice model prostacyclin prevents lung cancer (31). Unlike prostacyclin, prostaglandin E_2 promotes cancer initiation and lung cancer migration (32) and activates cardiac maladaptive remodeling (33).

All together these studies show that inflammation is one of the shared mechanisms of both HF and cancer. The important question is whether diminishing inflammation can reduce the development rate of CVD and cancer. Use of anti-inflammatory drugs (e.g., low-dose methotrexate, colchicine, and canakinumab) in the large clinical trials should answer this question.

Metabolic remodeling

The healthy tissues can derive energy from various circulating substrates. However, metabolic alterations due to accumulation of toxic intermediates and utilization of unbalanced substrates can alter the cardiac cell homeostasis and cancer growth. Indeed, recent studies have shown that onco-metabolic dysregulation can promote cardiac dysfunction (34).

Metabolic reprogramming occurs as an adaptive event in both cancer (35) and cardiac cells (36) in response to pathophysiological insult and stress, indicating that both cells share the same metabolic pathways. In HF and cancer, glucose oxidation and glycolysis are central metabolic pathways to generate energy in the form of adenosine triphosphate (ATP) (37, 38). Cancer cells are dependent on aerobic glycolysis that facilitates the incorporation of nutrients into biomass such as nucleotides, amino acids, and lipids to maintain cancer cell proliferation. Aerobic glycolysis is also needed for the adaptive hypertrophy in cardiomyocytes (39). In cancer cells glutamine is the essential carbon source for aspartate synthesis (40). However, in the damaged heart glycolysis and glucose oxidation are predominant over fatty acid oxidation and are required for pentose phosphate pathway (41).

Because of the upregulated glucose utilization in many solid tumors (42), as well as in the failing heart (43), glucose transporter 1 (GLUT1) becomes an important target for the treatment of cancer and HF. Additionally, it has been shown that sodium glucose co-transporter 2 (SGLT2) inhibition has beneficial effects on the heart as well as in pancreatic and prostate cancers. Thus, inhibition of glucose transports may prevent cardiac hypertrophy (44) and reduce cancer growth (45). However, more extensive researches are required to define their beneficially effects during development and progression of different cancer types and CVDs, before their clinical applications.

De novo lipogenesis leading to lipo-expediency has also emerged as common mechanisms of HF and cancer. Upregulation of fatty acid synthase (FAS), a key enzyme of *de novo* lipogenesis, has been found in both cancers (46) and HF patients (47). FAS inhibitors have anti-neoplastic properties in solid cancers and represents a potential therapeutic target for HF.

Changes in mitochondrial metabolism is also a common mechanism of HF and cancer development. Inhibition of mitochondrial electron transport chain (ETC) complex I can decrease mitochondrial ATP delivery (48), thereby limiting glucose availability. Additional to ETC inhibitory effects, metformin has been shown to reduce plasma levels of insulin and insulin-like growth factor 1 (IGF-1) to limit glucose availability in the glycolysis-dependent cancer cells (49). In cancer cells (e.g., pancreas cancer), an increased utilization of glutamine, a major substrate for respiration, is required in supporting macromolecule synthesis and maintain the redox homeostasis to contribute cancer growth (50). Thus, inhibitors of glutaminase, a catalyzer of the conversion of glutamine to glutamate, regulate redox balance, and autophagy, induce

apoptosis *via* mTOR signaling, and promote growth arrest. On the other hand, oxidative stress upregulates glutaminase 1 and promotes glutaminolysis in the heart. Inhibition of glutaminase improves maladaptive cardiac remodeling and improves sustain activation of autophagy-mediated reduced cardiac contractility (51).

The common maladaptive metabolism pathways in cancer and HF can provide opportunities to the discovery of new biomarkers, and development of juncture strategies and therapies to battle these diseases, and may anticipate to the metabolic phenotyping of diseases in the precision medicine in the field of cardio-oncology.

Angiogenesis

Angiogenesis is involved in the pathophysiology of both development of HF (52) and cancer (53). Angiogenesis is crucial for tumor growth and metastasis (54), whereas vascular refraction in the maladaptive sustained pressure overload contributes to the transition from compensated hypertrophy to HF (55). Because of increased oxygen demand in tumor or ischemic hearts, hypoxia upregulates HIF1a that promotes expression of angiogenic factors, such as vascular endothelial growth factor (VEGF) (56), angiopoietin-1 and -2 (57), and prokineticin (58, 59) to stimulate microvascular expansion.

The pharmacological or genetic inhibition of VEGF, and other key angiogenic signaling pathways accelerate the transition from adaptive cardiac remodeling to HF (60), while anti-angiogenic therapy beneficial to cancer (e.g., metastatic colon cancer, non-small cell lung cancer, breast cancer). However, cancer cells develop adaptive resistance to the anti-angiogenic therapy as well as severe cardiotoxicity, leading to development of ischemic CVD and HF (61). Thus, angiogenesis delineates an auspicious substrate for both cancer and HF.

Clonal hematopoiesis

Genetic assets leading to hematologic malignancies such as somatic mutations in hematopoietic stem cells are the potent risk factors for CVD and cancer (62). The mutation on the genes encode for key epigenetic regulators of hematopoiesis leads to the abnormal expansion of clonally derived hematopoietic stem cells (63). A higher frequency of accumulation of hematopoietic mutations in DNA methyltransferase 3 alpha (DNMT3α), Ten-eleven translocation-2 (*TET2*), additional sex combs like 1 (ASXL1), Janus kinase 2 (JAK2), and tumor protein 53 (TP53) (64) have been found in individuals with lymphoid or solid tumors who are exposure to genotoxic stress (65). Hematopoietic mutations in *DNMT3a*, *TET2*, and $JAK2^{V671F}$, can accelerate atherosclerosis and the increase risk of CVD by generating a pool of myeloid cells with an augmented proinflammatory profile (66). These mutations are associated with worse outcomes in patients with ischemic HF (67, 68).

Identification of the mechanisms linking somatic mutation-driven clonal hematopoiesis to CVDs is off interest specifically in personalized medicine. There are some questions needs to be addressed such as whether clonal hematopoiesis also contribute to CVD in cancer survivors (69) and whether these mutations can be predictive markers of cardiovascular risk and therapeutic responsiveness.

Cardiogenetic: Cardiac-associated genetic variant to cancer predisposition

Recent studies have demonstrated that 50% of non-ischemic cardiomyopathies caused by more than gene variants encoding for cytoskeleton, ion channels, nuclear envelope, intercellular junctions sarcolemma and sarcomeric proteins (70). A genetic predisposition to therapy-induced cardiomyopathy has been observed in families with history of hypertrophic, dilated and arrhythmogenic cardiomyopathies (71). Patients with cardiomyopathy or asymptomatic carriers of inherited cardiac diseases have a potential increased risk for cardiotoxicity induced by anticancer treatment (72).

Interestingly, several of these genes associated with familial cardiomyopathies harbor relevant genetic variants in somatic cancer cells. A high prevalence of somatic mutations in Titin (*TTN*), Dystrophin (*DMD*), and Desmoglein 2 (*DSG2*) have been associated with different stages of carcinogenesis process.

The KEGG (Kyoto Encyclopedia of Genes and Genomes terms) analyses have identified the total of 33 genes and 25 links with 17 metabolic pathways that can be implicated in interaction between genetic cardiomyopathies and molecular pathways of cancer. The genes involved in both cardiomyopathy and carcinogenesis include Protein tyrosine phosphatase, non-receptor 11 (*PTPN11*) and LMNA, another 12 genes from sarcomeric (thin and thick filament), desmosomal (*PKG/JUP*), metabolic (*PRKAG2, LAMP2, GLA*), and calcium handling (*PLN*) (73). For example, the RAS family of small Guanosine Triphosphate (GTP)-binding proteins (G proteins) plays a key role in intracellular signal transduction required for normal cardiac growth, development of hypertrophic cardiomyopathy and HF as well as cancer (74). RASopathies are single-gene inheritance disorder caused by germline mutations in genes that encode constituents or regulators of the RAS/mitogen-activated protein kinase (MAPK) pathway. RASopathies are accompanied with the higher risk of hematologic or solid cancer and congenital CVD (75). For example, hypertrophic cardiomyopathy development during childhood can be triggered by genetic mutations in *PTPN11*, KRAS, Son of

sevenless homolog 1 (*SOS1*), a RAS effector (*RAF1*), genes which are also involved in cancer developments (73).

The signaling pathway of wingless-related integration site (Wnt) controls proliferation and differentiation processes in different types of cancer. Indeed, in patients with desmosomal mutations on the Wnt pathway exhibit the histological fibro-adipose differentiation, a characteristic of arrhythmogenic cardiomyocardiopathy (76).

Additional mechanistic studies on damaging gene variants in CVD and cancer can unravel prognostic biomarkers and new treatment strategies for both disorders.

Cardiovascular drug: Promoter or suppressor of cancer incidence?

HF results in the hyperactivity of neurohormonal systems, including the renin–angiotensin–aldosterone (RAA) system and the sympathetic nervous system (77). Interestingly, noradrenaline and angiotensin II also play an important role in modulation of tumor microenvironment and tumor development (78). Contrarily, several studies demonstrated that patients treated with angiotensin-converting enzyme (ACE) inhibitors but not angiotensin receptor blocker (ARB) for more than 5 years have a higher lung cancer incidence (79). However, subgroup analysis has demonstrated a significant association between ARB and cancers in male genital organs (80). In contrary, the recent study demonstrated an association between the ARB and decreased risk of overall cancer and several site-specific cancers (81). Patients treated with hydrochlorothiazide, a diuretic drug, had a higher prevalence to have basal and squamous cell carcinoma (82). A large meta-analysis on hypertensive patients treated with all types of anti-hypertensive drugs (ARBs, ACEi, β-blockers, diuretics, and calcium channel blockers), demonstrated a 5.0–10.0% increase in the risk of cancer or cancer-related death (83). ARB use in patients with type 2 diabetes demonstrated a negative association for losartan (ARB), but a positive association for candesartan and telmisartan with the overall occurrence of cancer (84). Despite some contradictory conclusions about ARB mediated cancer risk, a recent study considering the exposure-risk relationship and using data from all 15 trials and randomized controlled trials has resulted in a very significant correlation between the degree of cumulative exposure (greater than 3 years) to ARBs and risk of all cancers especially lung cancers (85). In this study, patients with lower cumulative exposure to ARBs did not exhibit an increased risk of all cancers combined or lung cancer, explaining the heterogeneity in the results of randomized trials, due to terms of cumulative exposure to ARB (**Figure 2**).

The other cardiovascular drug is aspirin that leads to antiplatelet effects *via* inhibition of platelet cyclooxygenase (COX-1) and blockade of the production of thromboxane A2. Aspirin uses both cyclooxygenase-dependent and cyclooxygenase-independent mechanisms in cancer (86). Low-dose aspirin did not lower the cancer incidence in a low or medium CVD risk population (87, 88). The U.S. Preventive Services Task Force (USPSTF) recommended that use of low dose aspirin can reduce risk of CVDs and colorectal cancer among the people at the age of 50–60 (89). Thus, these benefit effect of aspirin may not translate to older adults (90). Currently, the mechanism of beneficial effects of aspirin is not known. The appropriate pre-clinical models are emerging to discover the molecular and cellular mechanism aspirin and ARBs in patient with cancer and CVD (**Figure 2**).

Pre-clinical models to study bilateral interaction between heart failure and cancer

Despite most of epidemiological studies showed high prevalence of cancer development in HF patients, they cannot prove direct interconnection between HF and cancer. There are some preclinical studies aimed to explore the bilateral relationship between HF and cancer development.

Cancer to heart failure

Cancer or anti-cancer drug-mediated cachexia and cancer-secretoms, adverse effects of anticancer drugs may induce several organ dysfunctions and HF as outlined in **Figure 3**.

Cancer cachexia promotes cardiac atrophy

Cachexia is defined as a state of involuntary weight loss. The symptoms of cancer cachexia such as fatigue, shortness of breath, and impaired exercise capacity are also symptoms of HF. Cachexia prompts metabolic changes in the metabolisms of lipids and proteins, leading to a negative nitrogen balance and reduction of the protein levels, causing insulin resistance, and anemia. Cachexia affects approximately 32% of cancer patients within half a year at the time of diagnosis, and causes one third of cancer deaths (91). Patients with pancreatic, gastro-oesophageal, lung, head and neck and colorectal cancers often have cachexia with a prevalence between 40 and 70% (92). It also co-occurs with metastasis in 80% of late-stage cancer patients (93).

The mechanism of cancer cachexia is not known, but Indeed, cancer patients, with and without cachexia exhibit the high levels of serum brain natriuretic peptide (BNP), renin and aldosterone (94). These altered levels of hormones increase sensitivity to infections, and decrease responsiveness to both chemotherapy and radiation treatment. They also cause loss of muscle protein that promotes muscle weakness and fatigue, and cardiac or respiratory failure (95). Moreover, the heart becomes atrophic in cancer patients. There are also several direct experimental evidence showing that cancer-mediated

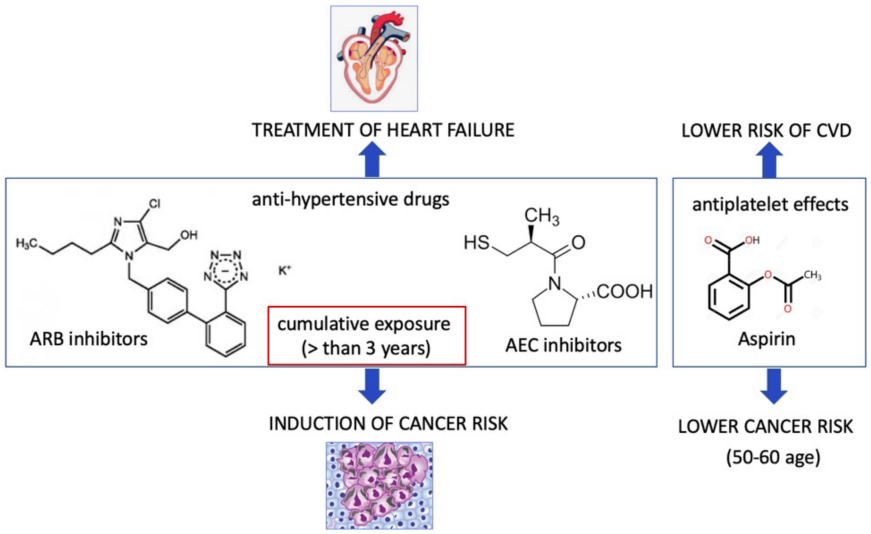

FIGURE 2
Cardiovascular drugs may promote or suppress cancer incidence. Angiotensin receptor II blocker that are used for treatment of hypertension at the cumulative exposure more than 3 years may induce cancer risk. Aspirin may lower cancer risk.

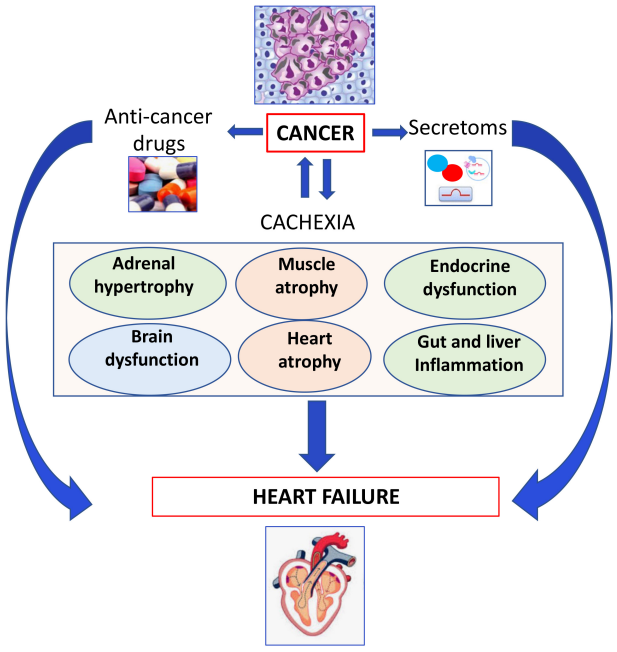

FIGURE 3
Role of cachexia in development of heart failure. Cancer or anticancer drug-mediated cachexia may induce several organ dysfunctions leading to heart failure.

muscle wasting and cachexia (96) can be key players of cancer-related death. Cancer itself can result in cardiac atrophy as well (97).

Cancer cachexia-mediated a waste of skeletal muscle and adipose tissue has been observed more severely in men more than women. The mechanisms of the sex differences in cancer cachexia have been experimentally studied by Cosper and Leinwand (98). In this study the tumor bearing (CD2F1) mice with injection of Colon-26 adenocarcinoma (C-26) has displayed a rapidly and increasingly loss of cardiac mass during the course of tumor progression. Significant differences in the disease phenotype have also been observed between males and

females. For example, male mice exhibit more waste in body weight, skeletal, and cardiac muscle and the higher cardiac dysfunctions than females. The decrease in all myofibrillar proteins, at the expenses of myosin heavy chain (MyHC) in heart is due to autophagy as a main proteolytic pathway. In this study, the estrogen receptor signaling has been shown to protect females against the loss of body weight and cardiac mass. Indeed, it appears that activation of Ca^{2+} dependent atrophy is also involved in wasting in skeletal muscle and heart of male Wistar rat bearing Yoshida AH-130 ascites hepatoma cells for 6 days as a cancer cachexia models (99).

Springer's group using the rat hepatoma model (AH-130-bearing rats) demonstrated that weight loss affects predominantly skeletal muscle and myocardium associated with left ventricular-dysfunction, fibrotic remodeling, and increased mortality (100). They found that several key anabolic and catabolic pathways were dysregulated in the cachectic hearts. These detrimental effects of the tumor on the heart and on survival can be alleviated by treatment with the β-blocker bisoprolol or the aldosterone antagonist spironolactone (101). Incoherent to this study, Toledo et al. have shown that the administration of a highly potent β2-adrenoceptor-selective agonist, formoterol, to cachectic tumor-bearing rats caused a significant reduction of muscle weight loss, an increase in lean body mass probably due to preventing muscle apoptosis and the increased muscle regeneration (102). The clinical trial phase 2 has shown *f*ormoterol has beneficial effects in patients with advanced cancer (103). Costelli et al. in AH-130-bearing rats have shown that β2-adrenoceptor agonists, clenbuterol, prevents skeletal muscle waste, however, it has no effect parenchymal organs (104).

Same group has shown that the mechanisms of muscle depletion is due to increased proteasome- and calpain-dependent proteolysis in tumor bearing rats treated with TNF-alpha synthesis inhibitor, or an antiprotozoal drug blocking the IL-6 and TNF-alpha action (105).

A variety of cytokines have also been proposed to trigger cancer-induced cardiac muscle wasting. Zhou X 's group has studied involvement of a high affinity activin type 2 receptor (ActRIIBa), that binds to TGF-β family ligands (myostatin, activin, Growth differentiation factor 11), utilizing two animal models; the tumor-bearing mice (colon 26, human G361 melanoma and TOV-21G ovarian carcinoma) and inhibin-knockout mice (106). Indeed, activation of ActRIIB pathway enhances ubiquitination of muscle proteins that are key pathways in muscle wasting. Indeed, inhibition of the ActRIIBa in this study has fully restored the loss of muscle during cancer cachexia, without altering the high levels of the inflammatory cytokine levels.

The emerging question in this area is whether animal models consistent with clinical cancer cachexia and the potential drugs can have beneficial effects in cancer patients (107).

Cancer cells derived secreted factors (secretoms) promote cardiac atrophy

Cancer cells release secreted factors (secretoms) result in developing cardiac atrophy and metabolic changes, but the exact signaling pathways in cardiomyocytes are still poorly understood (108). Cancer cells-mediated systemic metabolic alterations may impair cardiac function (39). Alterations in metabolic fueling of the heart as well as metabolic intermediates can alter gene expression, protein function and provoke epigenetic modifications thereby stemming in ventricular remodeling.

Somatic mutations in metabolic regulator genes could be the mechanism of cancer-mediated cardiac dysfunction. For example, somatic mutation in dehydrogenase (IDH1/2) gene causes in a gain-of-function, thereby allowing synthesis of 2-hydroxyglutarate (2-HG) that is structurally similar to α-ketoglutarate (α-KG), a key regulatory enzyme of cellular energy metabolism and an intermediate of the tricarboxylic acid (TCA) Krebs cycle (109). An increased circulating levels of 2-HG triggers dilated cardiomyopathy and contractile defects by impairing α-KG pathway, and leading to mitochondrial damage and myocardial glycogen accumulation (39).

More studies are required to identify novel factors that can be important to stratify the risk of development of CVD in cancer patients.

Anti-cancer-induced cardiotoxicity

The anticancer-drug induced cardiotoxicity has been widely studied, which can occur during, shortly after, or many years after cancer therapy. Cardiotoxicity can range from subclinical myocardial dysfunction to irreversible HF. Thus, in the long-term, the risk of death can be due to cardiovascular dysfunctions rather than tumor recurrence (39–41). The cancer therapy related-cardiovascular complications are listed in **Figure 4** and **Table 1** (110).

One of the important reasons for cardiotoxicity is that the anticancer drugs use similar pathways and targets in both cancer and heart cells to exert their cytotoxic effects as described in **Figure 5**. The anthracycline group of anticancer drugs use the same signaling pathways to induce cytotoxicity in both cancer and cardiac cells, leading to HF-related morbidity and mortality. The mechanism of anthracycline-mediated cardiotoxicity has been widely studied and recently reviewed by Nebigil and Désaubry (111). Targeted therapies such as tyrosine kinase inhibitors have also adverse effects on cardiovascular system. For example, heregulin receptor, HER2, express in both cancer and cardiac cells. Inhibition of HER2 by antibodies blocks the cancer cell proliferation, but it also blocks an important survival pathway in heart (112). Binding of VEGF to its receptors in endothelial cells activates angiogenesis. VEGF inhibitors can destroy the tumor angiogenesis and bring the tumor to avascular stage, whereas it can be detrimental in the heart due to reduced systemic angiogenesis (113).

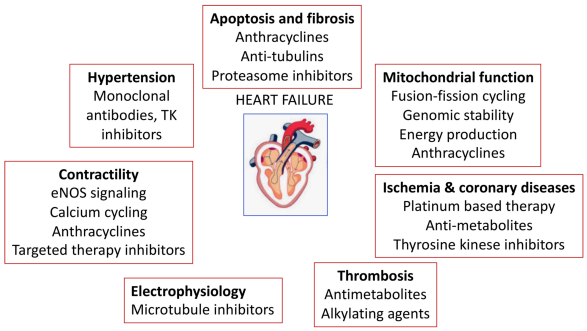

FIGURE 4
Anticancer drugs-mediated cardiac adverse effects. Anticancer drugs can induce hypertension, cardiovascular damage, ischemia and coronary diseases, thrombosis, contractility defects and arrhythmias.

TABLE 1 Some of the anticancer drugs-mediated adverse effects in cardiovascular system.

Anticancer drugs	Clinical manifestation
Anthracyclines (e.g., doxorubicin)	LV-dysfunction, contractile defects, ischemia, thromboembolism
Targeted therapy/TK inhibitors (e.g., bevacizumab, sorafenib, nilotinib) and anti-angiogenic therapy (e.g., VEGF inhibitor)	Hypertension, Bradycardia, QT-prolongation, contractile defects, Ischemia, and coronary diseases, venous thromboembolism
Immune check point inhibitors (e.g., ipilimumab, nivolumab)	Myocarditis/pericarditis

Checkpoint inhibitors induce T-cell activation. Monoclonal antibodies that are used to block immune inhibitory checkpoints target cytotoxic T-lymphocyte antigen 4 (CTLA-4 (ipilimumab, nivolumab, pembrolizumab), or anti-programmed cell death 1 (PD1) (atezolizumab, avelumab, durvalumab). The mechanisms of the adverse effects of immune checkpoints inhibitors are (1) release of cytolytic molecules (e.g., tumor necrosis factor-α, granzyme B, interferon-γ) that kill tumor cell and promote autoimmune lymphocytic myocarditis, (2) the PD-L1 and CTLA-4 are also expressed in heart and tumors and can share antigens that recognize by the same T-cell clones. Thus, activated T cells attack not only on tumor cells but also on cardiomyocytes, and destroys hearts (114).

Thus, the paracrine effect of anticancer drugs-mediated cardiotoxicity remains to be widely investigated.

Heart failure to cancer

Myocardial infarction mediated secretoms and innate immune system promotes cancer development

Kitsis et al. investigated the interaction between HF and cancer by creating MI-induced HF in a precancerous murine model, adenomatous polyposis coli $(APC)^{-/-}$mouse strain (115). These mice carried a non-sense mutation in *APC* leading to persistence of beta-catenin that induces spontaneous intestinal adenoma formation (1). The experimental HF on those mice resulted in increased tumor formation and tumor growth, 6 weeks after the MI procedure. To rule out the possibility that hemodynamic impairments lead to tumor growth, the failing heart has been transplanted into these precancer mice as a heterotopic murine (HFTx) heart model. Indeed, this model further proved that HF can contribute to tumor formation and progression. The candidate secreted molecules of HF were then identified based on meta-analyses from databases of proteins secreted from myocardium, and allied to the proteins previously associated with new-onset colorectal cancer. Five potential secreted proteins have been discovered, namely: α-1-antitrypsin (SerpinA1), α-1-antichymotrypsin (SerpinA3), fibronectin, ceruloplasmin, and paraoxonase 1. Indeed, serpinA3 promoted proliferation of the colon cancer cell that was associated with Akt-S6 phosphorylation. Moreover, Kitsis et al. found that the increased levels of serpinA3 and A1, fibronectin, ceruloplasmin, and paraoxonase 1 in patients with chronic HF (115).

Further studies need to establish whether (1) these secretoms can be used as cancer biomarkers to stratify the cancer risk in HF patients, (2) these secretoms can promote tumor formation as well as tumor progression.

The possible role of the immune system in HF to cancer has also been off great interest. Indeed, monocytes and monocyte-derived macrophages play key roles in cancer, such as promoting angiogenesis, tumor cell proliferation, migration, invasion as well as tumor immune evasion. On the other hand, post MI provokes sympathetic signaling such β3 adrenergic stimulation together with IL-1β) release, thereby activating leukocyte progenitors in the bone marrow, and monocytes, in the circulation (116). Recently using 2 mice model of breast cancers: C57BL/6J female mice orthotopically

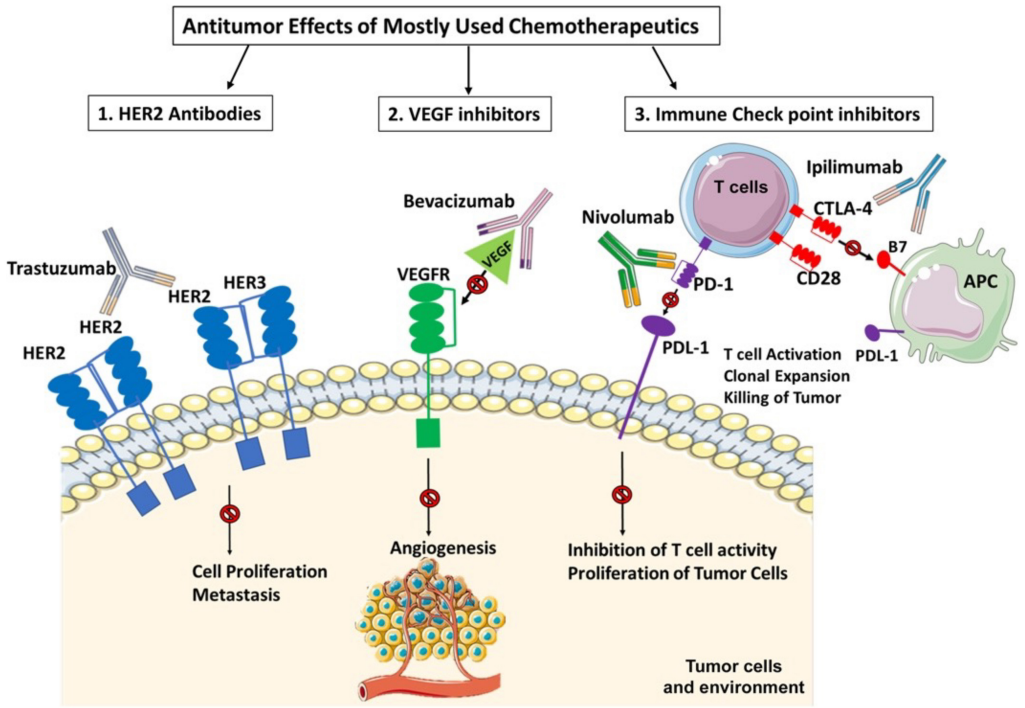

FIGURE 5
Three widely used anticancer drug mediated cytotoxicity in cancer and heart. Clinically used targeted therapies/tyrosine kinase (TK) inhibitors mainly focuses on inactivation of heregulin receptor (HER2) or vascular endothelial factor (VEGF) signaling in cancers. HER2 signaling is important in heart and cancer. HER2 activation by its ligand heregulin stimulates proliferation pathway in the tumor cells. The inhibition of HER2 by its antibody, trastuzumab, in cancer cells blocks the cancer proliferation but in heart it blocks an important survival pathway in heart. Binding of VEGF to its receptors in endothelial cells activates angiogenesis. Inhibition of VEGF by its antibody, bevacizumab, can destroy the tumor angiogenesis and bring the tumor to avascular stage, whereas it can be detrimental in the heart due to reduced systemic angiogenesis. Indeed, the immunotherapy targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4). anti-programmed cell death 1 (PD1) or its receptor (PDL-1) relies on cancer destruction through the activation of the host immune system. However, PD-L1 is also expressed in the non-immune cells to maintain self-tolerance. Nivolumab and Ipilimumab activate T cells and promotes T cell clonal expansion to kill tumor cells. However activated T cells also recognize shared antigens and destroy cardiomyocytes as well.

implanted the murine mammary cancer cell line (E0771) and genetically engineered mouse breast cancer model (MMTV-PyMT), Koelwyn et al. demonstrated that MI is an acute pathologic stimulus that induces the innate immune system, to accelerate breast cancer growth and metastasis as well as cancer-associated mortality in mice and humans (116). More specifically, MI epigenetically reprograms Ly6Chi monocytes to give rise to an immunosuppressive phenotype in the bone marrow. In parallel, MI increases circulating levels of Ly6Chi monocytes and recruitment of these monocytes to tumors. Furthermore, depletion of these cells abolishes MI-induced tumor growth. Interestingly, epidemiological studies showed that early stage breast cancer patients who had cardiovascular disorders before the treatment with the chemotherapeutics had increased risk of reappearance of tumor and cancer-specific death.

These preclinical and clinical results are important to understand host comorbidities and their impact on cancer progression.

Transverse aortic constriction mouse model of pathological hypertrophic cardiomyopathy promotes cancer

Avraham et al. investigated whether pathologic hypertrophic cardiomyopathy can alter tumor growth and progression, using transverse aortic constriction (TAC) as a mice model of pressure overload–induced cardiac hypertrophy in 2 type mouse syngeneic tumor models: a breast orthotopic cancer model (mouse mammary tumor virus–polyomavirus middle T antigen) and a lung cancer model (Lewis lung carcinoma) (117). This experimental TAC resulted in increased tumor growth and metastatic colonization. After TAC, the tumor implanted mice had a cardiac hypertrophy, and increased volume of tumors. Avraham et al. also investigated the role of host immune system in TAC-associated an increase in tumor growth, using immunodeficient NOD/SCID mice, which lack T and B lymphocytes, with reduced natural killer cell function. Indeed, increased tumor growth after TAC was also observed, eliminating the possible role of the adaptive immune system.

Transcriptomic profiling of the mice TAC-hearts revealed a number of upregulated secretoms known as pro-tumorigenic, such as connective tissue growth factor and periostin. Indeed, depletion of periostatin from the sera of TAC-mice abolished the proliferation of polyomavirus middle T antigen cells. Exogenous addition of periostin increased polyomavirus middle T antigen and Lewis lung carcinoma cell proliferation *in vitro*, showing that periostin in sera of the TAC-operated mice plays key role in cancer cell proliferation. Unfortunately, there was no studies showing that periostin inhibition *in vivo* reduces the tumor growth. Future *in vivo* studies are necessary to determine whether periostin and other secreted factors, promote hypertrophic cardiac remodeling-mediated tumorigenesis.

The mechanisms by which these secretoms exert tumorigenic effect need to be studied. These preclinical studies may open an avenue for the discovery of the heart-specific tumor markers and new therapeutic options.

Perspective

Recent clinical studies have shown that the mortality of certain cancer patients results from CVD such as HF, hypertension aneurysm of blood vessels and stroke (118). The risk of CVD mortality occurs during an acute phase (early risk) and a chronic phase (late risk) (119). Accordingly, cancer survivors who were diagnosed cancer before the age of 55 years displayed ten-fold higher CV-dependent mortality compare to the general population (118). On the other hand, the cancer risk incidence is high in the patients with HF (120).

Several limitations of the clinical studies showing a link between cancer and HF should be considered as well. For example, in some clinical studies the type of treatments the patients received have not been known to evaluate whether these therapies have adverse effects. Some of these studies have lack of information on co-illnesses and risk factors (e.g., smoking, alcohol consumption, obesity). The socioeconomic status should also be taken in account in these epidemiological studies. Moreover, these studies have been mostly performed on western population, therefore the percentage of risks may range in the different populations.

Preclinical studies are important to unravel the molecular pathways and targets involved in bilateral interaction between CVD and cancer. Cardiac- and cancer-secretoms have potential utility as biomarkers that can be used to identify risks in patient with HF in terms of cancer risk or vice versa. These data will exemplify the importance of understanding the development of comorbidities and help to implementation of strategies for better management of these patients and identify the cardiovascular or cancer-specific mortality.

Nevertheless, the cardio-oncology care should assess regularly the risk of development cancer in HF patients, and CVD in the cancer patients before starting chemotherapy.

Author contributions

MG and NT prepared the illustration, organized and partially wrote the manuscript. MS-M, JG, and CN wrote and edited the manuscript. 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

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Effects of the Chinese herbal medicine Hong Huang decoction, on myocardial injury in breast cancer patients who underwent anthracycline-based chemotherapy

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Objective: To assess the effects of Hong Huang Decoction (HHD), a Chinese herbal medicine, on myocardial injury in breast cancer patients who underwent anthracycline (ANT)-based chemotherapy.

Methods: A total of 51 patients with breast cancer who underwent an ANT-based chemotherapy program and met the inclusion/exclusion criteria were allocated to the treatment or placebo groups using a random number generation process. Patients in the treatment group received liquid HHD twice a day. Treatment was given from 1 day prior to chemotherapy up to the end of chemotherapy (after 6 months). Participants in the placebo group received a placebo over the same schedule. Left ventricular ejection fraction (LVEF), global longitudinal strain (GLS), diagnostic markers of acute myocardial infarction [e.g., lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), and B-type natriuretic peptide (BNP)], nitric oxide (NO), superoxide dismutase (SOD), as well as pro-inflammatory cytokines [e.g., tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and human C-reactive protein (CRP)], and anti-inflammatory cytokine interleukin-10 (IL-10), were outcome measures assessed before chemotherapy, 3 and 6 months after chemotherapy.

Results: Compared to the placebo group, the GLS value was significantly higher in the treatment group (19.95 ± 1.16 vs. 19.06 ± 1.64, $P \leq 0.001$). Significant differences were also noted for levels of SOD (689.71 ± 203.60 vs. 807.88 ± 182.10, $P < 0.05$), IL-6 (58.04 ± 22.06 vs. 194.20 ± 40.14, $P \leq 0.001$), IL-10 (237.90 ± 94.98 vs. 68.81 ± 32.92, $P \leq 0.001$), NO (75.05 ± 26.39 vs. 55.83 ± 19.37, $P \leq 0.005$), and TNF-α (301.80 ± 134.20 vs. 680.30 ± 199.60, $P \leq 0.001$) in the patients before chemotherapy compared to 6 months after initiating chemotherapy.

Conclusion: HHD regulated the levels of IL-6, IL-10, SOD, NO, and TNF-α. The results demonstrated that GLS is a better indicator of early myocardial injury compared to LVEF, and HHD could modulate oxidative stress to protect against ANT cardio toxicity.
Clinical trial registration: Chinese Clinical Trial Registry, identifier ChiCTR1900022394. Date of registration: 2019-04-09.

KEYWORDS
breast cancer, chemotherapy, anthracycline, Hong Huang decoction, GLS

Introduction

Breast cancer is one of the leading causes of morbidity and mortality worldwide (1). Advances in early diagnosis and treatment of breast cancer have contributed to the steady increase in the number of cancer survivors. Nonetheless, such effective cancer therapies have led to a noticeable increase in cardiovascular complications in a significant proportion of cancer survivors (2). Furthermore, cardiovascular complications have become the most common cause of death in breast cancer patients (3). As such, during comprehensive treatment of breast cancer, cardiotoxicity resulting from the chemotherapy agents and targeted therapies are worthy of further investigation. Anthracyclines (ANTs), such as daunorubicin, doxorubicin, idarubicin, and epirubicin, are chemotherapeutic agents used to treat diverse types of cancer (4). Oxidative stress plays an important role in cardiotoxicity induced by antineoplastic drugs and participates in toxic reactions (5). The mechanisms underlying ANT-induced cardiotoxicity are complex, multifactorial, may involve genetic mutations and continue to be a mystery. The most well-known mechanism is the generation and accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that account for lipid peroxidation and DNA damage in cardiomyocytes. These cells are particularly susceptible to free radical damage due to a deficiency in antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) or glutathione peroxidase (GSH-Px) (6, 7).

Cardiac ultrasound or echocardiography is routinely employed to evaluate cardiac function in cardiovascular disease (8). Speckle-tracking echocardiography (STE) is a modern, well-validated, and reproducible method of assessing left ventricular (LV) longitudinal deformation and providing a sensitive assessment of myocardial contractibility (9). Laboratories mainly record LV strain patterns in the long axis and use LV global longitudinal strain (GLS) calculated as the average from all segments, as a measure of global LV function (10).

Traditional Chinese medicine (TCM), with a history of thousands of years of clinical practice, has noticeably attracted the attention of clinicians (11, 12). Hong Huang Decoction (HHD) is a type of TCM composed of *Rhodiola rosea*, astragalus, turmeric, and rhubarb and is able to suppress oxidative stress *in vivo* (13). Flavonoids in traditional Chinese medicine can inhibit inflammatory factors such as IL-6 and reduce inflammatory responses (14). Moreover, Emodin can inhibit the inflammatory response enhancing cholesterol efflux, destroying lipid valves as well as inhibiting proinflammatory factors and chemokines (15). Our previous study revealed that HHD improves cardiac function in breast cancer patients undergoing chemotherapy (16). The composition and efficacy of HHD are shown in Table 1.

The present study aimed to assess the effects of HHD on myocardial injury in breast cancer patients who had undergone chemotherapy with ANT.

Methods

Study design

This randomized placebo-based trial was conducted in accordance with the CONSORT-SPI 2018 checklist. Eligible patients were randomized to either the HHD group or placebo group.

Study subjects

Inclusion criteria

i) Female patients who were diagnosed with breast cancer based on pathology;
ii) Patients aged between 18 and 70 years old;
iii) Normal cardiac function defined as left ventricular ejection fraction (LVEF) > 55% and New York Heart Association (NYHA) functional class I; cardiac troponin levels within the normal range;
iv) Patients who received at least 4 sessions of ANT chemotherapy.

Exclusion criteria

i) Patients who were not recommended for chemotherapy owing to various underlying problems;
ii) Patients who participated in other clinical trials;

TABLE 1 HHD composition.

Name (botanical, common PinYin names), traditional daily dose (grams)	Active compounds	Clinical and pharmacological effects	Adverse effects/toxicity
Rhodiola crenulata, Root of Kirilow Rhodiola, Hong jingtian, 3–10 g	Salidroside were detected in Rhodiola (17)	In a clinical study of 60 breast cancer patients, Zhang found that salidroside can provide a protective effect on epirubicin-induced early left ventricular regional systolic dysfunction in patients with breast cancer, and this effect may be induced by antioxidants (18)	No reported adverse events
Rheum Palmatum L., Rhubarb root and rhizome, Da Huang, 3–15 g	Aloe emodin, Rhein. Emodin, chrysophanol are introduced (19)	It has anti-microbial and anti-oxidative effects (20, 21)	The toxicity includes hepatotoxicity and nephrotoxicity. No adverse reactions were observed in mice with dose of 400 mg/kg (22)
Curcuma Longa L., Turmeric, Jianghuang, 3–10 g	The Turmeric contains curcumin (23)	An myocardial injury model in mice (*in vivo*) was established by He et al. the cardioprotective effects of Cur determined by its antioxidation (24)	No reported adverse events
Astragalus Membranaceus (Fisch.) Bge., Astragalus root extract, Huangqi, 9–30 g	Astragaloside iv and Pistil isoflavone glucoside were detected in AM (25)	Anti-inflammatory and anti-oxidative effects was observed in AM (26) As an antioxidant, attenuates DOX-induced cardiomyopathy through the suppression of NADPH oxidase (27)	No reported adverse events

iii) Pregnant and lactating women;
iv) Patients who did not sign the written informed consent form;
v) Patients with a history of alcohol or drug abuse.

Interventions

All patients received 4–8 rounds of chemotherapy with ANT. The ANT dose was administered based on the patient's body surface area and on the day of treatment, antacid, and antiemetic drugs (e.g., omeprazole) were routinely given. In addition, guided by results from routine blood tests as well as liver and kidney function tests, other required medications may have been prescribed. The chemotherapy protocols and management of adverse events unrelated to cardiac toxicity were in accordance with the Guidelines of the Chinese Society of Clinical Oncology (CSCO) on Diagnosis and Treatment of Breast Cancer (28).

Preparation of HHD

HHD was acquired from Jiangsu Provincial Hospital of TCM (Nanjing, China). The total weight of the crude herbs was 56 g. The herbs were blended in 400 mL of double-distilled water (1:8, w/v) for 1 h and heated at 100°C for 2 h. After continuous boiling for 2 h, the sample volume was reduced to 200 mL. Subsequent preparation steps were completed at Jiangsu Provincial Hospital of TCM. Each patient ingested 200 mL HDD as a split dose, twice daily during the whole chemotherapy period. HHD and placebo were provided in liquid form in similar non-transparent bags to ensure the patients were not privy to which treatment was being administered apart from a potential difference in taste.

Outcome measures

Primary outcome measures

Two-dimensional speckle tracking echocardiograph (STE) was performed with the GE Vivid E9 system (General Electric Healthcare, Milwaukee, WI, USA). LVEF was assessed by two-dimensional echocardiography using the modified Simpson's method (29). LVEF was measured at three time points (before chemotherapy, 3 months after the first round of chemotherapy, and 6 months after the first round of chemotherapy).

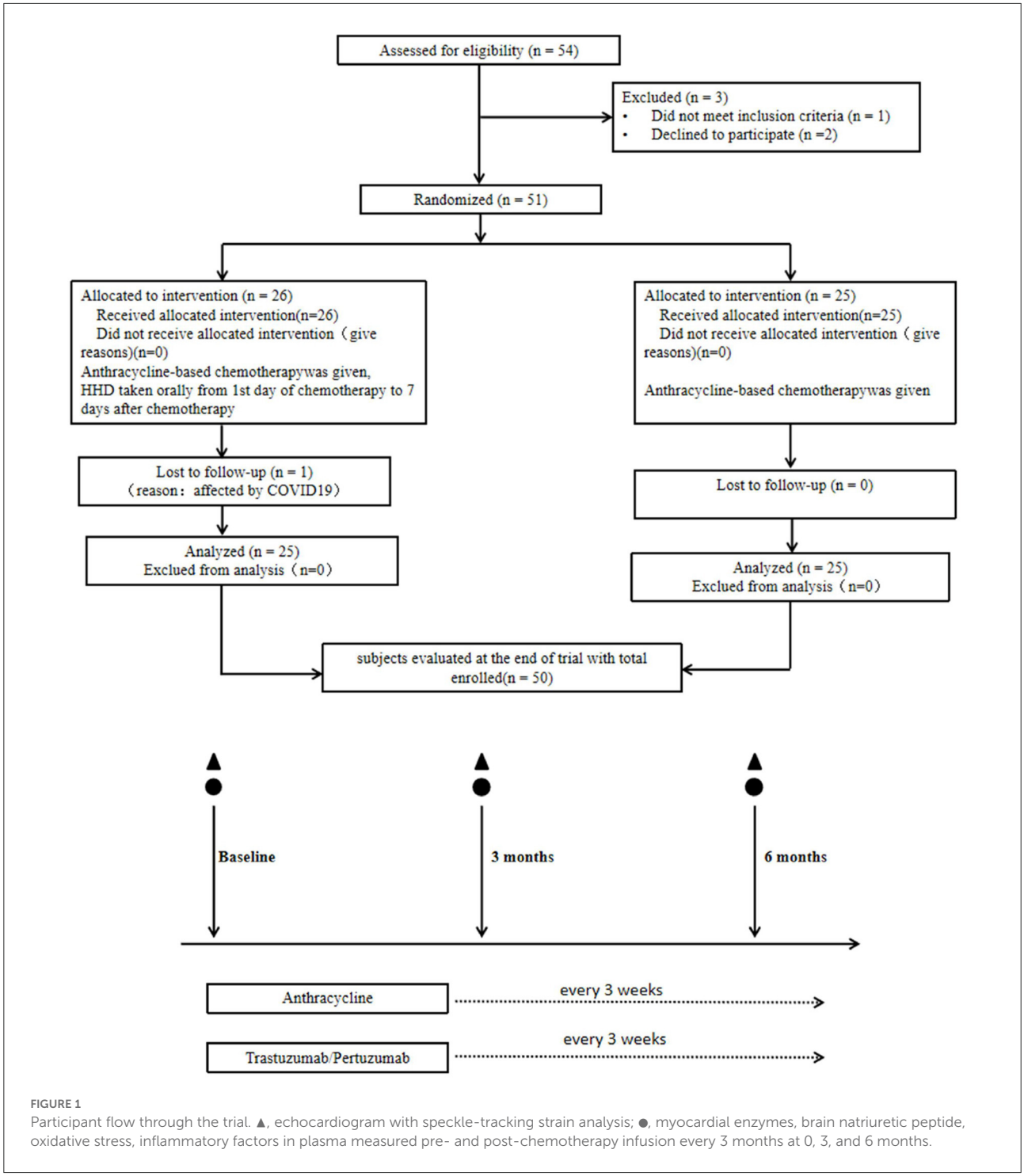

FIGURE 1
Participant flow through the trial. ▲, echocardiogram with speckle-tracking strain analysis; ●, myocardial enzymes, brain natriuretic peptide, oxidative stress, inflammatory factors in plasma measured pre- and post-chemotherapy infusion every 3 months at 0, 3, and 6 months.

Secondary outcome measures

GLS was measured with the above STE using the GE Vivid E9 system. After LVEF measurements were completed, the sector angle was adjusted and the settings optimized. The apical four-chamber section was selected and continuous three-dimensional dynamic images of four cardiac cycles were collected. The three-dimensional strain speckle tracking analysis software automatically calculated the GLS based on the collected images.

TABLE 2 The general information of recruited patients.

General data		Treatment group	Placebo group	*P*-value
Age (year)		48.5	47.5	0.652
Tumor size	≥2 cm	16	15	0.771
	<2 cm	9	10	
Neoadjuvant chemotherapy		7	8	0.785
Operation mode	*SM + SNLB	11	11	0.885
	*MRM	11	12	
	*BC	3	2	
Lymphatic metastasis (number)	0	13	12	0.943
	1–3	6	6	
	≥4	6	7	
Targeted therapy		4	4	1
Chemotherapy regimens	#EC-T	6	6	0.948
	#EC	9	8	
	#EC_{2W}-T	10	11	
Radiotherapy		10	10	1

*SM + SNLB, simple mastectomy + sentinel lymph node biopsy; MRM, modified radical mastectomy; BC, Breast-conserving surgery.
#Patients receive either four cycles of EC (Epirubicin 90 mg/m^2; Cyclophosphamide 600 mg/m^2, day 1, every 3 weeks), or eight cycles of EC-T (four cycles of Epirubicin 90 mg/m^2; Cyclophosphamide 600 mg/m^2, day 1, every 3 weeks, followed by four cycles of docetaxel 75 mg/m^2, day 1, every 3 weeks), or eight cycles of EC2W-T (four cycles of Epirubicin 90 mg/m^2; Cyclophosphamide 600 mg/m^2, day 1, every 2 weeks, followed by four cycles of docetaxel 75 mg/m^2, day 1, every 2 weeks).

Measuring diagnostic markers of acute myocardial infarction

Blood samples (5 ml) were extracted 1 day before undergoing chemotherapy and again at 3 and 6 months after initiation of chemotherapy. The samples were subjected to measurements of diagnostic markers for acute myocardial infarction which were lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB) and B-type natriuretic peptide (BNP).

Detection of the levels of inflammatory cytokines

The blood samples were also drawn in blood collection tubes without anticoagulant and sera were prepared by centrifugation at 500 g for 10 min at room temperature. The sera samples were then analyzed for levels of inflammatory cytokines. Serum levels of nitric oxide (NO) and SOD were measured at 550 nm using the Shimadzu-UV-160 spectrophotometer (Shimadzu Corp., Tokyo, Japan) and levels of NO and SOD were calculated. Levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10) and human C-reaction protein (CRP) in the sera were evaluated by enzyme-linked immunosorbent assay (ELISA; Multisciences (Lianke) Biotech Co. Ltd., Hangzhou, China).

Randomization

Patients were randomly assigned to study groups by non-stratified randomization. The random sequence was generated using the random number function of the Microsoft® Excel software. The randomization list was kept on a password-secured computer. All patients, study site personnel, raters, and contract research organization staff members were blinded to the group assignment.

Ethical statement and informed consent

The research protocol was approved by the Institutional Review Board of the Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (Nanjing, China; Approval No. 2018nl-164-02). From April 2019 to December 2020, patients who were admitted to the breast cancer department of our hospital were screened and enrolled in the current study. All the eligible patients signed the written informed consent form prior to commencing the study. The research protocol was registered at the Chinese Clinical Trial Registry (Reg. No. ChiCTR1900022394).

Statistical analysis

Data obtained from the samples collected over different time points were compared using the Student's *t*-test, and differences were compared using the *t*-test or the Student's *t*-test where appropriate, based on the data distribution. Categorical variables were expressed as percentages, and differences were compared using the chi-square or Fisher's exact test, where appropriate, based on the expected counts. All statistical analyses were performed using the SPSS 17.0 software (IBM Corp., Armonk, NY, USA).

TABLE 3 Echocardiographic measurements ($\bar{X} \pm$ SD) (%).

Indices	Groups	Before	3 months		6 months	
LVEF	Treatment	66.28 ± 3.02	66.26 ± 2.41	0.970	64.96 ± 2.69	0.094
	Placebo	66.76 ± 1.95	65.68 ± 2.34	0.045	65.48 ± 3.12	0.060
	P-value	0.596	0.392		0.531	

The values in the fifth column mean differences between 3 months after the start of chemotherapy compared to before chemotherapy; the values in the seventh column mean differences between 6 months after the initiation of chemotherapy relative to values before chemotherapy.

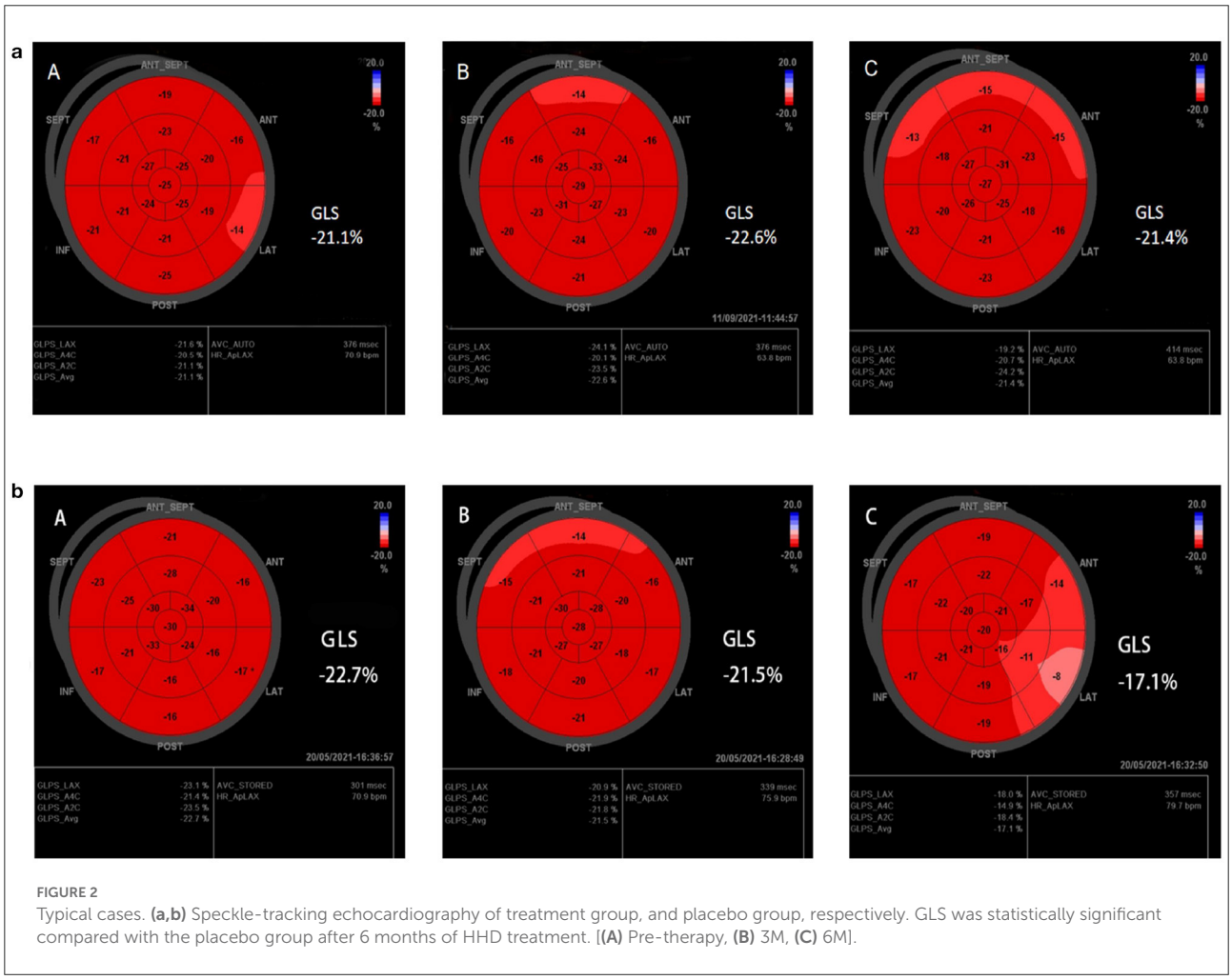

FIGURE 2
Typical cases. **(a,b)** Speckle-tracking echocardiography of treatment group, and placebo group, respectively. GLS was statistically significant compared with the placebo group after 6 months of HHD treatment. [**(A)** Pre-therapy, **(B)** 3M, **(C)** 6M].

Results

Patients' baseline demographic and clinical characteristics

A total of 54 patients were assessed for eligibility, of which 51 cases were enrolled as study subjects. Figure 1 shows the flowchart followed for patient selection. Patients' demographic and clinical characteristics at the beginning of the study are presented in Table 2. There was no significant difference in age, type of surgery, neoadjuvant chemotherapy, and chemotherapy regimen between the HHD-administered and placebo groups ($P > 0.05$).

LVEF

The LVEF value for the two groups of patients decreased after undergoing chemotherapy but the difference was not statistically significant (treatment group: $P = 0.094$; placebo group: $P = 0.060$). After 6 months of chemotherapy, the LVEF value did not significantly change (Table 3). This indicated that

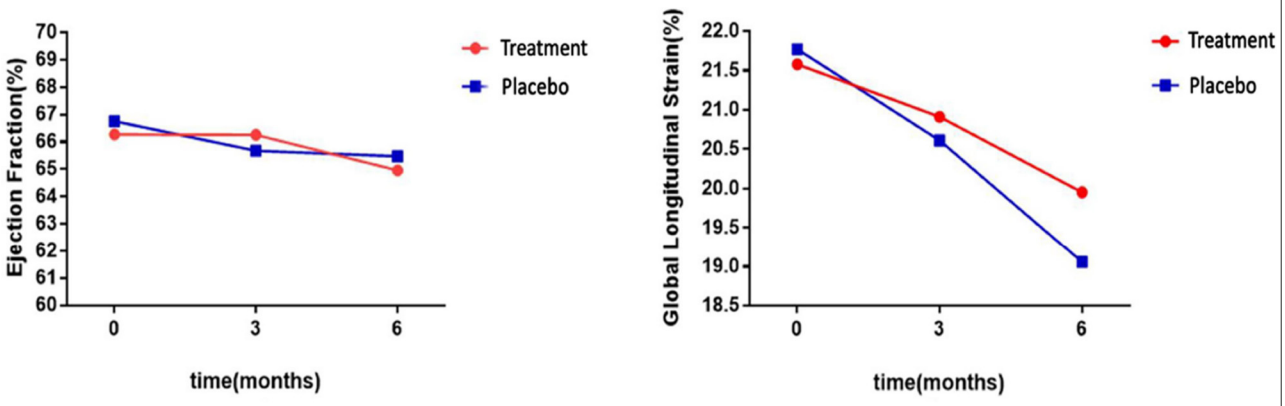

FIGURE 3
Statistical analyses of LVEF and GLS differences between groups. LVEF levels were not significantly different after 6 months of HHD treatment, but GLS decreased significantly.

TABLE 4 Echocardiography speckle tracking imaging (X ± SD).

Indices	Groups	Before	3 months		6 months	
GLS	Treatment	−21.58 ± 1.21	−20.91 ± 2.44	0.22	−19.95 ± 1.16	≤0.001
	Placebo	−21.77 ± 1.35	−20.61 ± 2.03	0.003	−19.06 ± 1.64	≤0.001
	P-value	0.613	0.638		0.03	

The values in the fifth column mean differences between 3 months after first chemotherapy and before chemotherapy; the values in the seventh column mean differences between 6 months after first chemotherapy and before chemotherapy.

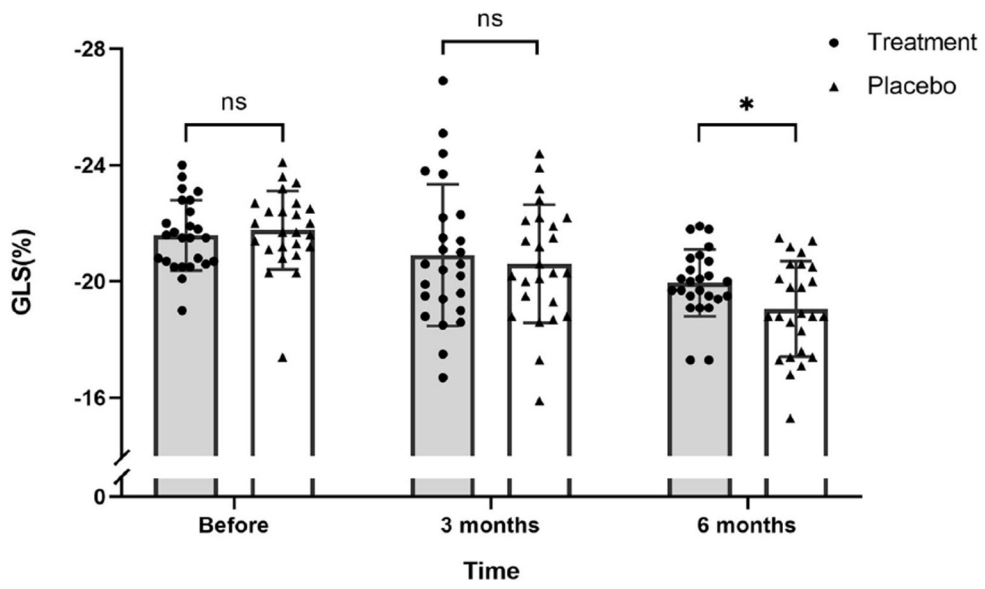

FIGURE 4
Scatter plot for GLS values of 51 patients on the day before chemotherapy, 3 months after the first round of chemotherapy and 6 months after the first round of chemotherapy. There was no significant difference in GLS values between the treatment and placebo groups before and after 3 months of chemotherapy. After 6 months of chemotherapy, the GLS values of the treatment group was significantly higher than the placebo group (ns, not significant; $^*P \leq 0.05$).

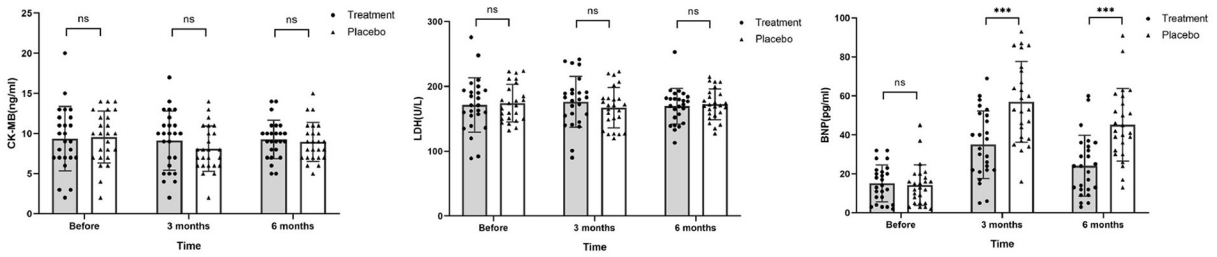

FIGURE 5
Levels of CK-MB, LDH, and BNP before chemotherapy, 3 and 6 months after chemotherapy. Before and after chemotherapy, the values of CK-MB and LDH in both groups decreased to a certain extent, but the difference was not statistically significant. BNP values in the placebo group were significantly higher than that in the treatment group after chemotherapy (ns, not significant; $^{***}P \leq 0.001$).

TABLE 5 Myocardial enzyme lists and related hormones (X ± SD).

Indices	Groups	Before	3 months		6 months	
CK-MB (ng/ml)	Treatment	9.36 ± 4.01	9.12 ± 3.69	0.715	9.28 ± 2.39	0.896
	Placebo	9.56 ± 3.24	8.12 ± 2.82	0.1	8.96 ± 2.42	0.505
	P-value	0.847	0.287		0.640	
LDH (U/L)	Treatment	171.40 ± 42.11	176.30 ± 39.24	0.595	169.52 ± 27.60	0.777
	Placebo	174.16 ± 28.88	167.30 ± 31.12	0.461	172.48 ± 23.76	0.840
	P-value	0.788	0.371		0.686	
BNP (pg/ml)	Treatment	15.04 ± 9.42	34.96 ± 17.31	≤ 0.001	24.08 ± 15.63	0.014
	Placebo	14.16 ± 10.40	56.96 ± 20.71	≤ 0.001	45.20 ± 18.65	≤ 0.001
	P-value	0.692	≤0.001		≤0.001	

The values in the fifth column mean differences between 3 months after first chemotherapy and before chemotherapy; the values in the seventh column mean differences between 6 months after first chemotherapy and before chemotherapy.

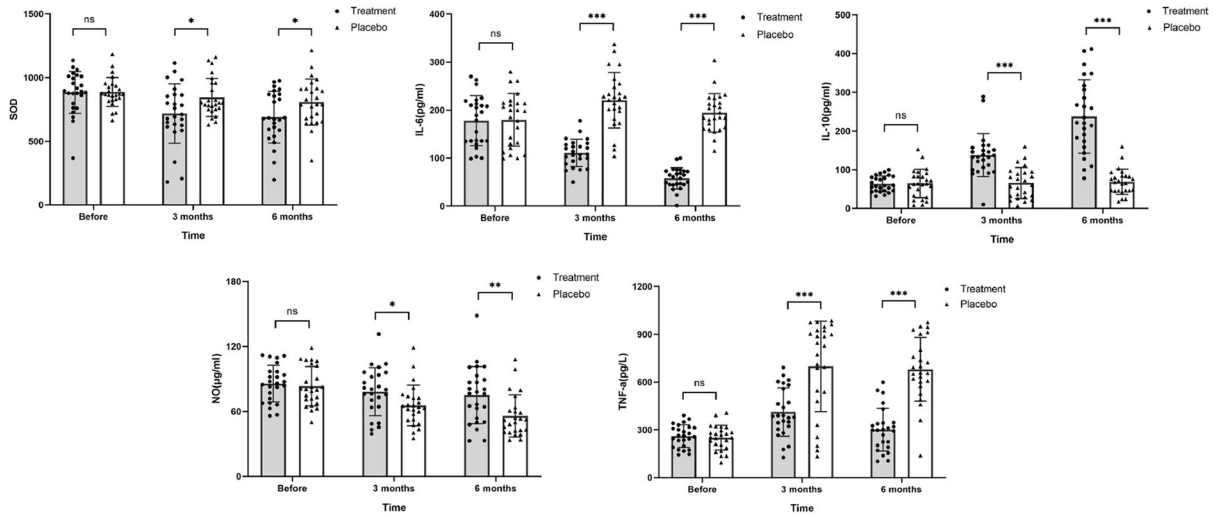

FIGURE 6
Changes in SOD, IL-6, IL-10, NO, and TNF-α levels after chemotherapy. The values of SOD, IL-6, and TNF-α in the treatment group were higher than those in the placebo group, while levels of IL-10 and NO were noticeably lower than those in the placebo group after chemotherapy (ns, no significance difference; $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$).

TABLE 6 Oxidative stress and inflammatory plasma markers.

Indices	Groups	Before	3 months		6 months	
SOD	Treatment	883.50 ± 164.26	717.48 ± 233.11	0.003	689.71 ± 203.60	≤ 0.001
(u/L)	Placebo	887.04 ± 114.26	844.56 ± 150.04	0.228	807.88 ± 182.10	0.109
	P-value	0.930	0.026		0.036	
IL-6 (pg/ml)	Treatment	177.90 ± 52.22	110.80 ± 28.57	≤ 0.001	58.04 ± 22.06	≤ 0.001
	Placebo	179.60 ± 54.89	220.50 ± 57.8	≤ 0.001	194.20 ± 40.14	0.171
	P-value	0.912	≤0.001		≤0.001	
IL-10 (pg/ml)	Treatment	63.44 ± 20.24	137.60 ± 55.70	≤ 0.001	237.90 ± 94.98	≤ 0.001
	Placebo	64.68 ± 36.82	65.64 ± 40.23	0.905	68. ± 32.92	0.581
	P-value	0.883	≤0.001		≤0.001	
NO (μmol/L)	Treatment	85.64 ± 16.90	78.18 ± 22.22	0.049	75.05 ± 26.39	0.043
	Placebo	83.22 ± 18.19	65.58 ± 18.73	≤ 0.001	55.83 ± 19.37	≤ 0.001
	P-value	0.630	0.035		0.005	
TNF-α (pg/L)	Treatment	261.10 ± 70.55	412.20 ± 152.10	≤ 0.001	301.80 ± 134.20	0.164
	Placebo	251.00 ± 77.49	698.70 ± 284.50	≤ 0.001	680.30 ± 199.60	≤ 0.001
	P-value	0.634	≤0.001		≤0.001	

The values in the fifth column mean differences between 3 months after first chemotherapy and before chemotherapy; the values in the seventh column mean differences between 6 months after first chemotherapy and before chemotherapy.

although the LVEF value could be an important index to evaluate the cumulative ANT cardiotoxicity, our results showed that there was no significant difference in the LVEF value over a short-term (≤6 months), confirming that LVEF would not reveal any early decline in cardiac function.

Echocardiography speckle tracking imaging (GLS)

Interestingly, GLS value of a typical case in the treatment group was significantly higher than the placebo group after 6 months of chemotherapy (−21.4 vs. −17.1%) (Figure 2). At the same time, the GLS values for both treatment and placebo groups decreased after 6 months of chemotherapy compared with the pre-chemotherapy measurement (Figure 3) (treatment group: −21.58 ± 1.21 vs. −19.95 ± 1.16, $P \leq 0.001$; placebo group: −21.77 ± 1.35 vs. −19.06 ± 1.64, $P \leq 0.001$; Table 4). This suggests that GLS is a better indicator of cardiotoxicity caused by ANT compared to LVEF (Figure 4). These results showed that HHD had a protective effect on the early treatment of myocardial injury caused by ANT during chemotherapy. The utility of early strain changes to predict subsequent cardiotoxicity is as shown in Figure 2.

Measurement of diagnostic markers of acute myocardial infarction

After chemotherapy, the plasma levels of CK-MB and LDH in both groups were reduced compared to those recorded before chemotherapy was initiated. In addition, BNP levels increased after 3 months of chemotherapy for both groups (Figure 5). However, levels for the HHD-treatment group were significantly lower than those in the placebo group ($P < 0.05$, Table 5).

The levels of inflammatory cytokines

SOD levels decreased to within 100 U/L after 3 months of chemotherapy in the HHD-treated group. On the other hand, NO levels were significantly reduced after chemotherapy in the both groups with the reduction more pronounced in the placebo group than that in the treatment group. After 3 months of chemotherapy, TNF-α levels was elevated to nearly 3 times higher than that before chemotherapy in the placebo group (698.70 ± 284.50 vs. 251.00 ± 77.49, $P \leq 0.001$), while levels were elevated 1.6 times in the treatment group (412.20 ± 152.10 vs. 261.10 ± 70.55, $P \leq 0.001$). IL-6 and IL-10 are important indicators of inflammation. After 6 months of chemotherapy, IL-6 levels decreased significantly in the treatment group (58.04 vs. 177.90, $P < 0.05$), whereas IL-10 levels were markedly elevated (237.90 vs. 63.44, $P < 0.05$). The results indicate that HHD provides antioxidant and anti-inflammatory effects on the body during chemotherapy. Before chemotherapy, there was no significant difference in the levels of IL-6 and IL-10 between the two groups. After 6 months of chemotherapy, there was a significant difference in the levels of IL-6 and IL-10 between the two groups ($P < 0.05$; Figure 6). Combined with the changes in GLS in the two groups, HHD may improve the repair of early myocardial injury caused by ANTs during chemotherapy by reducing oxidative stress and inflammation. Further data are listed in Table 6.

Discussion

Cardiotoxicity is a potential complication of breast cancer chemotherapy and attempts to attenuate the complication may lead to the use of reduced dosage and subsequent loss of clinical effectiveness. This in turn would have an impact on patients' morbidity, mortality, and quality of life, which are independent of the original prognosis.

Improved breast cancer survival together with better awareness of the later-stage effects of cardiotoxicity has led to growing recognition for surveillance of ANT- treated cancer survivors with early intervention to treat or prevent heart failure (30). Echocardiography is the most frequently used method to detect cardiac injury in breast cancer patients (31). Our study revealed that for breast cancer patients, LVEF values decreased after 6 months of ANT chemotherapy with no significant difference between post- and pre-chemotherapy. Similarly, treatment with HHD did not provide any significant difference in LVEF values when compared to the placebo group. Moreover, the GLS value was markedly reduced after 6 months of chemotherapy although significantly higher in the HHD treated group compared to the placebo group of patients. Current guidelines recommend the quantification of LVEF before and after chemotherapy with additional scanning for high-risk patients. Studies have shown that cardiac imaging with echocardiographic measurement of GLS can detect myocardial injury in the early stage prior to the development of left ventricular dysfunction (32). Our study also found that GLS had a more noticeable effect than LVEF on ANT-induced myocardial injury in the early stages (within 6 months) of chemotherapy. After 6 months of chemotherapy, breast cancer patients are generally in the early stages of follow-up, and assessment of GLS value during this follow-up period is key to identifying myocardial injury in the patients.

Our results showed that the GLS values of the two groups decreased after 6 months of chemotherapy, especially in the placebo group. HHD improved the GLS value but not to normal levels. This may be a result of insufficient treatment time or patients suffering from subclinical myocardial injury (as indicated by the GLS value) at that stage of follow-up, which had not yet developed into more serious cardiac dysfunction (33).

Early myocardial injury is also related to inflammation caused by IL-6, IL-10, and TNF-α cytokine storm (34–36). After myocardial injury, there are two different inflammatory phases: one is the initial pro-inflammatory phase of eliminating damaged cells, and the other is the anti-inflammatory reparative phase leading to wound healing and scar formation. IL-6 and IL-10 participate in these two respective phases (37). In particular, IL-6 is a valuable biomarker for predicting future adverse events in patients with myocardial injury (38). From our study, it is evident that after 6 months of chemotherapy, the level of pro-inflammatory factor IL-6 decreased significantly and the level of anti-inflammatory factor IL-10 increased significantly in the treatment group. This further validates that HHD has a superior anti-inflammatory property. Combined with the above GLS value, it can be inferred that HHD protects the myocardium by regulating the inflammatory response.

A number of potential cardioprotective drugs have been explored (39). Dexrazoxane is the only drug for patients with advanced breast cancer. Dexrazoxane's cardioprotective mechanism against ANT was proposed to be due to iron chelation, preventing ANT-iron binding and ROS generation. Angiotensin-converting enzyme (ACE)-inhibitors, angiotensin-receptor blockers (ARBs) and beta-blockers have also been used to prevent ANT cardiotoxicity (2, 4). A meta-analysis showed that there was no robust evidence to support the routine use of cardiac protective agents or liposomal formulations. The study also highlighted that there was a need to improve cardiac monitoring during oncology trials (40). Captopril and carvedilol are commonly used for the assessment of cardiotoxicity in clinical trials, however, their effects have not been fully explored (41). Our study has shown that HHD had a cardioprotective effect on early-stage breast cancer patients.

ANT cardiotoxicity is associated with DNA damage, inhibition of protein synthesis, mitochondrial biogenesis, induction of apoptosis, inflammation, and generation of ROS (5, 42). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-2 (NOX2) is abundant in inflammatory cells, where it is suggested to contribute to oxidative stress (43). The NOX2 generating ROS promotes the expression of inflammatory cytokines. Oxidative stress is associated with the process of inflammation. Oxidation aggravates the inflammatory reaction, and inflammation promotes oxidation through inflammatory mediators (44–47).

Studies have confirmed that TCM can regulate the state of oxidative stress and inflammation and hence, regulate and reduce ANT cardiotoxicity. Curcumin is an anti-oxidant (48) while Salidroside in Rhodiola can regulate SOD levels and protect Lipopolysaccharide-induced myocardial infarction through the ROS mediated PI3K/Akt/mTOR signaling pathway. This indicates that Salidroside in Rhodiola can protect cardiomyocytes through anti-oxidation (49), and salidroside can mediate TNF-α MAPK and NF-κB activation in induced cardiac microvascular endothelial cells (CMECs) to reduce the inflammation of heart and blood vessels (50). This is also reflected by the changes for inflammatory factors IL-6, IL-10, and TNF-α observed in our study (Figure 6).

In summary, our study showed that HHD could regulate the levels of IL-6, IL-10, SOD, NO, and TNF-α and GLS was a better indicator of early-stage myocardial injury triggered by ANT. The results also suggest that HHD could modulate oxidative stress to protect against ANT cardiotoxicity.

Data availability statement

The data presented in the study are deposited in the Resman IPD, accession number ChiCTR1900022394.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Affiliated Hospital of Nanjing University of traditional Chinese medicine (Jiangsu Hospital of traditional Chinese Medicine). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SC and JX contributed to conception and design of the study and wrote the first draft of the manuscript. SC, JX, LC, and YH carried out case collection and organized the database. ML and JZ conducted outcome measures. MF and HW performed the statistical analysis. LC and YH wrote sections of the manuscript. CY gave academic and technical guidance. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.921753/full#supplementary-material

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Left ventricular ejection fraction and cardiac biomarkers for dynamic prediction of cardiotoxicity in early breast cancer

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Background/Purpose: This study aims to quantify the utility of monitoring LVEF, hs-cTnT, and NT-proBNP for dynamic cardiotoxicity risk assessment in women with HER2+ early breast cancer undergoing neoadjuvant/adjuvant trastuzumab-based therapy.

Materials and methods: We used joint models of longitudinal and time-to-event data to analyze 1,136 echocardiography reports and 326 hs-cTnT and NT-proBNP measurements from 185 women. Cardiotoxicity was defined as a 10% decline in LVEF below 50% and/or clinically overt heart failure.

Results: Median pre-treatment LVEF was 64%, and 19 patients (10%) experienced cardiotoxicity (asymptomatic n = 12, during treatment n = 19). The pre-treatment LVEF strongly predicted for cardiotoxicity (subdistribution hazard ratio per 5% increase in pre-treatment LVEF = 0.68, 95%CI: 0.48–0.95, p = 0.026). In contrast, pre-treatment hs-cTnT and NT-proBNP were not consistently associated with cardiotoxicity. During treatment, the longitudinal LVEF trajectory dynamically identified women at high risk of developing cardiotoxicity (hazard ratio per 5% LVEF increase at any time of follow-up = 0.36, 95% CI: 0.2–0.65, p = 0.005). Thirty-four patients (18%) developed an LVEF decline $\geq$ 5% from pre-treatment to first follow-up ("early LVEF decline"). One-year cardiotoxicity risk was 6.8% in those without early LVEF decline and pre-treatment LVEF $\geq$ 60% (n = 117), 15.9% in those with early LVEF

decline or pre-treatment LVEF < 60% (n = 65), and 66.7% in those with early LVEF decline and pre-treatment LVEF < 60% (n = 3), (Gray's test $p < 0.0001$).

Conclusion: Cardiotoxicity risk is low in two thirds of women with HER2+ early breast cancer who have pre-treatment LVEF ≥ 60% and no early LVEF decline > 5% during trastuzumab-based therapy. The longitudinal LVEF trajectory but not hs-cTnT or NT-proBNP allows for a dynamic assessment of cardiotoxicity risk in this setting.

KEYWORDS

cardiotoxicity, breast cancer, trastuzumab, risk assessment, left ventricular ejection fraction, cardiac biomarkers

Introduction

Cardiotoxicity is an increasingly recognized complication of antineoplastic therapy that leads to morbidity, cancer treatment discontinuation, and potentially worse long-term outcome (1). Patients with human epidermal growth factor receptor 2-positive early breast cancer (HER2+ eBC) are particularly at risk for cardiotoxicity, because modern treatment schedules for this tumor rely on two antineoplastic agent classes of significant cardiotoxic potential, namely, anthracyclines and HER2-targeted therapies (2, 3). In clinical practice, routine monitoring of left ventricular ejection fraction (LVEF) during the course of treatment is a widely adopted strategy to survey this population for cardiotoxic treatment effects. However, whether LVEF as a single measurement in time or as a trajectory over time is truly sufficient to identify patients at high risk for cardiotoxicity is currently debated (1). In addition, recent studies demonstrate that biomarkers of cardiac injury, including cardiac troponins I (cTnI), cardiac troponin T (cTnT), and N-terminal pro-brain natriuretic peptide (NT-proBNP), are correlated with LVEF measurements in patients with cancer and may predict cardiotoxic complications (4, 5).

While it is natural for physicians to re-assess prognosis and clinical management based on emerging imaging and laboratory data, quantifying the relationship between a longitudinal string of measurements and a clinical outcome is not straight-forward from a technical perspective. So-called joint models of longitudinal and time-to-event data were developed to address this issue (6). These models lend themselves both to estimating an association between a longitudinal biomarker trajectory and a clinical outcome, as well as to outcome risk predictions for individual patients conditional on their previous longitudinal biomarker trajectory (7). In this study on a large single-center population of patients with HER2+ eBC, we applied joint models of longitudinal and time-to-event data to quantify the prognostic association between longitudinal LVEF measurements and cardiotoxicity, and assessed whether LVEF trajectory can be used for dynamically predicting individual cardiotoxicity risk. Moreover, we investigated related longitudinal changes in the cardiac biomarkers high-sensitive cTnT (hs-cTnT) and NT-proBNP, and their prognostic potential in predicting cardiotoxicity independently and when added to LVEF.

Materials and methods

Study cohort, design, and echocardiography measurements

In this retrospective cohort study, we included all patients with HER2+ eBC who initiated neoadjuvant and/or adjuvant HER2-directed antineoplastic therapy (trastuzumab ± pertuzumab ± chemoendocrine therapy) in a curative intent between February 2006 and January 2016 in a single academic center in Europe (Division of Oncology, Medical University of Graz, Graz, Austria) and had a baseline echocardiography report and at least one follow-up echocardiography report available. Thus, n = 19 women without an available pre-treatment echocardiography report and n = 2 women without any on-treatment echocardiography reports (n = 2) were excluded from the analysis. As part of established care pathways in this center, echocardiography is performed every 3 months during treatment and every 6–12 months thereafter. LVEF was measured with Teichholz, Simpson, and 3D methods according to the preference of the echocardiography examiner. Echocardiography data, demographic data, tumor data, and outcome data were extracted from the hospital information system (HIS) MEDOCs as previously described, as well as from the echocardiography documentation systems at the local Division of Cardiology. Cardiotoxicity was defined as a 10% decline in LVEF below 50% and/or overt clinical heart failure as assessed by treating physicians, and could be either symptomatic or asymptomatic

(8). Time-to-cardiotoxicity was defined as the time from first administration of HER2-directed treatment to the occurrence of cardiotoxicity or censoring alive on the last echocardiography date, whichever came first. The conduct of the study was approved by the local institutional review board before any data collection took place (ethics committee of the Medical University of Graz, ethikkommission@medunigraz.at, vote number: EK-Nr. 31-287 ex 18/19).

Laboratory measurements

As part of the structured biobanking program of our institution, one 7-ml vial of whole blood was drawn into an appropriate sampling tube (VACUETTE® CAT Serum Sep Clot Activator, Greiner Bio-One International GmbH) every 3–6 months in routinely indicated blood collections. The biobanking samples were further processed to gain serum, which was then aliquoted and deep-frozen at −80°C. We only included samples from patients who gave written informed consent. We quantified the concentrations of NT-proBNP and hs-cTnT on a cobas® 8000 e801 analyzer (Roche Diagnostics, Mannheim, Germany) using automated electrochemiluminescence immunoassays (ECLIA) from the same manufacturer.

Statistical methods

All the statistical analyzes were performed by FP using Stata 15.0 (Stata Corp., Houston, TX, United States). Continuous variables were reported as medians [25th–75th percentile], whereas count data were summarized as absolute frequencies (%). Medians [25th–75th percentile] instead of means ± standard deviations were chosen as measures of central tendency, because many of our examined continuous variables had skewed distributions. The distribution of data between patients with and without cardiotoxicity was evaluated by rank-sum tests, χ^2-tests, and Fisher's exact tests, as appropriate, whereas Spearman's ρ was used for univariate correlation analyzes. Median follow-up was estimated with the reverse Kaplan-Meier estimator (9). Simulation of LVEF values in% from uniform distributions was used to handle discrete LVEF readouts (**Supplementary Paragraph 1**). The cumulative incidence of cardiotoxicity was computed with competing risk cumulative incidence estimators, treating death from any cause as the competing event of interest. Follow-up time in all the cardiotoxicity risk analyzes was truncated at 1 year after the first dose of HER2-targeted therapy. Distant recurrence-free survival was obtained with a Kaplan-Meier estimator. Cumulative incidences between two or more groups were compared by Gray's test (10). Uni- and multivariable modeling of cardiotoxicity subdistribution hazards was performed with Fine and Gray competing risk regression models (11). Changes in LVEF and cardiac biomarkers over time were analyzed with linear mixed models with up to a cubic specification for follow-up time, a random intercept for each patient, and a random effect for linear follow-up time (12). To quantify the association between trajectories of LVEF and cardiac biomarkers with subsequent risk of cardiotoxicity, we fitted joint models of longitudinal and time-to-event data (6). Briefly, joint models estimate the association between a longitudinal process (in our case continuous longitudinal measurements of LVEF or cardiac biomarkers) and a time-to-event process (in our case the rate of cardiotoxicity). This association is represented by the association parameter α. As previously described (7), we used a linear mixed model for LVEF and cardiac biomarkers, a Weibull model for cardiotoxicity rates, and examined two specifications of α, namely, the "current parameter" specification (to be interpreted as the association between a biomarker at any time of follow-up and cardiotoxicity risk), and the "1st derivate" specification (to be interpreted as the association between the rate of change in a biomarker and cardiotoxicity risk). Missing LVEF and cardiac biomarker readings were allowed in both linear mixed models and joint models. Personalized predictions of cardiotoxicity risk conditional on LVEF and biomarker trajectories were obtained from joint models based on the dynamic prediction approach of Rizopoulos (13). The full analysis code is available upon request to FP.

Results

Study cohort and cardiotoxicity event rates

A total of one-hundred-eighty-five women with HER2+ eBC were included in the analysis (**Table 1**). Briefly, the median age of the cohort was 55 years [25th–75th percentile: 49–65], 61 (34%) patients had node-positive disease, and 103 (56%) were treated in the neoadjuvant setting. Median echocardiographic follow-up was 1.1 years, with 75 and 25% of the study cohort being followed-up with echocardiography for at least 0.9 and 1.7 years, respectively. During this interval, 19 patients (10%) experienced cardiotoxicity. All 19 cardiotoxicity events occurred during HER2-targeted treatment. However, only one-thirds of these events was symptomatic (n = 7, 37%), and most of the patients required modification/termination of their HER2-targeted cancer treatment (n = 14, 74%). In detail, the HER2-targeted treatment was not changed in n = 5 women with cardiotoxicity (26%), modified in n = 2 women with cardiotoxicity (11%), and permanently terminated in n = 12 women with cardiotoxicity (63%). Median time to cardiotoxicity was 6.7 months (25th–75th percentile: 3.4–10.2), and median maximum LVEF decline in patients with cardiotoxicity was 18% (25th–75th percentile: 14–22,

TABLE 1 Baseline characteristics of the study population (n = 185).

Variable	n (% miss.)	Overall (n = 185)	No cardiotoxicity during F/U (n = 166)	Cardiotoxicity during F/U (n = 19)	P
Demographics					
Age (years)	185 (0%)	55 [49–65]	54 [48–64]	62 [54–69]	0.066
Female sex	185 (0%)	185 (100%)	166 (100%)	19 (100%)	N/A
Body Mass Index (kg/m^2)	140 (24%)	25 [22–30]	25 [22–29]	27 [26–31]	0.020
Tumor and treatment characteristics					
HER2 positive	185 (0%)	185 (100%)	166 (100%)	19 (100%)	N/A
Estrogen receptor positivity	184 (< 1%)	124 (67%)	112 (68%)	12 (63%)	0.68
Progesteron receptor positivity	184 (< 1%)	109 (59%)	99 (60%)	10 (53%)	0.54
Ki-67 (%)	142 (23%)	35 [23–45]	35 [20–45]	36 [28–53]	0.51
Tumor grade G3	179 (3%)	118 (66%)	104 (64%)	14 (82%)	0.13
TNM cT3-4	181 (2%)	12 (7%)	11 (7%)	1 (6%)	0.99
TNM cN +	180 (3%)	61 (34%)	52 (32%)	9 (50%)	0.13
TNM cM0	185 (0%)	185 (100%)	166 (100%)	19 (100%)	N/A
Left-sided breast cancer	185 (0%)	95 (51%)	84 (51%)	11 (55%)	0.730
Neoadjuvant therapy	185 (0%)	103 (56%)	92 (55%)	11 (58%)	0.84
Adjuvant radiotherapy	185 (0%)	144 (78%)	129 (78%)	15 (79%)	0.90
Selected comorbidities					
Coronary artery disease	185 (0%)	3 (2%)	3 (2%)	0 (0%)	0.999
Arterial hypertension	185 (0%)	55 (30%)	46 (27%)	9 (47%)	0.076
Diabetes mellitus	185 (0%)	9 (5%)	8 (5%)	1 (5%)	0.932
Study variables					
Baseline LVEF (%)	185 (0%)	64 [60–68]	65 [60–70]	62 [57–63]	0.016
Baseline hs-cTnT (pg/mL)	70 (62%)	5 [2–8]	5 [2–8]	9 [2–17]	0.34
Baseline NT-proBNP (pg/mL)	70 (62%)	94 [59–191]	94 [59–191]	151 [33–447]	0.69

Data are medians [25th–75th percentile] for continuous variables, and absolute frequencies (%) for count data. n (%miss.) reports the number of patients with an observed record for the respective variable (% missing). F/U, follow-up; N/A, not applicable; HER2, human epidermal growth factor receptor 2; Ki-67, proliferation index Ki-67; TNM, tumor node metastasis classification; cT, clinical tumor size according to TNM system; cN +, clinically positive nodes according to TNM system; cM0, no clinical indication of metastasis according to TNM system; LVEF, left ventricular ejection fraction; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-brain-natriuretic peptide.

range: 10–35). Overall, the 19 events corresponded to 3-, 6-, and 12-month cardiotoxicity risks of 2% (95% CI: 1–6), 4% (2–9), and 11% (7–17), respectively (Supplementary Figure 1). During an oncologic median follow-up interval of 4.5 years, 22 patients developed distant metastasis, and 9 patients died. This corresponded to a 5-year distant-recurrence-free survival (dRFS) experience of 85% (95% CI: 78–90, Supplementary Figure 2). Relatively few missing data were present (Table 1), except for baseline hs-cTnT and baseline NT-proBNP, which were both only available in 70 patients (62% missing). Among baseline variables other than LVEF and cardiac biomarkers, higher BMI but not radiotherapy for left-sided breast cancer was associated with higher cardiotoxicity risk (Table 1).

Pre-treatment LVEF, hs-cTnT, and NT-proBNP for cardiotoxicity risk prediction

At baseline, median pre-treatment LVEF was 64% [60–68], and median pre-treatment levels of hs-cTnT and NT-proBNP were 5 [2–8] and 94 pg/ml [59–191], respectively (Table 1). Baseline hs-cTnT and NT-proBNP were moderately positively correlated with each other (Spearman's ρ = 0.32, p < 0.001); baseline LVEF was only minimally correlated with hs-cTnT (ρ = -0.1, p = 0.042) but is not correlated with NT-proBNP (ρ = -0.03, p = 0.61). A lower pre-treatment LVEF predicted for increased cardiotoxicity risk. In detail, 12-month cardiotoxicity risk was 8% (95% CI: 4–14) in 37 patients with an LVEF < 60%, and 24% (13–42) in 148 patients with an LVEF ≥ 60% (Gray's test p = 0.007, Figure 1), which corresponded to a 3.3-fold relative increase in the subdistribution hazard of cardiotoxicity for LVEF < 60% (Table 2). In the subset of 70 patients with observed pre-treatment cardiac biomarkers, patients with hs-cTnT levels > the 75th percentile of its distribution (n = 14) experienced a 12-month cardiotoxicity risk of 19%, whereas the corresponding estimate for those patients with ≤ this cut-off (n = 56) was 2% (Gray's test p = 0.043, cut-off at > 8 pg/ml, Supplementary Figure 3A). The results were even more pronounced when using a cut-off of 14 pg/ml, although subgroup sizes were small (Supplementary Figure 3B). No association between hs-cTnT and cardiotoxicity risk was observed when modeling the variable as a continuous parameter in a time-to-event model (Table 2). Otherwise, cardiotoxicity risks were not statistically significantly different between patients with NT-proBNP levels ≤ (n = 48) and > (n = 22) the established upper limit of normal (ULN) at 150 pg/ml (12-month risks: 3% vs. 11%, Gray's test p = 0.23, Supplementary Figure 4 and Table 2). Higher age and higher BMI were

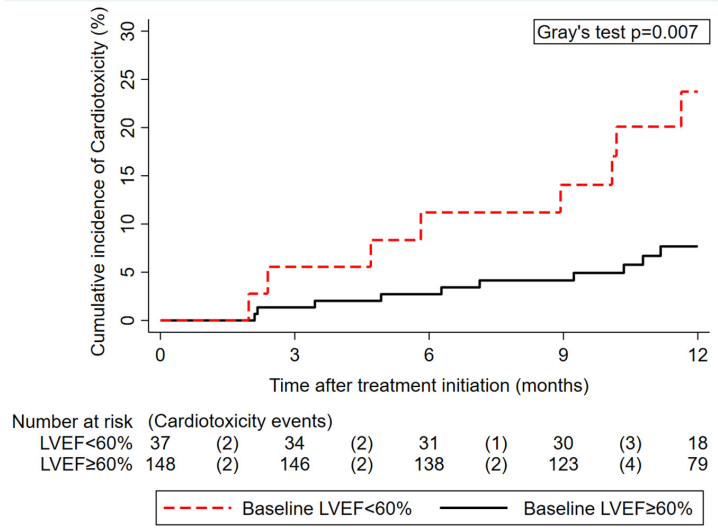

FIGURE 1
Cumulative 12-month incidence of cardiotoxicity according to pre-treatment left ventricular ejection fraction (LVEF) (n = 185). The numbers below the x-axis represent a risk table, with the number of patients at risk for cardiotoxicity at the beginning of each interval and the number of patients who developed cardiotoxicity during the pertinent interval in round brackets.

numerically but not statistically significantly associated with higher cardiotoxicity (**Table 1**).

Longitudinal evolution of left ventricular ejection fraction and cardiac biomarkers

Within 1 month before and 12 months after the first administration of the HER2-targeted therapy, we analyzed 792, 326, and 326 measurements of LVEF, hs-cTnT, and NT-proBNP. The median number of measurements per patient was 4.3 [range: 1–11] for LVEF, 3.8 [range: 1–9] for hs-cTnT, and 3.8 [range: 1–9] for NT-proBNP. In detail, average LVEF declined by.2%/month in a linear fashion (95%CI: 0.1–0.3, $p < 0.0001$, no evidence for improved fit of quadratic model), whereas hs-cTnT and NT-proBNP showed non-linear changes (**Supplementary Figure 5**). Patients with and without cardiotoxicity had a strong separation in LVEF trajectory (**Figure 2A**). In contrast, separation was more modest for NT-proBNP trajectories, and absent for hs-cTnT trajectories (**Figures 2B–C**).

Cardiotoxicity risk and longitudinal trajectories of left ventricular ejection fraction, hs-cTnT, and NT-proBNP

The longitudinal trajectory of LVEF harbored prognostic information on cardiotoxicity risk beyond a single baseline measurement. In joint modeling, patients without cardiotoxicity had an average decline in LVEF of.1%/month (95%CI: 0.0–0.3, $p = 0.017$), while the average of those with cardiotoxicity was 1.4%/month (1–1.7, $p < 0.001$) (absolute difference in monthly decline = 1.2%, $p < 0.001$). LVEF trajectory and cardiotoxicity risk were strongly associated. Here, a 1% increase in LVEF at any time of follow-up was associated with a.8-fold relative reduction in the risk of cardiotoxicity (association parameter α = hazard ratio (HR) = 0.81, 95%CI: 0.72–0.92, $p = 0.001$). Importantly, the prognostic association between LVEF trajectory and cardiotoxicity risk prevailed upon multivariable adjustment for baseline LVEF (adjusted association parameter α = 0.79, 95% CI: 0.64–0.97, $p = 0.028$). The rate of change in LVEF (i.e., 1st derivative of association parameter α) was numerically but not statistically significantly associated with cardiotoxicity risk beyond LVEF trajectory (**Table 2**). In contrast to LVEF, trajectories in cardiac biomarkers were not consistently prognostic for cardiotoxicity risk (although these analyzes were only possible for a subgroup of n = 96 patients with available biomarker data). In detail, increasing in hs-cTnT trajectory was not associated with cardiotoxicity risk (**Table 2**), whereas increase in NT-proBNP trajectory predicted for increased cardiotoxicity (HR for cardiotoxicity per 100 pg/ml increase in NT-proBNP at any time of follow-up = 1.23, $p = 0.004$, **Table 2**). However, the prognostic association between increasing in NT-proBNP trajectory and cardiotoxicity did not prevail after the multivariable adjustment for baseline NT-proBNP (adjusted HR per 100 pg/ml increase = 0.44, $p = 0.5$), suggesting that NT-proBNP trajectory does not add prognostic information on cardiotoxicity risk beyond a single baseline measurement.

TABLE 2 Associations between baseline and longitudinal left ventricular ejection fractions, high-sensitivity troponin T, N-terminal pro-brain natriuretic peptide, and cardiotoxicity.

Variable	(S) HR	95%CI	*P*
Baseline variables			
Baseline LVEF (per 5% increase)	0.68	0.48–0.95	0.026
Baseline LVEF < 60%	3.31	1.31–8.40	0.012
Baseline hs-cTnT (per 5 pg/mL increase)	1.64	0.73–3.70	0.23
Baseline hs-cTnT > 75th percentile	8.05	0.73–88.89	0.089
Baseline hs-cTnT > 14 pg/mL	9.51	0.86–105.19	0.066
Baseline hs-cTnT (per doubling)	1.63	0.53–4.96	0.39
Baseline NT-proBNP (per 50 pg/mL increase)	1.03	0.85–1.25	0.75
Baseline NT-proBNP > 150 pg/mL	3.94	0.36–43.52	0.26
Baseline NT-proBNP (per doubling)	1.16	0.48–2.81	0.75
Trajectory variables			
LVEF trajectory (per 5% increase)	0.36	0.20–0.65	0.001
LVEF rate of change (per 1%/month greater change in LVEF trajectory)	0.12	0.01–23.15	0.43
hs-cTnT trajectory (per 5 pg/mL increase)	1.25	0.81–1.94	0.31
hs-cTnT rate of change (per 1 pg/mL/month greater change in hs-cTNT trajectory)	0.64	0.23–1.79	0.40
NT-proBNP trajectory (per 100 pg/mL increase)	1.23	1.07–1.42	0.004
NT-proBNP rate of change (per 5 pg/mL/month greater change in NT-proBNP trajectory)	0.72	0.48–1.08	0.11

Results for the baseline variables are from univariable Fine and Gray competing risk regression models. Results for trajectory variables are from joint models of longitudinal and time-to-event data. (S)HR, (subdistribution) hazard ratio; 95% CI, 95% confidence interval; p, Wald test *p*-value; LVEF, left ventricular ejection fraction; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide.

Personalized prediction of cardiotoxicity risk based on left ventricular ejection fraction trajectories

The joint models allowed for a highly personalized, dynamic prediction of cardiotoxicity risk for individual patients conditional on their previous LVEF trajectory. This finding is illustrated according to two sample patients (**Figure 3**). Patient "ID #2" (left panel) is a 67-year-old lady receiving neoadjuvant chemoimmunotherapy with liposomal doxorubicin, docetaxel, and trastuzumab for triple-positive locally advanced left-sided breast cancer. Throughout the treatment, she had 4 LVEF measurements that were oscillating within the normal range. Conditional on her previous LVEF trajectory, the model predicted a subsequent 6-month cardiotoxicity risk from her last visit at 12 months after a treatment initiation of below 10%. Patient "ID #63" is a 69-year-old lady with triple-positive locally advanced left-sided breast cancer receiving neoadjuvant chemoimmunotherapy with 5-fluorourcil, epirubicin, cyclophosphamide, paclitaxel, and trastuzumab, followed by adjuvant therapy with trastuzumab and letrozole and adjuvant radiotherapy. This patient had declining LVEF during neoadjuvant therapy, and the model predicted a roughly 20% risk of cardiotoxicity for the subsequent 6 months after her last visit. Indeed, she developed asymptomatic cardiotoxicity (LVEF 45% with diffusely impaired contractility) 3 months after starting the adjuvant treatment.

Risk stratification toward cardiotoxicity based on baseline and dynamic left ventricular ejection fraction cut-offs

In an exploratory analysis of early LVEF decline, thirty-four patients (18%) developed an LVEF decline of at least 5% from baseline to first follow-up ("early LVEF decline"). Combining the cut-off at 60% for baseline LVEF and 5% for early LVEF decline, one can obtain the following empiric risk stratification: 1-year cardiotoxicity risk was 6.8% in those without early LVEF decline and a baseline LVEF $\geq$ 60% (n = 117, "group 1"), 15.9% in those with an early LVEF decline or a baseline LVEF < 60% (n = 65, "group 2"), and 66.7% in those with an early LVEF decline and a baseline LVEF < 60% (n = 3, "group 3") (Gray's test $p < 0.001$, **Figure 4**).

Exploratory analysis: Biomarker-based surveillance in patients at low risk for cardiotoxicity

In the above-defined "group 1" with low cardiotoxicity risk, n = 62 patients had a total of 156 hs-cTnT and NT-proBNP readings available after the second LVEF measurement. During the ensuing 12 months, 4 cardiotoxicity events occurred. Within the limitations of these small absolute numbers of patients, readings, and cardiotoxicity events, we did not observe an association between the cardiac biomarker

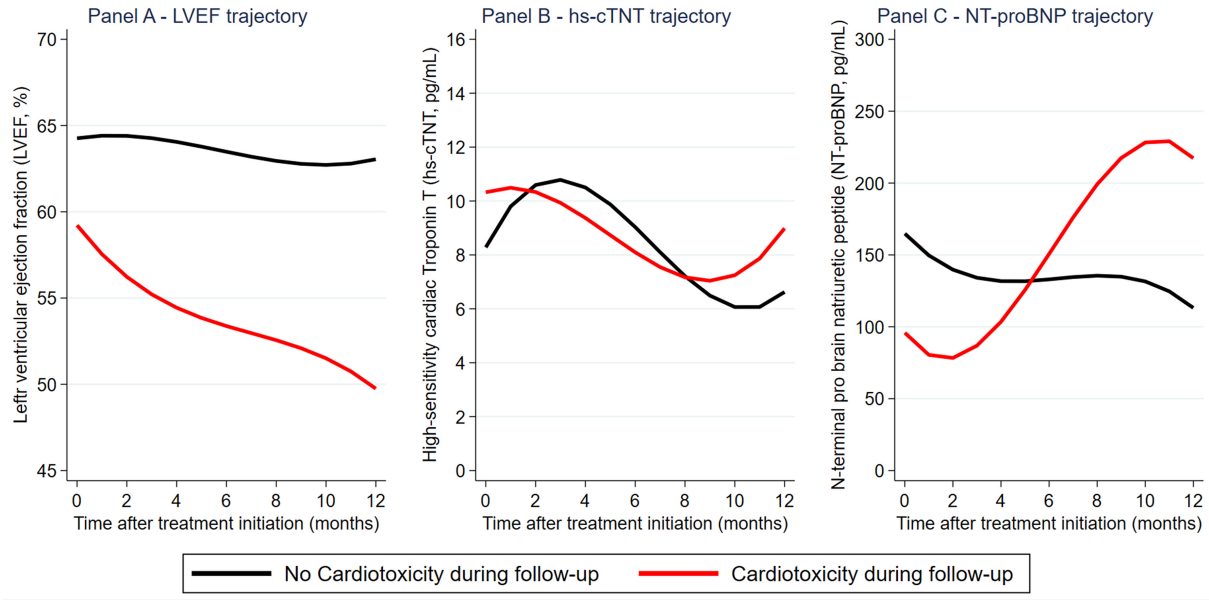

FIGURE 2
Longitudinal evolution of left ventricular ejection fraction (LVEF), hs-cTnT, and NT-proBNP during trastuzumab-based therapy: Distribution by cardiotoxicity outcomes. Reported graphs are from marginal means predicted at monthly intervals from a mixed effects model.

trajectories of the low-risk patients and future cardiotoxicity risk (not shown).

Discussion

In this study, we examined the utility of LVEF and cardiac biomarkers hs-cTnT and NT-proBNP for dynamic assessment of cardiotoxicity risk in women with HER2+ eBC undergoing trastuzumab-based therapy. Our specific aim was to examine the three variables as a longitudinal trajectory to understand whether monitoring of these markers during therapy informs cardiotoxicity prognosis beyond a single measurement prior to treatment initiation. We found that LVEF harbors strong prognostic information on subsequent cardiotoxicity risk not only as a single baseline measurement but also as a longitudinal trajectory. This allowed for us to develop a joint model for personalized dynamic prediction of cardiotoxicity conditional on each woman's prior LVEF trajectory. An early decline in LVEF after treatment initiation was particularly prognostic for cardiotoxicity. We could therefore identify a cut-off-based risk stratification rule with baseline LVEF and the first follow-up LVEF. This simple rule could delineate a large subgroup of women who had low cardiotoxicity risk. Regarding hs-cTnT and NT-proBNP, our findings did not support the hypothesis that the two cardiac biomarkers have a strong utility for dynamic cardiotoxicity risk assessment in this setting.

Interpretation of findings and comparison with the literature: Left ventricular ejection fraction

The association between pre-treatment LVEF and cardiotoxicity risk in the oncologic setting is well-established, and guidelines from leading societies in the field recommend considering periodic LVEF assessment in patients with cancer undergoing cardiotoxic therapy (1, 14). Our study confirms and quantifies the added prognostic information that can be gained from longitudinal LVEF assessment in the oncological important population of women with HER2+ eBC. This can reassure breast oncologists that routine echocardiographic assessment of LVEF prior to treatment initiation and at 3-month intervals during treatment is a meaningful strategy to dynamically identify women with HER2+ eBC at high risk of cardiotoxic complications from trastuzumab-based therapy. As appropriate cut-offs for LVEF and its change are not defined in this setting (1), we derived a risk stratification rule based on pre-treatment LVEF and first follow-up LVEF. This rule could delineate two-thirds of HER2+ eBC patients who have a low risk of cardiotoxicity. We can hypothesize that this large subgroup may be a meaningful population for reducing the intensity of echocardiographic follow-up without compromising cardiac safety. However, a successful prospective and external validation of the risk stratification rule is required before such a hypothesis can be translated to routine clinical care. Moreover, the joint model of longitudinal and time-to-event data allowed

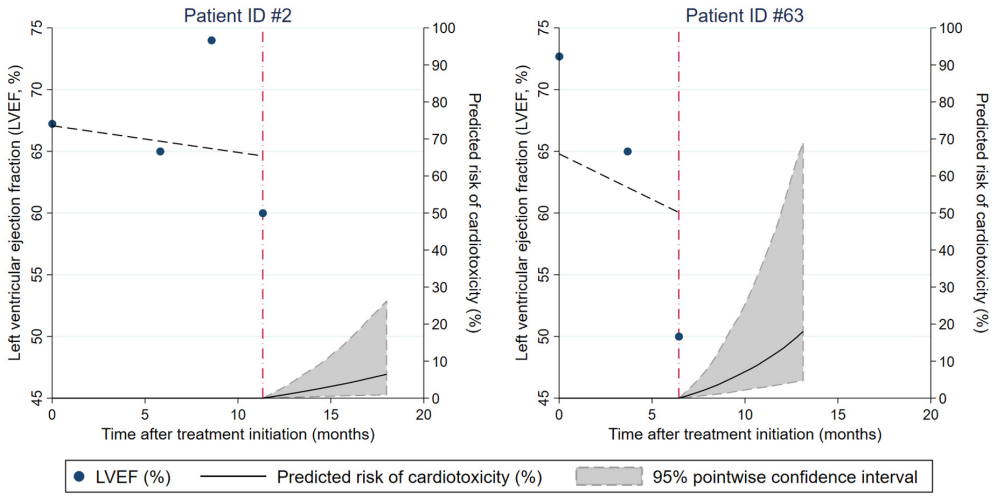

FIGURE 3
Dynamic prediction of cardiotoxicity risk for individual patients conditional on their prior left ventricular ejection fraction (LVEF) trajectory. Results are from a joint model of longitudinal and time-to-event data. The characteristics of patients ID#2 and ID#63 are described in the Results section, paragraph "Personalized prediction of cardiotoxicity risk based on LVEF trajectories."

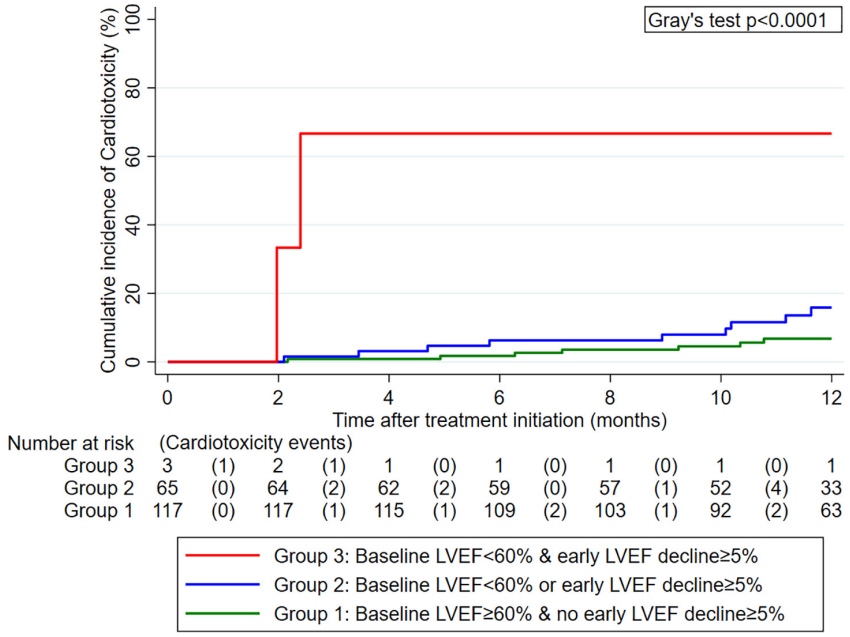

FIGURE 4
Cumulative 12-month incidence of cardiotoxicity according to three groups defined by pre-treatment left ventricular ejection fraction (LVEF) and early LVEF change after treatment initiation (n = 185). Data were estimated with competing risk cumulative incidence estimators, treating death-from-any-cause as the competing event of interest. The numbers below the x-axis represent a risk table with the number of patients at risk for cardiotoxicity at the beginning of each interval and the number of patients who developed cardiotoxicity during the pertinent interval in round brackets.

for us to obtain highly personalized dynamic cardiotoxicity risk predictions for individual patients based on their LVEF trajectory. It is conceivable that an online implementation of this model could provide breast oncologists and cardio-oncologists with continuously updated cardiotoxicity risk predictions at the point of care, which could then inform the subsequent intervals of echocardiography. Statistical extensions of the joint model to define patient-specific optimal spacing of follow-up intervals based on a longitudinal biomarker trajectory have been developed (15, 16), but again, this would require

prospective external validation before clinical implementation in this setting.

Interpretation of findings and comparison with the literature: Cardiac biomarkers

In contrast to the robust findings on LVEF, the results regarding cardiac biomarkers hs-cTnT and NT-proBNP were mixed. The pre-treatment cardiac biomarkers were correlated poorly with LVEF, elevated pre-treatment hs-cTnT predicted for cardiotoxicity when used with previously described cut-offs (17) but not as a continuous variable, and elevated pre-treatment NT-proBNP showed no association with cardiotoxicity risk. In terms of their longitudinal trajectory, hs-cTnT did not dynamically predict for cardiotoxicity, while a prognostic association between increase in NT-proBNP during treatment and higher cardiotoxicity risk was not independent of baseline NT-proBNP. In a further attempt to study whether longitudinal hs-cTnT or NT-proBNP monitoring may be a valid substitute for echocardiography in two-thirds of the HER2+ eBC patients who were identified as having a low risk of cardiotoxicity by our risk stratification rule, we did not observe the two biomarkers to be associated with cardiotoxicity risk in this subpopulation. Thus, our findings do not provide consistent evidence that pre-treatment cardiac biomarkers hs-cTnT and NT-proBNP or their longitudinal trajectory have a convincing utility for cardiotoxicity risk assessment in HER2+ eBC patients undergoing trastuzumab-based therapy.

Although we may have found stronger cardiotoxicity associations of these biomarkers with a larger sample size, the mixed findings mirror the available literature on this topic. For example, Cardinale et al. and Zardavas et al. found elevated pre-treatment troponins to be predictive of cardiotoxicity in patients with breast cancer undergoing trastuzumab-based therapy, but this finding was confined to patients that had previously received anthracyclines (17–19). Previous anthracycline therapy was not a relevant factor in our cohort of newly diagnosed eBC patients, which could explain the absence of association between hs-cTnT and cardiotoxicity. The literature on NT-proBNP is similarly inconclusive. Feola et al. found elevated pre-treatment BNP in patients with cardiotoxicity (8), while Dodos et al. and Zardavas et al. did not observe such an association (17, 20). In patients with metastatic renal cell carcinoma treated with tyrosine kinase inhibitors, increases in NT-proBNP during treatment were observed, but these increases were not correlated with subsequent cardiotoxic events (21). Increases in BNP subsequent to radiotherapy for left-sided breast cancer were also reported, although their relevance to subsequent cardiotoxicity has not been explored (22).

Generalizability of study results beyond HER2+ eBC

While trastuzumab-based therapy for breast cancer is the model disease setting for cardiotoxicity in oncology, it needs to be discussed that many studies in the field address biologically and clinically heterogenic spectra of patients across treatment settings and cancer entities, such as patients with and without prior anthracyclines, patients during or after trastuzumab-based therapy or chemotherapy, patients during or after radiotherapy, patients being treated with tyrosine kinase inhibitors for non-breast cancer entities, or patients being treated in the adjuvant, neoadjuvant, or metastatic setting. As the prognostic potential of cardiac biomarkers may strongly differ between, e.g., a cohort of newly diagnosed eBC patients with little comorbidity undergoing adjuvant trastuzumab and a cohort of heavily pretreated patients with metastatic breast cancer on palliative immunochemotherapy, more stringent oncologic delineations of study populations could help in clarifying the utility of cardiac biomarkers for cardiotoxicity risk assessment in oncology.

Limitations

Finally, several limitations of this study need to be discussed. First, our results apply to women with HER2+ eBC undergoing trastuzumab-based therapy in the curative setting but do not necessarily generalize to other cancer entities or treatment settings. Second, although our cohort underwent a stringent echocardiographic follow-up and was selected in a pre-specified manner from a single academic cancer center, the retrospective nature of the study can be associated with information bias. Third, pre-treatment cardiac biomarkers measurements were only available for a subgroup of our study cohort. This may have led to an underestimation of the association between pre-treatment cardiac biomarkers and cardiotoxicity risk or a limited power to detect true associations between the pre-treatment biomarkers and cardiotoxic outcomes. Fourth, the proposed risk stratification rule must not be implemented in clinical practice before successful external validation. Fifth, consistent with the aim of our study to quantify the utility of LVEF and cardiac biomarker monitoring for eBC patients undergoing trastuzumab-based curative therapy, follow-up was confined to 1 year after treatment initiation. We therefore cannot analyze any long-term cardiotoxic complications occurring years or decades after completion of therapy (23). These complications can be relevant for women with HER2+ eBC who have contemporary cure rates and long-term survival above 80% (3). Fifth, because of the complexity of time-dependent confounding, data on the prescription of relevant co-medications, such as RAAS-blockers, aldosterone antagonists, and beta-blockers, could not be included in the present analysis. This also applied to the

potential cardiotoxic effects of concurrent anthracyclines, which were given to a non-negligible proportion of women in this cohort and may have further modified cardiotoxicity risk but also biomarker trajectories. Finally, emerging cardio-oncologic echocardiography techniques, including measurements of global longitudinal strain by speckle tracking or diastolic dysfunction (20, 24–27), were not available.

Conclusion

In an era of increasing numbers of trastuzumab-based therapies, our results support the routine use of LVEF monitoring as an adequate tool to identify women with HER2+ eBC. We confirm the utility of LVEF for pre-treatment cardiotoxicity risk assessment and define its potential for personalized dynamic risk assessment when used as a longitudinal trajectory during treatment. Cardiotoxicity risk is low in two-thirds of the women with HER2+ eBC who have pre-treatment LVEF $\geq$ 60% and no early LVEF decline $>$ 5% during trastuzumab-based therapy. In this study, the cardiac biomarkers hs-cTnT and NT-proBNP did not appear to have a convincing utility for cardiotoxicity risk assessment in this setting, either as a single pre-treatment measurement or as a longitudinal trajectory. Future studies should prospectively validate these findings and examine their generalizability to other cancer entities and oncologic treatment settings.

Data availability statement

The datasets presented in this article are not readily available because public reposition of the data underlying this study is not possible under the current institutional review board decision. However, the data may be shared as part of research cooperation upon reasonable request to the corresponding author. Requests to access the datasets should be directed to PR, peter.rainer@medunigraz.at.

Ethics statement

This study involved human participants and was reviewed and approved by Ethikkommission der Medizinischen Universität Graz. The patients/participants provided their written informed consent to participate in this study where this was legally required and mandated by the institutional review board mandate (i.e., biobank sampling).

Author contributions

FP, MP, and PR: idea. FP and PR: conception and design. FP, TG, SF, FM, and EK: data collection. TN, TG, and PR: sample analysis and biobank extraction. FP: statistical analysis and wrote the first draft of the manuscript. PR: revised the first draft of the manuscript. All authors interpreted the data, critically reviewed the manuscript draft and contributed to its writing, agreed with the manuscript's conclusions and submission in the present form, and authorship criteria according to ICMJE editors met.

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Conflict of interest

PJ reports a consulting or advisory role, received honoraria, research funding, and/or travel/accommodation expenses from: Abbvie, Bayer, Boehringer, Novartis, Pfizer, Servier, Roche, BMS, and Celgene.

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

Supplementary material

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Cardiogenic shock among cancer patients

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Sophisticated cancer treatments, cardiovascular risk factors, and aging trigger acute cardiovascular diseases in an increasing number of cancer patients. Among acute cardiovascular diseases, cancer treatment, as well as the cancer disease itself, may induce a cardiogenic shock. Although increasing, these cardiogenic shocks are still relatively limited, and their management is a matter of debate in cancer patients. Etiologies that cause cardiogenic shock are slightly different from those of non-cancer patients, and management has some specific features always requiring a multidisciplinary approach. Recent guidelines and extensive data from the scientific literature can provide useful guidance for the management of these critical patients. Even if no etiologic therapy is available, maximal intensive supportive measures can often be justified, as most of these cardiogenic shocks are potentially reversible. In this review, we address the major etiologies that can lead to cardiogenic shock in cancer patients and discuss issues related to its management.

KEYWORDS

heart failure, cardiogenic shock, cancer patient, cardio-oncology, cancer therapy

Introduction

Cancer and cardiovascular diseases are the two most prevalent diseases worldwide and the leading cause of death in modern countries (1). However, due to advances in medicine, the mortality rate among cancer patients (CPs) has decreased dramatically over the last three decades (2, 3). Therefore, more of these CPs who also share many cardiovascular risk factors (4) develop fatal heart diseases (5), such as cardiogenic shock (CS) which can be caused by many distinct etiologies (Figure 1).

Cardiogenic shock may result from cancer itself, directly or indirectly through increased coronary risk and thromboembolic events, tamponade, or paraneoplastic syndrome. In these situations, CS belongs to cardio-oncology syndrome type one in the new classification of cardio-oncology syndromes published in the *Journal of the International Cardio-Oncology Society* (ICOS) (6). Cardiogenic shock may also result

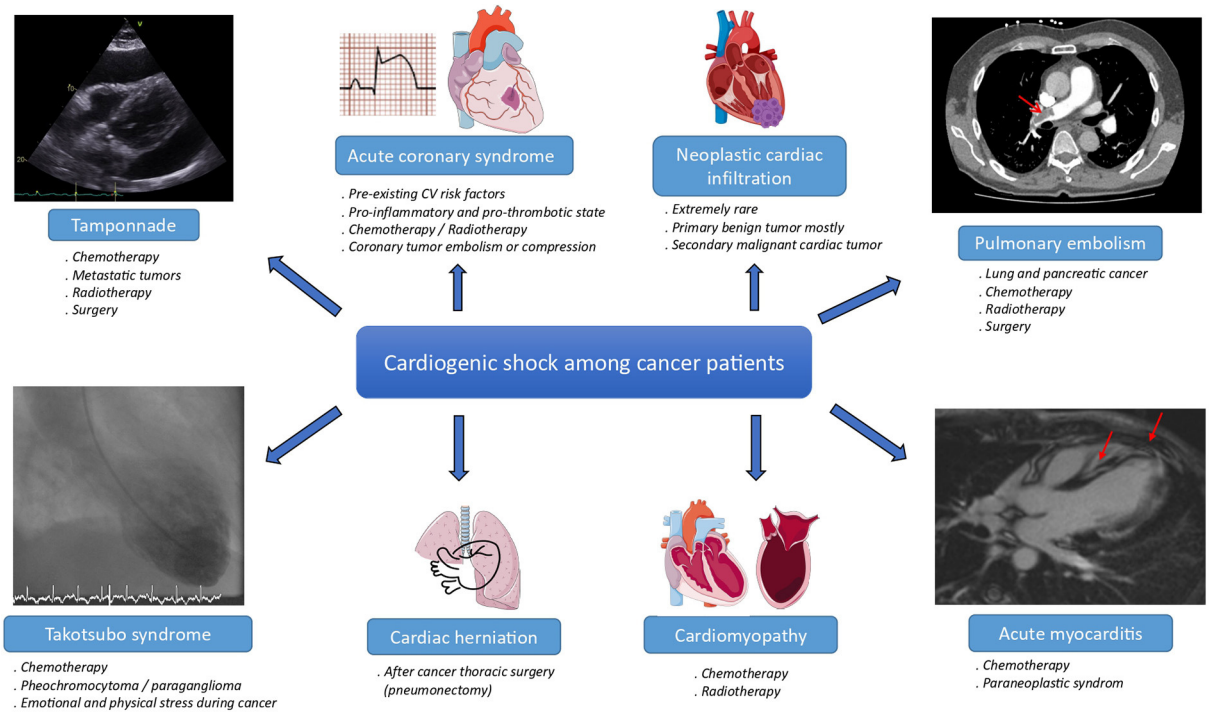

FIGURE 1
Main etiologies of cardiogenic shock among cancer patients. CV, cardiovascular. The meaning of the red arrow is "Magnetic resonance imaging of acute myocarditis".

from collateral side effects of cancer treatments, which belong to cardio-oncology syndrome type two (6). The inflammation in cardio-oncology type two syndrome has been described as a hallmark of cancer therapy-induced cardiovascular complications, whether through an increase in proinflammatory cytokines such as interleukin-1 or inflammasome (7).

Cancer treatments are improving, but many therapies such as surgery, radiotherapy, chemotherapy, targeted therapies (e.g., hormone therapies, angiogenesis inhibitors), and immunotherapy might have significant cardiotoxicity. Consequently, cardio-oncology has emerged as a new and fast-growing subspecialty in recent years (8) with cardio-oncology teams and cardio-oncology services being progressively implemented in hospitals (9).

Abbreviations: 5-FU, 5-fluoro-uracil; ACS, acute coronary syndrome; AFP, axial flow pump; CP, cancer patient; CS, cardiogenic shock; ESC, European Society of Cardiology; HER2, Human Epidermal Growth Factor Receptor-2; IABP, intra-aortic balloon pump; ICI, immune checkpoint inhibitors; ICOS, International Cardio-Oncology Society; InterTAK, International Takotsubo Registry; MCS, mechanical circulatory support; PE, pulmonary embolism; TKIs, tyrosine kinase inhibitors; TTS, Takotsubo syndrome; VA-ECMO, venoarterial-extracorporeal membrane oxygenation; VTE, venous thromboembolic events.

Cardiogenic shock is defined as a primary cardiac dysfunction with low cardiac output and without hypovolemia leading to critical organ hypoperfusion and tissue hypoxia. Diagnostic criteria include persistent hypotension and signs of compromised end-organ perfusion (10, 11). In recent times, CS remains one of the greatest challenges in cardiology and intensive care medicine. Compared to acute heart failure, CS has a 10-fold higher in-hospital mortality rate, remaining >40% despite medical and surgical advances (12). Given the specificities of different cancers or their treatments, management of CS in this particular setting requires a comprehensive knowledge of the various determinants in addition to a multidisciplinary collaboration between intensivists, cardiologists, cardiac surgeons, and oncologists. To help physicians evaluate and manage CP with acute cardiovascular disease, recent guidelines were published by a Task Force including the Association of Acute Cardiovascular Care (ACVC) and the Council of Cardio-Oncology (CO council) of the European Society of Cardiology (ESC) in 2021 (13).

In this review, we aim to summarize the latest evidence on CS among oncological patients to provide an overview of research areas and knowledge that are often at the crossroads of several medical specialties.

TABLE 1 Main cancer therapies that can induce acute coronary syndrome in cancer patients.

Therapy	Mechanisms	Time of onset
Antimetabolites	Vasospasm	Within 2 to 5 days
5-FU		
Capecitabine		
Alkylating agents	Oxidative stress,	Within 3 months
Cisplatin	endothelial dysfunction	
TKIs	Endothelial, platelets and	Within 2 years
Sunitinib	coagulation activation	
Nilotinib		
Anti-microtubule	Vasospasm, cellular	Within 2 weeks
agents	hypoxia	
Paclitaxel		
Docetaxel		
VGEF inhibitors	Acute thrombosis	Within 3 months
Radiotherapy	Oxidative stress, fibrosis,	15–30 years following
	and direct endothelial	treatment
	injury accelerated CAD	

TKIs, tyrosine kinase inhibitors; 5-FU, 5-fluorouracil; VEGF, vascular endothelial growth factor; CAD, coronary artery disease.

Epidemiology

Epidemiology data underlying the relationship between CS and cancer are scarce, as most of the published data are case reports. In the largest European prospective multicenter study, which included CS patients from a broad spectrum of etiologies, 7% were CPs. In this registry, 30-day mortality was 29.4% in CPs. Interestingly, in this study, cancer was not an independent variable associated with 30-day mortality (14).

In contrast, in the administrative Nationwide Inpatient Sample Database which includes around 500,000 patients hospitalized for CS between 2004 and 2011, cancer was independently associated with poor outcomes. Having a solid tumor, with or without metastases, accounted for the most disadvantageous prognostic factors with an odds ratio (OR) of 2.05 and 1.50 respectively (15). In another study, based on the same database but for the period 2010 and 2014, CS was higher in patients with colon cancer and lower in patients with multiple myeloma, while patients with lung cancer had the highest risk of dying from CS (16).

Cardiogenic shock related to acute coronary syndromes

In the largest published registry of acute coronary syndrome (ACS) in CP, the proportion of ACS among patients with cancer on treatment was ∼3%. This number has been increasing since the early 2000s and the most common types of associated malignancies are lung, prostate, and breast cancers (17). The risk of ACS in patients with a current or historical diagnosis of cancer is more than two times higher as compared to the general population (18). Indeed, CPs are often older and may share traditional cardiovascular risk factors (4). In addition, cardiovascular toxicity induced by cancer therapies may cause ACS through different pathophysiological mechanisms (Table 1). Coronary vasospasm is one of the most described mechanisms and is typically caused by 5-fluoro-uracil (5-FU) or its prodrug capecitabine (19). Other mechanisms may include plaque rupture resulting from cisplatin and vinca alkaloids (20), or coronary thrombosis due to pro-inflammatory and prothrombotic conditions associated with increased platelet aggregability induced by specific cancer therapies (e.g., cisplatin, vascular endothelial growth factor (VEGF) signaling pathway inhibitors and cyclophosphamide) (21). Direct endothelial injury associated with accelerated coronary artery disease induced by radiotherapy (22) can cause ACS typically within 10 to 30 years following treatment but rarely during treatment (23). Moreover, mortality caused by ACS is higher in oncological patients (24, 25) with malignancy being considered an independent predictor of increased risk of repeated revascularization and stent thrombosis (17). Patients with ACS and malignancies might also be more likely to experience CS (26), even if this additional risk is not found in all studies (27).

Clinical presentation and diagnostic algorithms of ACS in CPs are relatively close to those in patients without cancer. However, ACS symptoms can frequently be atypical in CPs, mistaken with cancer symptoms such as cancer-related anemia (28), with less than one-third of them experiencing chest pain, and less than half of them having dyspnea (29).

Acute coronary syndrome management in CS remains almost identical to non-CPs, although sometimes complicated by increased comorbidities in CPs (30) or cytopenia. Chemotherapy-induced thrombocytopenia may be caused by DNA synthesis inhibition in megakaryocyte development, leading to megakaryocyte progenitor cell death due to alkylating agents or by oxaliplatin-dependent antibodies cross-reaction with platelet antigens (31). Consensus statements for ACS management in CPs advise early echocardiography to evaluate left ventricular function and exclude cancer or cancer therapy-related complications (13). Cardiotoxic cancer therapy should be at least temporarily interrupted after multidisciplinary discussion, especially if a causal relation is suspected (13). Experts advise that if a stent is needed, a drug-eluting stent (preferred over balloon angioplasty or bare metal stent) together with shortened dual antiplatelet therapy duration (mandatory for only 1 month for instance) is probably the safest choice in CPs undergoing percutaneous coronary intervention (PCI) who do not need short-term surgery. Aspirin and clopidogrel should be preferred to ticagrelor and prasugrel because of the high

bleeding risk and limited data regarding both efficacy and safety in patients with active cancer (13). Thrombocytopenia due to cancer or cancer therapy is observed in about 10% of patients, which makes ACS management challenging because of an increased risk of bleeding complications. In CPs, experts advise that aspirin and clopidogrel can be administered if platelets are >10,000 and >30,000/μL, respectively. Experts also suggest that a minimum platelet count of 30,000 and 50,000/μL is required for PCI and coronary artery bypass grafting respectively (13).

Cardiogenic/obstructive shock related to acute pulmonary embolism

Incidence of venous thromboembolic events (VTE) in CPs is around 15% (32). Cancer patients have an estimated seven-fold increased risk for VTE compared to the general population, especially in the first few months after cancer diagnosis (33). Thus, VTE is the second leading cause of death after cancer progression (34). Lung and pancreatic cancer are the most common malignancies associated with VTE (35, 36). In addition, lung and colorectal cancers are associated with the highest thromboembolic risk leading to pulmonary embolism (PE) (37).

The association between cancer and hypercoagulable state, sometimes eponymously referred to as Trousseau syndrome, has been known for more than a century. In addition to the usual risk factors, the hypercoagulable state of cancer is driven by activation of the coagulation cascade and platelet aggregation caused by tumor expression of procoagulant proteins released into the circulation such as tissue factor, plasminogen activator inhibitor-1, and podoplanin (38). Furthermore, cancer treatments such as surgery, hospitalization, central venous catheters, anti-tumor drugs [e.g., cisplatin, 5-fluorouracil (5-FU), tamoxifen], as well as supportive therapies (e.g., erythropoietin), may increase thrombosis via still incompletely understood mechanisms (39).

The risk of acute PE is therefore relatively high in CPs and its incidence is increased by surgery, chemotherapy, radiotherapy, and disease progression (40). In a retrospective study assessing the routine histologic examination of more than 1,300 surgical embolectomies in a general population, of whom 30% had a history of malignant disease, direct neoplastic emboli accounted for <1%. Among these <1% of patients, neoplastic embolism was the first manifestation of an underlying neoplasm in most cases, mainly caused by unknown lung cancer (41). This implies that CPs are much more likely to develop non-neoplastic thromboembolism than neoplastic emboli, although few rare cases of neoplastic arterial embolism leading to fulminant pulmonary hypertension complicated by right ventricular failure and cardiogenic shock have been identified (42, 43).

In a recent study, acute PE in CPs was associated with a 90% increase in all-cause inpatient mortality (44). These patients are less likely to present with chest pain (45) but most of them report dyspnea (40).

Guidelines recommend the use of thrombolysis for high-risk PE unless contraindicated (46). The only absolute contraindication for thrombolysis in cancer is central nervous system neoplasm, for which pulmonary embolectomy is recommended (46). Even though thrombolysis is not formally contraindicated in CPs, they are less likely to receive thrombolysis (47, 48) due to bleeding risk concerns and probably the intensity of cancer care.

Recent results on a retrospective database showed that surgical thrombectomy in patients with cancer is complicated with worse in-hospital outcomes, including mortality and more post-procedural bleeding than thrombolysis (49). Hence, another interesting option that should be considered, according to recent French and European guidelines, is embolectomy *via* percutaneous catheter-directed treatment for patients with high-risk PE in whom intravenous thrombolysis is contraindicated (46, 50).

Mortality due to PE is more important during the first 3 months but even CPs who survive beyond this period have an increased risk of death compared to the general population (51). However, with a median survival time of more than 2 years, maximal treatment intensity during the acute phase should be considered in CPs with PE (52), especially in young patients with few comorbidities and early-stage cancer.

Finally, cancer-associated arterial thromboembolism is a less frequent but key part of Trousseau syndrome. In the CATS cohort, a cohort of 1,880 patients with active newly diagnosed or relapsed cancer, the frequency of arterial thromboembolism was 2.6% during a median prospective observation time of 2 years (53). This was mainly caused by lung and kidney cancer. Thus, in CPs, the development of arterial thromboembolism has been reported to be associated with a three to five-fold increased risk of death (54).

Cardiogenic shock related to acute cardiomyopathy

Cardiomyopathy-associated ventricular dysfunction is another typical clinical scenario that may lead to CS in CPs, mostly due to cardiotoxic anticancer agents. Numerous guidelines emphasize the need to identify patients with an increased risk of developing cardiovascular toxicity (55–57). Although slightly different, all definitions include patients with previous heart disease and abnormal left ventricular function, elevated cardiac biomarkers before initiation of anticancer therapy, prior mediastinal radiotherapy, and patients with prior or ongoing anthracycline treatment or HER2 (Human Epidermal Growth Factor Receptor-2) targeted agents such as trastuzumab and trastuzumab-derived antibody-drug conjugates (58).

Among chemotherapy drugs that are most likely to induce cardiotoxicity, anthracyclines (e.g., doxorubicin) have been used since the late 1950s to treat solid and hematologic cancers such as lymphoma, leukemia, sarcoma, and breast cancer. Anthracyclines are associated with several cardiovascular toxicities, including dilated cardiomyopathy with left ventricular systolic dysfunction leading to heart failure (59). In an Italian prospective study assessing more than 2,600 patients, the overall incidence of anthracycline-induced cardiotoxicity was 9% (60). This cardiotoxicity was dose-dependent, with the highest incidence observed during the first year after the completion of chemotherapy in 98% of the cases. However, early detection and heart failure therapy allow full or partial recovery in 82% of patients (60). Notably, in this study, the more severe the cardiotoxicity was, the more tendency there was for life-threatening arrhythmias or conduction disturbances requiring pacemaker implantation (60).

Anthracycline-associated cardiotoxicity is now thought to occur at the time of first exposure, a hypothesis supported by the finding of troponin release after administration, mainly as a consequence of increased intracellular Ca2+concentration, oxidative stress, DNA damage, and impairment of DNA repair through inhibition of the topoisomerase II, activation of cell senescence, and cell death (61). Recent evidence points to the involvement of many mechanisms mainly converging toward mitochondrial dysfunction (62).

In the case of anthracycline-induced CS, treatment is based on the empiric management of CS. Through antioxidant effects (*via* decreased NO production) and reduction of intracellular Ca2+ in cardiomyocytes, sodium-glucose cotransporter 2 (SGLT-2) inhibitors, also called gliflozins, may be a promising cardioprotective strategy in anthracycline-associated cardiotoxicity (63).

However, although described in case reports, rapid recovery of cardiac function appears to be rare (64); some case reports even reported bridges to recovery months after long-term cardiac support device implantation (65, 66).

Besides anthracyclines, anticancer agents including chemotherapies and certain targeted therapies have been associated with acute heart failure (Table 2). Indeed, the transmembrane receptor HER4 partners with HER2 in cardiomyocytes, the latter being a target in cancer therapy. Of note, a number of anti-HER2 agents are approved in HER2-positive breast [e.g., monoclonal antibodies, antibody-drug conjugates, and tyrosine kinase inhibitors (TKIs)] and gastric cancer (e.g., monoclonal antibodies, antibody-drug conjugates) (67). Among them, trastuzumab, a targeted therapy to the HER2 receptor in breast cancer and all its derivatives such as trastuzumab emtansine or trastuzumab deruxtecan, can be responsible for acute cardiac toxicity, which is usually reversible as opposed to the aforementioned anthracyclines (59). For patients receiving cardiotoxic chemotherapy, such as anthracycline or other anti-HER2 therapy, guidelines recommend a three-monthly left ventricular ejection fraction monitoring (68).

TABLE 2 Main cancer therapies that can induce cardiomyopathies in cancer patients.

Therapy	Mechanisms	Time of onset
Anthracyclines	Oxidative stress-induced DNA damage activation of senescence and cell death	Within the first year
Doxorubicin		
Alkylating agents	Oxidative stress, endothelial dysfunction	Within 1 to 2 weeks
Carboplatin		
Cisplatin		
Cyclophosphamide		
Monoclonal Antibodies	Cardiomyocytes stunning and hibernation	Within 4 to 8 weeks
Trastuzumab (anti-HER2)		
TKIs	Oxidative stress, inhibition of NO cell apoptosis	Within the first year
Imatinib		
Sunitinib		
Sorafenib		
Proteasome inhibitors	Not fully understood	Within 2 years
Bortezomib		
Radiotherapy	Oxidative stress, fibrosis and endothelial cell damage	15–30 years following treatment

TKIs, tyrosine kinase inhibitors; HER2, human epidermal growth factor receptor-2; DNA, deoxyribonucleic acid; NO, nitric oxide.

Cardiogenic shock related to myocarditis

Myocarditis is an inflammatory condition leading to inflammatory cell infiltration into the myocardium. It can be a consequence of anticancer treatment (Table 3) or paraneoplastic syndrome, which can lead to CS in cancer patients (69).

Identified many years ago, myocarditis secondary to the antimetabolite 5-FU is a very rare complication related to an inflammatory response, driven by apoptosis of myocardial and endothelial cells (70). Cyclophosphamide, a nitrogen mustard alkylating agent increasingly used to treat various types of cancers and autoimmune conditions, can rarely lead to myocarditis, occurring within 1–3 weeks, usually after high doses (>1.5 g/m^2/day) (71).

Most recently, novel therapies harnessing the immune system, such as immune checkpoint inhibitors (ICI), have been proven to be associated with myocarditis. Although rare (less than 1%), it often occurs about 1 month after the first dose (72). ICI-related myocarditis usually appears in a fulminant presentation with a high fatality rate of almost 50% (73).

TABLE 3 Main cancer therapies that can induce myocarditis in cancer patients.

Therapy Time of onset	Mechanisms	Frequency/
Antimetabolites	Dysregulated inflammatory response	Extremely rare/At the beginning
5-FU		
Alkylating agents	Not fully understood	Unknown/within 1-3 weeks
Cyclophosphamide		
ICIs	*T*-cells could target an antigen	<1%/within the the first month
Ipilimumab (CTLA-4 inhibitor)	potentially shared by the tumor and	
Atezolizumab (PDL-1 inhibitor)	cardiomyocytes	
Nivolumab (PD-1 inhibitor)		

ICIs, immune checkpoints inhibitors; 5-FU, 5-fluorouracil; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PDL-1, programmed death ligand-1; PD-1, programmed cell death protein-1.

The histopathological features of ICI-associated myocarditis imply myocardial infiltration of T-lymphocytes, both CD4+ and CD8+, and macrophages leading to myocyte death, without B-lymphocytes being present (74). A potential pathophysiological hypothesis for ICI-myocarditis is that cardiomyocytes may share targeted antigens with the malignancy, thus becoming targets of the same activated T-cells clones, leading to lymphocytic infiltration of the myocardium (75).

Besides cancer therapy, paraneoplastic syndromes are other possible triggers of myocarditis in CP. Catecholaminergic myocarditis has been associated with pheochromocytoma (76, 77), giant-cell myocarditis with lymphoma, sarcoma, lung cancer, and thymomas (78), and eosinophilic myocarditis has been associated in eosinophilic leukemia and lung cancer (79).

As in other causes of myocarditis, empirical treatments of myocarditis are often based on immunosuppressive therapies such as high-dose corticosteroids (61).

In specific ICI-associated myocarditis, high-dose intravenous corticosteroids and withdrawal of ICI are considered the first-line therapy (62), while abatacept (CTLA-4 agonist), alemtuzumab (anti-CD52 antibody), and anti-thymocyte globulin (anti-CD3 antibody) have been suggested in corticosteroid-resistant forms (63).

TABLE 4 Main cancer therapies that can induce Takotsubo syndrome in cancer patients.

Therapy
Antimetabolites
5-FU
Capecitabine
Monoclonal antibodies
Trastuzumab (anti-HER2)
Rituximab (anti-CD20)
Bevacizumab (anti-VEGF)
TKIs
Sunitinib
Ibrutinib
ICIs
Ipilimumab (CTLA-4 inhibitor)
Atezolizumab (PDL-1 inhibitor)
Nivolumab (PD-1 inhibitor)
Radiotherapy

TKI, tyrosine kinase inhibitors; ICI, immune checkpoint inhibitors; 5-FU, 5-fluorouracil; HER2, human epidermal growth factor receptor-2; VEGF, vascular endothelial growth factor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PDL-1, programmed death ligand-1; PD-1, programmed cell death protein-1.

Cardiogenic shock related to Takotsubo syndrome

Takotsubo syndrome (TTS) among CP has been mainly reported either as a cardiotoxic effect of antineoplastic treatment (Table 4), as a complication of specific tumors [such as pheochromocytoma and paraganglioma (80)], or as a complication of the significant emotional and physical stress that frequently accompanies cancer. TTS is a clinical syndrome that generally presents as chest pain mimicking ACS or as an acute heart failure marked by severe left ventricular systolic dysfunction mostly characterized by apical akinesis or ballooning with hyperdynamic basal segments, usually following an emotion or physical stressor, predominantly affecting post-menopausal women. TTS leading to CS occurs in ∼10% of all cases according to the International Takotsubo Registry (InterTAK), which is the largest registry to date (81).

Although the pathophysiology of the syndrome is still not well understood, commonly hypothesized mechanisms include circulating plasma catecholamines surge, inflammation,

estrogen deficiency, microvascular dysfunction, and spasm of the epicardial coronary vessels (82). In the latest International Expert Consensus Document on Takotsubo Syndrome, malignancy, chemotherapy, and radiotherapy are listed among its triggers (83). 5-FU or its prodrug (capecitabine) have been involved in up to 50% of reported cases of TTS leading to CS (84). Its hypothetical mechanism may be coronary vasospasm and direct cardiotoxicity through the production of free radicals and microthrombi related to 5-FU–mediated kallikrein stimuli (84).

Interactions between TTS and malignancy are probably more complex than initially thought, as many studies now report that cancer will frequently be diagnosed within a few years following TTS. In a 4-year follow-up study, 9.6% of TTS patients developed malignancies (85) while Burgdorf observed cancer diagnosis in up to 14% of TTS patients in a 3-year follow-up study (86). These data suggest shared environmental or genetic triggers. Thus, reports have suggested variations in common signaling pathways related to survival cascades and cardioprotective roles which are thought to be up-regulated in adrenergic stress, such as the phosphatidyl inositol-3 kinase/protein kinase-B activation (87) and BCL2-associated athanogene3 protein polymorphism (88) as a potential link between cancer and TTS.

Prevalence of cancer in patients presenting with TTS has been reported to account for up to 28.5% (89). Therefore, cancer screening may be useful for patients who do not have a clear TTS-triggering stressor. In comparison to patients without cancer, the co-existence of cancer and TTS results in increased hospital length-of-stay with increased risk of mechanical ventilation (90, 91), cardiac arrest (92), and all-cause in-hospital and long-term mortality (93, 94). However, in the latest analysis of the InterTAK Registry, CPs with TTS did not experience more CS than non-CP (90).

Solid tumors appear more likely to develop TTS compared to hematologic malignancies (95). According to the InterTAK Registry, breast cancer was the most prevalent type of malignancy-related TTS in 26.2% of the cohort followed by tumors affecting the gastrointestinal system and the respiratory tract with a prevalence of 16.1% and 15.4%, respectively. Hematological malignancies were less prevalent than solid tumors, affecting up to 10% of patients (90). These differences are probably also partly due to the specificities of cancer treatment used for each cancer type.

In addition to the tumor type, the stage of cancer is also an important factor to be considered, as TTS appears to be more prevalent in patients with advanced or recurrent disease (95). Although, this could be explained by treatment selection bias.

Even if TTS in CPs remains poorly understood and multifactorial, several hypotheses are currently considered, such as the emotional trauma of the cancer diagnosis, the inflammatory state of cancer, and the physical stress of various cancer treatments (96, 97).

Guidelines regarding TTS management are lacking as no prospective randomized clinical trials have been performed in this patient population. Nevertheless, a recent international expert consensus endorsed by the ESC advises avoiding inotropes such as adrenaline, noradrenaline, dobutamine, and milrinone (83) because TTS patients treated with catecholamine drugs suffer a 20% increased mortality (98), although this may represent a selection bias due to the initial presentation of the patients. Instead, in TTS leading to CS, experts suggest considering the Ca2+-sensitizer levosimendan or short-term mechanical circulatory support (MCS) such as an axial flow pump (AFP) (e.g., impella) or venoarterial-extracorporeal membrane oxygenation (VA-ECMO) (83).

Experts also suggest looking for the presence of left ventricular outflow tract obstruction, which occurs in about 20% of TTS patients with CS (99). In this situation, experts suggest intravenous fluid, short-acting beta-blocker, and AFP to avoid diuretics, nitroglycerin, or intra-aortic balloon pump (IABP) (83).

Finally, pheochromocytomas and paragangliomas are rare neuroendocrine tumors that can cause catecholamine-induced myocardial dysfunction that may be complicated with CS in 2% of patients (76). To be differentiated from the classic phenotype of TTS, these conditions are labeled as TTS phenocopies (82) and may share genetic predispositions (100). Even though there are no guidelines, VA-ECMO support could be a life-saving therapy, allowing myocardial recovery within a few days (77). After hemodynamic stabilization, treatment should include α-blockade to negate the effects of the excess hormones secreted by the pheochromocytoma and minimize intraoperative hemodynamic instability, with elective tumor removal scheduled under stable conditions (77).

Cardiogenic/obstructive shock related to cardiac tamponade

In 1935, thoracic surgeon Claude Beck first described the classic Beck triad in patients with acute cardiac tamponade including "hypotension, increased jugular venous pressure, and a small and quiet heart."

In recent years, cancer represents ∼25% of the cardiac tamponade etiology. Prognosis of cardiac tamponade is essentially related to the etiology, thereby patients with cancer and metastatic involvement of the pericardium usually have a bad short-term prognosis as it is the sign of advanced cancer. In a recent study, factors associated with poor prognosis at 2-years after pericardiocentesis for malignant effusions were age >65 years, platelet counts <20,000/μL, lung cancer, presence of malignant cells in the effusion, and drainage duration (101).

Pericardial effusion develops in up to 21% of patients with underlying malignancy (102). Cancers most frequently

presenting with involvement of the pericardium are mainly solid malignancies, such as advanced lung cancer (∼30% of patients with lung cancer present pericardial effusion), malignant melanoma (40–70% of patients), breast cancer (∼25% of patients), and less frequently hematological malignancies such as leukemia and lymphomas (about 15% of patients) (103).

Most malignancy-related pericardial effusions are caused by direct or metastatic invasion of a non-cardiac tumor. Primary malignant pericardial mesotheliomas or cardiac synovial sarcomas are very rare (104). It has been suggested that the pathophysiology of non-neoplastic effusions in CPs is related to obstruction of the mediastinal lymphatic system by tumor infiltration, which can also result from radiotherapy-induced fibrosis, especially after chemoradiotherapy for lung and esophageal cancers (105).

Effusions can also be paraneoplastic as a result of pericarditis (106). Cancer treatments themselves may affect the pericardium indirectly by increasing the risk of opportunistic viral infections-causing pericarditis or directly by causing pericarditis as with radiation therapy (23), chemotherapy (e.g., cyclophosphamide, anthracyclines), targeted therapy (e.g., TKIs), or immunotherapy (107).

Consensus statements for cardiac tamponade management in CP recommend that immediate echo-guided pericardiocentesis should be preferentially performed (13). Indeed, in a recent clinical report, percutaneous pericardiocentesis with extended catheter drainage was safe and effective in CPs, including those with thrombocytopenia managed by platelet transfusion support (101). A prolonged drainage (2–5 days) together with intrapericardial instillation of sclerosing agents (e.g., bleomycin) is suggested by experts to reduce the risk of recurrences (13). Since surgical pericardiotomy is less effective in CPs and associated with more complications, experts suggest that it should be conducted only when a safe percutaneous approach is not possible (13). In case of recurrent effusions, experts advise the creation of a pericardial window, surgically or *via* percutaneous balloon pericardiotomy, to reduce the risk of repeated interventions by allowing drainage into an adjacent space, usually the pleura (13), even if the outcome is poor in these situations.

Experts advise treating acute malignant pericarditis in the same way as in non-CPs with non-steroidal anti-inflammatory drugs and colchicine in the absence of contraindications (13) to relieve symptoms and reduce the risk of relapse as well as to avoid the development of constrictive pericarditis (108). Even if pericardial effusion in ICI-related pericarditis is not often associated with hemodynamic compromise and tamponade (109), experts suggest additional treatment with methylprednisolone (1 mg/kg/day) while temporarily discontinuing the ICI (13).

Physicians should be aware that neoplastic tamponades appear to be at the greatest risk for effusive-constrictive pericarditis (110) and pericardial decompression syndrome compared to non-CPs (111). Pericardial decompression syndrome is a very rare but potentially fatal complication following pericardial drainage manifesting with paradoxical hemodynamic deterioration and/or pulmonary edema, commonly associated with ventricular dysfunction. The onset of this syndrome after the procedure varied widely, ranging from "immediate" to 48 h (111).

Cardiogenic/obstructive shock related to cardiac herniation

Cardiac herniation is a very rare complication mainly encountered after cancer thoracic surgery with a high mortality rate (50–100%) (112). Though it was first reported in medical literature in 1948 after a pneumonectomy of the left lung for a carcinoma (113), this disease remains largely unknown (114).

Although it looks similar on both sides of the heart, the pathophysiology mechanism leading to hemodynamic failure is different (115). On the right side, the protrusion of the heart through an ignored or inadequately closed pericardial sac defect usually following a pericardiotomy may enable the heart to rotate its tip to the right around the superior vena cava/inferior vena cava axis, resulting in torsion of these large vessels and leading to a dramatic reduction of cardiac preload and thus cardiac output (116). On the left side, it involves protrusion and/or rotation of the left ventricular through the pericardial defect causing its strangulation (116).

Usually occurring within the first 24 h after surgery, a case report has described a sudden cardiac herniation up to 6 months after a right pneumonectomy (117). The only effective resuscitative treatment seems to be an emergency re-thoracotomy with the closure of the pericardial defect and restoration and fixation of the herniated heart to its normal position (118).

Cardiogenic shock related to neoplastic cardiac infiltration

Primary cardiac tumors are extremely rare, with an autopsy frequency ranging from 0.001 to 0.03% (104). Most of these primary lesions are usually benign (119) but can also be malignant, such as cardiac sarcoma, which accounts for ∼2% of primary cardiac tumors (120). In contrast, cardiac metastases are slightly more common (121), with up to 12% of CPs having metastases to the heart or pericardium at autopsy, although most of them remain clinically silent (122, 123). Thus, only 1% of total extracardiac malignancies have clinically symptomatic cardiac involvement, mainly caused by melanoma, lymphoma, leukemia, and carcinoma of the lung, breast, and esophagus (121, 124). The pathophysiology includes a direct extension

TABLE 5 Main etiologies related to cardiogenic shock in cancer patients.

	Cancer disease	Chemotherapy	Targeted therapy	Immunotherapy	Radiotherapy	Surgery
Acute coronary syndromes	X	X	X		X	
Acute pulmonary embolism	X	X			X	X
Acute cardiomyopathy		X	X		X	
Myocarditis	X	X		X		
Takotsubo syndrome	X	X	X	X		
Cardiac tamponade	X	X	X	X	X	X
Cardiac herniation						X
Neoplastic cardiac infiltration	X					

(e.g., lung carcinoma), hematogenous seeding (e.g., melanoma, lymphoma), venous extension (e.g., renal carcinoma), and retrograde lymphatic seeding (e.g., breast carcinoma) (119).

Although the literature is full of case reports and autopsy studies, the prevalence of cardiac infiltration leading to CS is unknown. The reference treatment for primary cardiac tumors remains cardiac resection surgery, if possible, with few exceptions (125). Resection is usually not indicated for secondary malignant cardiac tumors and treatment mainly relies on other anticancer therapies (126).

General issues for consideration regarding cardiogenic shock among cancer patients

Cancer and cardiovascular diseases are the leading causes of mortality worldwide. Evidence shows that these diseases have common risk factors, in an aging population, and are interconnected by adverse effects of cancer treatments on cardiovascular status (Table 5) (127). However, patients with cancer were excluded from most of the large cardiology studies and registries (128). Therefore, there is very little information on the impact of cancer in CS, even though data are emerging (5).

The occurrence of acute cardiovascular diseases in the cancer trajectory often causes interruption of potentially effective treatment, precluding completion of the therapy and influencing oncologic prognosis.

Core CS therapeutic principles do not differ substantially from non-CPs, even if there are some specificities such as those aforementioned. Complexity rather lies in the treatment intensity of CPs that can reasonably be implemented in the best interest of the patient.

According to a recent experts' review on critically ill oncology and hematology patients, no predefined criteria or prognostic scores of intensive care unit (ICU) or cardiac care unit triage for admission should be used (129). Each situation being different and challenging, the benefit-risk assessment must be discussed in an urgent multidisciplinary manner based on multiple criteria such as performance status (130) and frailty (131, 132) (Figure 2).

Experts also suggest that time-limited trials should be used for CPs, meaning unlimited ICU management with a full-code status for a limited period before a re-evaluation of the clinical situation (129).

The appropriate length of time for full-code status (doing everything that can be done, including cancer chemotherapy and short-term MCS) seems to be 1 week in CPs with solid tumors. To be noted, in the case of multiple organ failure, full-code management for 4 to 5 days leads to similar outcomes as unlimited aggressive care (133). Full-code status seems to be at least 2 weeks in hematology patients unless they are in multiple organ failure, in which case 1 week would be enough to provide the same survival as with unlimited aggressive care (133).

For instance, a full code status should be warranted in CS cases of newly diagnosed malignancies, PE in CPs with good performance status and no frailty, acute cardiac toxicity after complete cancer remission, and clinical response undetermined or still unpredictable (59). If needed, short-term MCS for refractory CS should be implemented before the onset of multi-organ failure in selected patients as a strategy to buy time for cardiac recovery (bridge-to-recovery strategy) or bridge-to-other therapies (bridge-to-decision strategy) (134).

Although evidence for the potential effect of cardioprotective drug therapy in preventing or mitigating the cardiotoxic effects of cancer therapy is incomplete to date, these therapies may play an important role in the future (135, 136).

Conclusion

Cancer and cardiovascular diseases are the most prevalent diseases worldwide. Cardiogenic shock among cancer patients is an issue that can occur as a result of various causes and is likely to increase in the coming years. With improvements in cancer therapy and intensive care medicine, cardiogenic shock in cancer

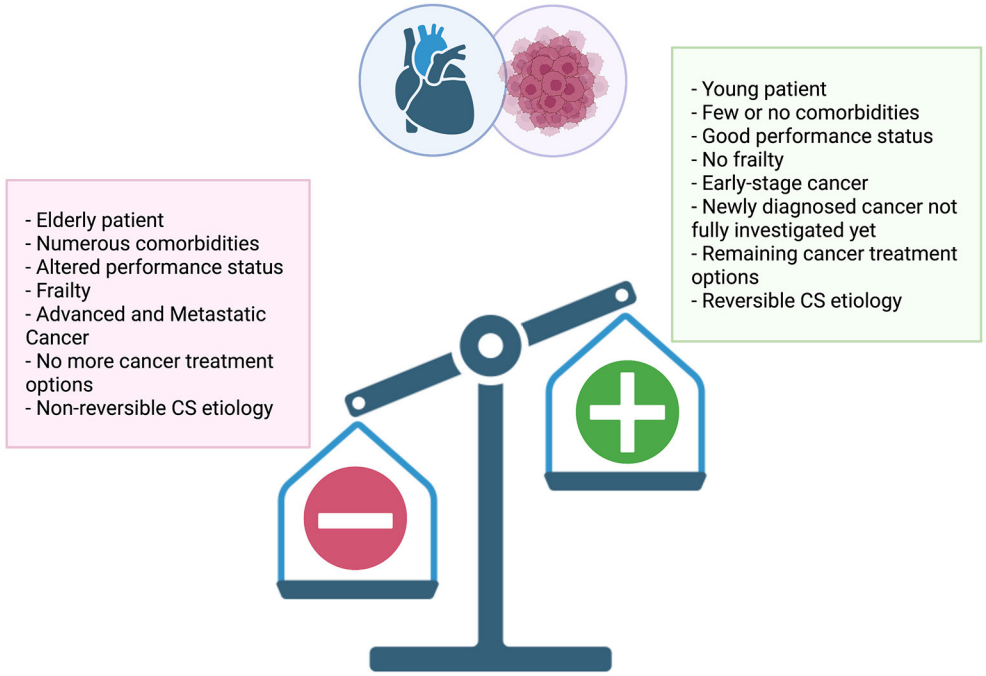

FIGURE 2
Multiple criteria to consider when assessing treatment intensity of cardiogenic shock in cancer patients.

patients no longer means poor survival prognosis and initiation of palliative care. Rather, full code status with unlimited intensive care management (no restriction) is indeed worthwhile in some very speific situations. Multidisciplinary collaboration between intensivists, cardiologists, cardiac surgeons, and oncologists is essential in these critical situations.

Author contributions

AC, FM, and HM wrote the manuscript, reviewed, and edited the article before submission. CD, JG, LZ, and MS revised the manuscript. AC and HM created the figure with BioRenders.com subscribed to HM. All authors approved the final manuscript.

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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Derivation, validation and assessment of a novel nomogram-based risk assessment model for venous thromboembolism in hospitalized patients with lung cancer: A retrospective case control study

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Purpose: This study aimed to develop and validate a specific risk-stratification nomogram model for the prediction of venous thromboembolism(VTE) in hospitalized patients with lung cancer using readily obtainable demographic, clinical and therapeutic characteristics, thus guiding the individualized decision-making on thromboprophylaxis on the basis of VTE risk levels.

Methods: We performed a retrospective case–control study among newly diagnosed lung cancer patients hospitalized between January 2016 and December 2021. Included in the cohort were 234 patients who developed PTE and 936 non-VTE patients. The patients were randomly divided into the derivation group (70%, 165 VTE patients and 654 non-VTE patients) and the validation group (30%, 69 VTE patients and 282 non-VTE patients). Cut off values were established using a Youden´s Index. Univariate and multivariate regression analyses were used to determine independent risk factors associated with VTE. Variance Inflation Factor(VIF) was used for collinearity diagnosis of the covariates in the model. The model was validated by the consistency index (C-index), receiver operating characteristic curves(ROC) and the calibration plot with the Hosmer-Lemeshow goodness-of-fit test. The clinical utility of the model was assessed through decision curve analysis(DCA). Further, the comparison of nomogram model with current models(Khorana, Caprini, Padua and COMPASS-CAT) was performed by comparing ROC curves using the DeLong's test.

Results: The predictive nomogram modle comprised eleven variables: overweight(24-28) defined by body mass index (BMI): [odds ratio (OR): 1.90,

95% confidence interval (CI): 1.19-3.07], adenocarcinoma(OR:3.00, 95% CI: 1.88-4.87), stageIII-IV(OR:2.75, 95%CI: 1.58-4.96), Central venous catheters (CVCs) (OR:4.64, 95%CI: 2.86-7.62), D-dimer levels≥2.06mg/L(OR:5.58, 95% CI:3.54-8.94), PT levels≥11.45sec(OR:2.15, 95% CI:1.32-3.54), Fbg levels≥3.33 g/L(OR:1.76, 95%CI:1.12-2.78), TG levels≥1.37mmol/L (OR:1.88, 95%CI:1.19-2.99), ROS1 rearrangement(OR:2.87, 95%CI:1.74-4.75), chemotherapy history (OR:1.66, 95%CI:1.01-2.70) and radiotherapy history(OR:1.96, 95%CI:1.17-3.29). Collinearity analysis with demonstrated no collinearity among the variables. The resulting model showed good predictive performance in the derivation group (AUC 0.865, 95% CI: 0.832-0.897) and in the validation group(AUC 0.904,95%CI:0.869-0.939). The calibration curve and DCA showed that the risk-stratification nomogram had good consistency and clinical utility. Futher, the area under the ROC curve for the specific VTE risk-stratification nomogram model (0.904; 95% CI:0.869-0.939) was significantly higher than those of the KRS, Caprini, Padua and COMPASS-CAT models(Z=12.087, 11.851, 9.442, 5.340, all P<0.001, respectively).

Conclusion: A high-performance nomogram model incorporated available clinical parameters, genetic and therapeutic factors was established, which can accurately predict the risk of VTE in hospitalized patients with lung cancer and to guide individualized decision-making on thromboprophylaxis. Notably, the novel nomogram model was significantly more effective than the existing well-accepted models in routine clinical practice in stratifying the risk of VTE in those patients. Future community-based prospective studies and studies from multiple clinical centers are required for external validation.

KEYWORDS

lung cancer, venous thromboembolism, risk assessment, nomogram model, thromboprophylaxis

Introduction

Venous thromboembolism (VTE), which manifests as deep vein thrombosis (DVT) and pulmonary thromboembolism (PE), is a major global burden of disease (1). DVT mostly affects the deep veins of the lower limbs. After the thrombi dislodge from clots in the deep veins of the lower limbs falls off, it can drift along with the blood flow and block the pulmonary arteries and its branches, resulting in PE. Hence, DVT and PE, collectively referred to as VTE, are the manifestations of the same disease at different stages. Of note, it has been established that there is a strong association between cancer and VTE events (2). On the one hand, patients with malignancy are at a high risk of VTE, account for approximately 20% of all patients complicated with vein thrombosis, and have a 4 to 7 fold increased risk of developing VTE compared to the general population (3). While on the other hand, cancer-associated thrombosis (CAT) is commonly associated with higher morbidity and mortality, increased hospital stay, reduced quality of life and higher medical costs (4, 5). Indeed, VTE is responsible for 9% of death in cancer patients, making it the second leading cause of death in cancer patients (6). As a result, VTE events continue to be common and potentially fatal complication in cancer inpatients.

However, the incidence of VTE might be underestimated due to the low rate of clinical detection, as well as the high rates of misdiagnosis and missed diagnosis (7). Several studies have reported that patients with lung cancer (LC) have a relatively higher of VTE development than patients with other solid tumors (8, 9). Furthermore, it has been recently recognized that VTE is surprisingly common in newly-diagnosed patients with LC and linked with poor prognosis (10). More importantly, considerable morbidity of long-term complications results from VTE, such as post-thrombotic syndrome and chronic thromboembolic pulmonary hypertension, which not only affect the treatment of patients with primary diseases but also reduce the patients' quality of life (11, 12). Given the diminishing evidence regarding the benefits of VTE thromboprophylaxis in low risk situations, overprophylaxis is clearly undesirable, and could result in an inherent risk of bleeding which may offset its clinical benefits (13). Consequently, early detection of high-risk

factors for lung cancer combined with VTE should be paid for particular attention. There is an urgent need for useful clinical tools to accurately predict the risk of VTE in hospitalized patients with LC and to guide individualized decision-making on thromboprophylaxis.

Presently, nomogram-based prediction model has been widely used as a user-friendly screening tool for the diagnosis and prognosis of diseases (14). Nomogram is a visual display of complex mathematical formulas, which integrates multiple prediction variables and then uses the line with scale according to a certain proportion, so that the probability of occurrence of predicted events can be simply determined. Currently, comprehensive treatments such as surgery, chemotherapy, radiotherapy and more recently immunotherapy have been recognized as additional risk factors for VTE in patients with lung cancer (15–17). In particular, the current analysis demonstrates that nomogram has good risk-prediction ability for VTE in postoperative lung cancer patients (18). However, studies on the use of a nomogram model for predicting the risk of CAT are limited, especially for patients with LC receiving first-line systemic therapy (19–21). Meanwhile, there is less risk assessment models(RAMs) to evaluate the risk of VTE exclusively for hospitalized cancer patients. Therefore, more attention should be paid to the construction of specific VTE risk assessment model to guide prophylaxis decisions for hospitalized LC patients.

Considering the differences in the patient population characteristics and treatment modalities, it is necessary to develop an accurate, objective, and practical tool to predicting VTE in lung cancer patients using available clinical parameters, which would be helpful in guiding clinical decision-making on prophylaxis. Therefore, the aim of this study was to develop and validate a specific risk-stratification nomogram model for the prediction of VTE in lung cancer patients to provide a theoretical basis for the individualized treatment on the basis of VTE risk levels, thus guiding the implementation of clinical prevention and treatment.

Materials and methods

Study design

This study was a matched case-control study. Data from a total of 10,053 newly diagnosed lung cancer patients admitted to The Affiliated Tumor Hospital of Xinjiang Medical University between January 2016 and December 2021 were collected and retrospectively analyzed. Patients enrolled in our study were all inpatients. The calculation of the sample size was based on demonstrating the probability of exposure among sampled control patients was 0.2 with 90% power and 5% statistical significance. Therefore, the obtained sample size of 234 VTE patients was adequate to address the study aims. To reduce potential selection bias between groups, a 1:4 ratio propensity score matching (PSM) method was performed with optimal full matching (22) with the covariates age, gender, and ethnicity. For each VTE patient, a matched sample of 4 non-VTE patients was also obtained. Thus, this sample of 936 non-VTE patients were selected out of the total 9819 control cases. Enrolled patients were further randomized into the derivation group (70%, 165 VTE patients and 654 non-VTE patients) and the validation group (30%, 69 VTE patients and 282 non-VTE patients) (Figure 1).

The study was conducted in accordance with the ethical standards revised in the 2013 Declaration of Helsinki. This study was approved by the Ethics Committee of The Affiliated Tumor Hospital of Xinjiang Medical University with the ethical approval number: 2019BC007. However, the requirement to obtain informed consent for any research utilizing patients' medical information was waived owing to the retrospective design of the study.

Patients and eligibility

The inclusion criteria for the VTE group were as follows: (a) 18 years or older; (b) length of hospital stay >3 days;(c) all primary lung malignant tumors confirmed by histopathological examination; (d) with DVT and/or PE events confirmed by objective imaging methods and (f) complete case data. The primary diagnosis of VTE (DVT and/or PE) and comorbidities were abstracted from electronic medical records (EMR) according to the International Statistical Classification of Diseases, 10th Clinical Modification (ICD-10 CM).

The exclusion criteria were as follows: (a) inpatients hospitalized for<72h; (b) second malignancy other than lung cancer; (c) patients with acute coronary syndrome or history of implantation of intracardiac devices (pacemakers, prosthetic valves, or implantable cardioverter-defibrillators, etc.) or history of VTE prior to admission; (d) prophylactic anticoagulation before VTE occurring during antitumor therapies; (e) patients receiving long-term therapeutic anticoagulation (at least 3 months) before hospitalization; (f) previous hematological diseases, rheumatoid arthritis, limited liver and kidney function and/or damage.

Controls were selected by propensity score matching (PSM) method from adult lung cancer inpatients (length of hospital stay>3 days) admitted into the same departments during the same period as cases, without an ICD-10 code for VTE (DVT and/or PE) at discharge. The same exclusion criteria used for

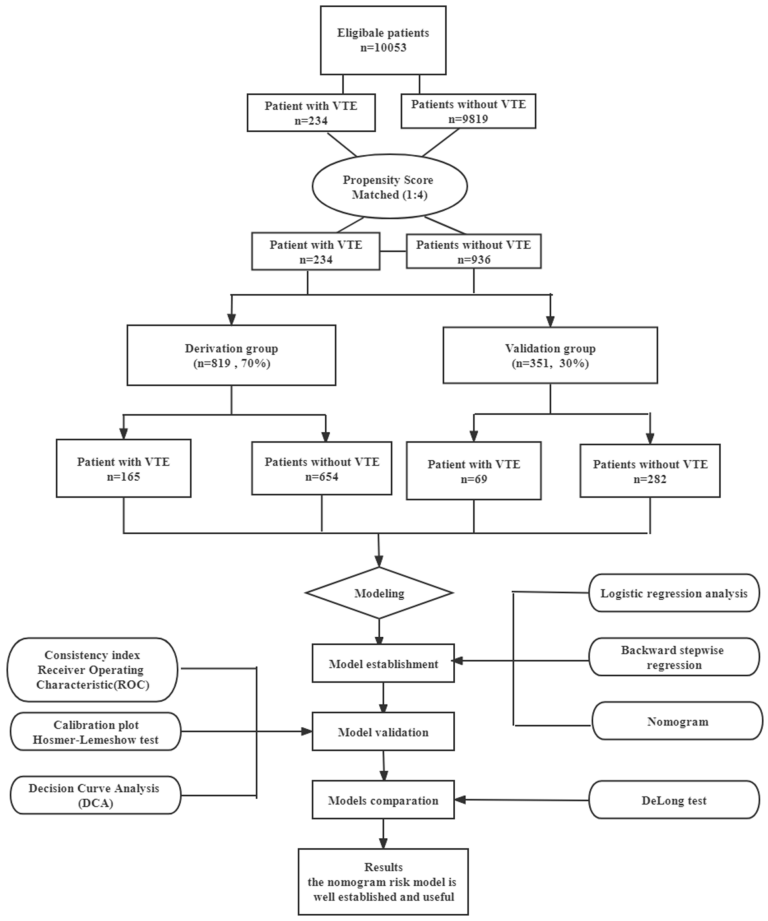

FIGURE 1
Flow chart of the study design and analysis. VTE, venous thromboembolism.

cases were also applied to controls. Controls were frequency-matched to cases at a ratio of 4:1.

VTE diagnosis

Symptomatic or incidental VTE that occurred within the first 6 months of cancer diagnosis during the patients'hospitalization was the primary outcome of the study, including DVT and PE. The diagnosis of PE was made by computed tomography pulmonary angiography(CTPA) according to the consensus guidelines, with single/bilateral/multi-lobar pulmonary artery embolism and its branches being the main type. The diagnosis of DVT was made by computed tomography(CT) angiography or complete compression venous ultrasonography(CUS) according to standard ultrasonographic criteria (23), with venous blood stasis in the upper- and lower-extremities. All VTE events were was independently reviewed by two experienced experts in the field of angiology and radiology.

Data collection and follow-up

The abstracted data were extracted from the electronic EMR retrospectively: patients' demographic and clinicopathological characteristics including age, gender, ethnicity, smoking history, blood type, body mass index (BMI) before initial treatment (baseline), Eastern Cooperative Oncology Group performance status (ECOG PS), central venous catheters(CVCs) indwelling history, tumor pathology(adenocarcinoma and non-adenocarcinoma) and clinical stage(early and advanced stage), PD-L1 expression(<50%, ≥50%), and diver genes status(EGFR and KRAS mutation, ALK and ROS1 rearrangement and wild type); Detailed information about historic treatment regimens including surgery, targeted therapy, chemotherapy, radiotherapy or immunotherapy during the follow-up period for case group and control group; Comorbid conditions including hypertension, diabetes and coronary heart disease. In order to avoid the effect of anticancer treatment on the value of the indicator, all laboratory examination data were obtained from

the pre-treatment baseline assessment after admission. All D-dimer levels were assayed in plasma using the Innovance D-dimer immunoturbidimetric method (Siemens Healthcare, Eschborn, Germany). Laboratory examination data including routine blood indicators(haemoglobin(Hb), leucocyte platelet (Plt), neutrophil-to-lymphocyte ratio(NLR), platelet-to-lymphocyte ratio(PLR)); Coagulation function indexs (prothrombin time(PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg), D-dimer (Ddi)); Biochemical routine (albumin, alanine transaminase(ALT), aspartate transaminase (AST), Lactate dehydrogenase (LDH), triglyceride(TG)); Pro-Brain natriuretic peptide (pro-BNP)) and tumor biomarkers (cytokeratin-19-fragment(CYFR121-1), carcinoembryonic antigen(CEA), carbohydrate antigen 125(CA125), Gastrin releasing peptide(Pro-GRP) and neuron-specific enolase (NSE)). Further, VTE risk was evaluated *via* Khorana Prediction Score, Padua Prediction Score, Caprini Risk Assessment model and COMPASS-cancerassociated thrombosis score (COMPASS-CAT), respectively. Predictor variables were identified from synthesis of the literatures about VTE risk (24–27). All patients were followed up by telephone or hospital visit until the occurrence of VTE, death or end of follow-up in March 2022.

Derivation and internal verification of the nomogram

The risk assessment model was developed in the derivation cohorts by binary multiple logistic regression analysis. Internal validation was performed in the internal validation cohorts. The Chi-square test for categorical variables were used to compare the baseline characteristics between the derivation and validation cohorts.

Logistic regression analysis for univariate and multivariate analyses and stepwise regression analysis were used to evaluate the independent factors influencing thrombosis in lung cancer patients. Variables with a *P*-value <0.05 in the univariate regression analysis were included in multivariate logistic regression analysis. Afterward, variables with clinical significance and those with $P < 0.05$ in the multivariate analysis were included in the backward stepwise logistic regression analysis. Backward stepwise selection was applied using the likelihood ratio test with Akaike's information criterion (AIC) minimum method as the termination rule (28). The effect measure of each variable on VTE was presented as odds ratios (OR) and corresponding 95% confidence intervals (CI). Variance Inflation Factor (VIF) was used for collinearity diagnosis of the covariates in the model. Then the nomogram was constructed by using the RMS package in the R (r4.1.3) software to visually score individual risk probabilities of VTE in lung cancer patients.

The reliability of internal validation was assessed using the bootstrap method with 1000 replicates. The discrimination of the nomogram model was evaluated by the consistency index (C-index) and receiver operating characteristic curve (ROC) analysis. Further, the area under the ROC curve (AUC) was obtained to quantitatively evaluate the discriminative ability of the nomogram to predict VTE in patients with lung cancer. The possible value for an AUC ranges from 0.5 (no better discrimination than chance) to 1.0 (perfect discrimination). Moreover, calibration curve was plotted to assess the calibration of the nomogram with the Hosmer-Lemeshow goodness-of-fit test, and a p-value of the Hosmer-Lemeshow test > 0.05 indicates that a model has high goodness of fit. Finally, decision curve analysis (DCA) was performed to assess the clinical utility of the predictive nomogram model for guiding clinical decision making of thromboprophylaxis in lung cancer (29).

Assessment of risk of bias and applicability

Risk of bias (ROB) and applicability was assessed using the Prediction Study Risk Of Bias Assessment Tool (PROBAST) (30). The assessment of ROB comprises four domains—participants, predictors, outcome, and analysis, questions are answered as "yes", "probably yes", "probably no", "no", or "no information". The degree of ROB and applicability were judged as "low", "high", or "unclear" for each domain. Risk of bias and applicability assessment was performed by one reviewer and checked by a further reviewer. Any disagreements were mediated by a third reviewer.

Diagnostic value of the nomogram

The diagnostic performance of new prediction model and current models (Khorana, Caprini, Padua and COMPASS-CAT) were evaluated assessed by calculating the AUC. The diagnostic value of the nomogram was assessed by calculating the sensitivity, specificity, positive predictive values (PPV) and negative predictive values(NPV). Further, the comparison of new prediction model with current models was performed by comparing ROC curves using the DeLong's test.

Statistical analysis

SPSS version 25.0 software (IBM, USA) and R version 4.1.3 software (https://www.r-project.org/) were performed for statistical analysis. PSM was performed with optimal full matching by the R package '*Matchlt*'. Multiple imputation with chained equations was used to replace missing data for BMI

values. Considering the model's extrapolation accuracy and clinical application, continuous variables were transformed into categorical variables by determining the optimal cut-off (OCF) value according to the maximum Youden index on the basis of the receiver operating characteristic(ROC) curves. The continuous BMI variable was categorized based on cut-off values routinely used in clinical practice for ease of interpretation. Categorical variables were presented as whole numbers and proportions. Comparisons between two groups were performed using the Chi-squared test for categorical variables. A two- sided p-value < 0.05 indicated statistical significance.

Results

Patient characteristics and VTE incidence

A total of 1170 patients were enrolled and randomly assigned at a ratio of 7:3, resulting in 819 patients assigned to the derivation group and 351 assigned to the validation group (Figure 1). Based on the current sample size and effect size, our study has a statistical power of 91%, which exceeds the minimal statistical power of 80% required for the adequacy of sample sizes. The control's lung cancer duration(time since cancer diagnosis) was ≥ the case's duration to ensure that the control would have equal exposure to the risk of VTE induced by cancer. The demographic and clinical characristics of patients in the derivation and validation cohorts are illustrated in Table 1, indicate that most characterisics were similarly distirbuted between the two cohorts. Overall, 165(20.1%) patients in the development cohort and 69(19.7%) patients in the validation cohort developed VTE, and there was no significant difference in VTE morbidity between the two cohorts (c^2=0.037, P = 0.848). Similarly, there were no significant differences of incidence in DVT alone(4.2% vs.3.1%), PE alone (14.9% vs.15.4%), and DVT&PE (1.1% vs.1.1%)(c^2=0.407, 0.046, 0.004, P=0.688, 0.830, 0.951, respectively) (Table 1)

Exploration of risk factors for VTE

The results of univariate and multivariate logistic regression analyses based on the factors associated with VTE are presented in Table 2. In the validation group, univariate analysis showed that the following factors were statistically significant: BMI, histology, clinical stage, CVC history, hypertension, NLR, coagulation function indexs(D-dimer, ATTT, PT and Fbg levels), biochemical routine indexs(albumin, LDH and TG levels), Pro-BNP, tumor biomarkers(CEA, CA125 and Pro-GPR levels), molecular driver status(ROS1 rearrangement and PD-L1 high expression) and historic treatment regimens(chemotherapy, radiotherapy, and immunotherapy history)(P<0.05).

Afterward, according to the multivariate and backward stepwise logistic analysis, the results showed overweight(24-28) defined by BMI[1.90(1.19-3.07)], adenocarcinoma[3.00(1.88-4.87)], stageIII-IV[2.75(1.58-4.96)], CVC history[4.64(2.86-7.62)], D-dimer levels≥2.06 mg/L[5.58(3.54-8.94)], PT levels≥11.45sec[2.15(1.32-3.54)], Fbg levels≥3.33 g/L[1.76(1.12-2.78)], TGlevels≥1.37mmol/L[1.88(1.19-2.99)], ROS1rearrangement[2.87(1.74-4.75)],chemotherapy history [1.66(1.01-2.70)] and radiotherapy history[1.96(1.17-3.29)] ROS1rearrangementwere considered to be independent risk factors for VTE in lung cancer, and these factors were eventually incorporated into the final model (Table 2). Furthermore, the collinearity diagnostic analysis demonstrated that the VIFs of those risk factors were less than 4, indicating that there is no strong indication of multicollinearity among variables. Thus, there were eleven variables included in the final multivariable prediction model as predictors (Figure 2).

Development of the nomogram model

Based on the regression coefficient of these risk factors, the risk score model of VTE[Logit (P)] was constructed as Logit(P) = -6.306+0.643×BMI+1.099×Pathology+1.011×TNM-stage+1.543×CVC history+1.719×D-dimer+0.766×PT+0.563×Fbg +0.631×TG+1.055×ROS1-rearrangement+0.505×chemotherapy history+0.674×radiotherapy history. For visualization and convenient clinical use of the predictive model, the mathematical risk prediction model was visualized as a nomogram to predict the likelihood of VTE in hospitalized patients with lung cancer (Figure 2). The probability of developing VTE can be determined by assigning points for each variable by drawing a line upward to the Points axis, summing all the points from the variables plotted on the total points axis and then drawing a vertical line from the total points axis straight down to the risk of VTE axis. For example, the application of this model to a 53-year-old patient with lung cancer would show the following results: BMI of 28 kg/m^2, histology of adenocarcinoma, IV stage, with CVC history, D-dimer of 5.97 mg/L, PT of 11.8 sec, Fbg of 2.48 sec, TG of 1.2g/L, ROS1 rearrangement(+), without chemotherapy and radiotherapy history. The total score of the above predictors was 38 + 64+59+89+100+44+0+0+61+0+0 = 455 and the corresponding risk probability of VTE was 0.81 (81%)

Performances of discrimination and calibration

The AUCs in the development and validation group were 0.865 (95% CI: 0.832-0.897) and 0.904 (95%CI: 0.869-0.939), respectively, which indicated the good prediction performance of the model (Figure 3). The proposed nomogram was validated

TABLE 1 Demographic and clinical characteristics of patients in derivation and validation cohorts.

Variables [n (%)]	Categories	Development group				Validation group			
		VTE (+)	VTE (-)	χ^2	*P* value	VTE (+)	VTE (-)	χ^2	*P* value
Age (years)				—	0.770*			—	0.699*
<40		2 (1.2)	17 (2.6)			2 (2.9)	6 (2.1)		
40-50		11 (6.7)	40 (6.1)			6 (8.7)	17 (6.0)		
50-60		42 (25.5)	176 (26.9)			21 (30.4)	78 (27.7)		
≥60		110 (66.7)	421 (64.4)			40 (58.0)	181 (64.2)		
Sex				0.057	0.817			0.645	0.422
Male		89 (53.9)	362 (55.4)			42 (60.9)	154 (54.6)		
Female		76 (46.1)	292 (44.6)			27 (39.1)	128 (45.4)		
Ethnicity				0.857	0.651			3.006	0.222
Han		138 (83.6)	528 (80.7)			53 (76.8)	233 (82.6)		
Uygur		15 (9.1)	65 (9.9)			7 (10.1)	30 (10.6)		
Others ethnic minorities		12 (7.3)	61 (9.3)			9 (13.0)	19 (6.7)		
VTE events type				—	—			—	—
DVT alone		34 (20.6)				11 (15.9)			
PT alone		122 (73.9)				54 (78.3)			
DVT&PT		9 (5.5)				4 (5.8)			
Blood type				28.881	<0.001			—	0.021*
A		39 (28.7)	122 (25.4)			17 (32.7)	56 (27.9)		
B		55 (40.4)	107 (22.3)			17 (32.7)	38 (18.9)		
AB		18 (13.2)	59 (12.3)			6 (11.5)	17 (8.5)		
O		24 (17.6)	192 (40.0)			12 (23.1)	90 (44.8)		
BMI[a] (kg/m^2)				9.363	0.009			6.986	0.030
Normal(<24.0)		72 (43.6)	370 (56.6)			25 (36.2)	146 (51.8)		
Overweight(24.0-28.0)		69 (41.8)	220 (33.6)			33 (47.8)	89 (31.6)		
Obesity (≥28.0)		24 (14.5)	64 (9.8)			11 (15.9)	47 (16.7)		
ECOG PS				2.189	0.139			0.987	0.320
0-1		159 (96.4)	607 (92.8)			66 (95.7)	257 (91.1)		
≥2		6 (3.6)	47 (7.2)			3 (4.3)	25 (8.9)		
Histology				15.449	<0.001			10.908	0.001
Adenocarcinoma		45 (27.3)	291 (44.5)			22 (31.9)	155 (55.0)		
Non-adenocarcinoma		120 (72.7)	363 (55.5)			47 (68.1)	127 (45.0)		
cTNM stage[b]				12.687	<0.001			13.715	<0.001
I-II		25 (15.2)	191 (29.2)			5 (7.2)	84 (29.8)		
III-IV		140 (84.8)	463 (70.8)			64 (92.8)	198 (70.2)		
Smoke history				0.005	0.943			8.239	0.004
Never		96 (58.2)	376 (57.5)			26 (37.7)	163 (57.8)		
Current and former		69 (41.8)	278 (42.5)			43 (62.3)	119 (42.2)		
CVC history		103 (62.4)	148 (22.6)	96.303	<0.001	42 (60.9)	72 (25.5)	29.976	<0.001
History of disease									
Hypertension		66 (40.0)	179 (27.4)	9.431	0.002	18 (26.1)	91 (32.3)	0.722	0.395
Diabetes mellitus		17 (10.3)	96 (14.7)	1.769	0.183	9 (13.0)	51 (18.1)	0.67	0.413
Heart disease		15 (9.1)	60 (9.2)	<0.001	1	8 (11.6)	41 (14.5)	0.193	0.661
Blood routine									
Hemoglobin(g/L)				2.963	0.085			—	0.190*
	<95	5 (3.0)	46 (7.0)			2 (2.9)	23 (8.2)		
	≥95	160 (97.0)	608 (93.0)			67 (97.1)	259 (91.8)		
Platelet(×109 g/L)				2.627	0.105			10.221	0.001

(Continued)

TABLE 1 Continued

Variables [n (%)]	Categories	Development group				Validation group			
		VTE (+)	VTE (-)	χ^2	*P* value	VTE (+)	VTE (-)	χ^2	*P* value
	<222	73 (44.2)	338 (51.7)			22 (31.9)	153 (54.3)		
	≥222	92 (55.8)	316 (48.3)			47 (68.1)	129 (45.7)		
NLR				9.726	0.002			4.987	0.026
	<3.42	64 (38.8)	345 (52.8)			25 (36.2)	147 (52.1)		
	≥3.42	101 (61.2)	309 (47.2)			44 (63.8)	135 (47.9)		
PLR				1.212	0.271			0.914	0.339
	<146.24	58 (35.2)	263 (40.2)			22 (31.9)	110 (39.0)		
	≥146.24	107 (64.8)	391 (59.8)			47 (68.1)	172 (61.0)		
Blood coagulation									
D-dimer(mg/L)				98.756	<0.001			64.215	< 0.001
	<2.06	58 (35.2)	497 (76.0)			20 (29.0)	224 (79.4)		
	≥2.06	107 (64.8)	157 (24.0)			49 (71.0)	58 (20.6)		
APTT(sec)				10.083	0.001			6.346	0.012
	<24.15	29 (17.6)	57 (8.7)			13 (18.8)	22 (7.8)		
	≥24.15	136 (82.4)	597 (91.3)			56 (81.2)	260 (92.2)		
PT(sec)				26.946	<0.001			21.062	< 0.001
	<11.45	44 (26.7)	324 (49.5)			11 (15.9)	133 (47.2)		
	≥11.45	121 (73.3)	330 (50.5)			58 (84.1)	149 (52.8)		
Fbg(g/L)				5.045	0.025			5.226	0.022
	<3.33	63 (38.2)	316 (48.3)			20 (29.0)	127 (45.0)		
	≥3.33	102 (61.8)	338 (51.7)			49 (71.0)	155 (55.0)		
Biochemical routine									
Albumin(g/L)				8.02	0.005			8.541	0.003
	<41	123 (74.5)	408 (62.4)			53 (76.8)	160 (56.7)		
	≥41	42 (25.5)	246 (37.6)			16 (23.2)	122 (43.3)		
ALT(IU/L)				0.666	0.414			0.265	0.607
	<9.05	25 (15.2)	81 (12.4)			10 (14.5)	32 (11.3)		
	≥9.05	140 (84.8)	573 (87.6)			59 (85.5)	250 (88.7)		
AST(IU/L)				1.509	0.219			2.218	0.136
	<18.05	70 (42.4)	241 (36.9)			31 (44.9)	97 (34.4)		
	≥18.05	95 (57.6)	413 (63.1)			38 (55.1)	185 (65.6)		
LDH(U/L)				12.007	0.001			8.629	0.003
	<258.25	112 (67.9)	528 (80.7)			46 (66.7)	235 (83.3)		
	≥258.25	53 (32.1)	126 (19.3)			23 (33.3)	47 (16.7)		
TG(mmol/L)				5.35	0.021			2.526	0.112
	<1.37	79 (47.9)	381 (58.3)			36 (52.2)	179 (63.5)		
	≥1.37	86 (52.1)	273 (41.7)			33 (47.8)	103 (36.5)		
Pro-BNP(pg/ml)				15.727	<0.001			11.3	0.001
	<263.75	113 (68.5)	541 (82.7)			46 (66.7)	240 (85.1)		
	≥263.75	52 (31.5)	113 (17.3)			23 (33.3)	42 (14.9)		
Tumor biomarkers									
CYFA21-1(ng/mL)				3.392	0.066			0.003	0.298
	<3.34	92 (55.8)	418 (63.9)			39 (56.5)	181 (64.2)		
	≥3.34	73 (44.2)	236 (36.1)			30 (43.5)	101 (35.8)		
CEA(ug/L)				4.186	0.041			4.267	0.039
	<3.18	58 (35.2)	290 (44.3)			27 (39.1)	152 (53.9)		
	≥3.18	107 (64.8)	364 (55.7)			42 (60.9)	130 (46.1)		

(Continued)

TABLE 1 Continued

Variables [n (%)]	Categories	Development group				Validation group			
		VTE (+)	VTE (-)	χ^2	*P* value	VTE (+)	VTE (-)	χ^2	*P* value
CA125(U/mL)				10.858	0.001			9.77	0.002
	<38.45	82 (49.7)	419 (64.1)			33 (47.8)	194 (68.8)		
	≥38.45	83 (50.3)	235 (35.9)			36 (52.2)	88 (31.2)		
Pro-GPR(pg/mL)				3.974	0.046			4.117	0.042
	<37.23	112 (67.9)	386 (59.0)			49 (71.0)	160 (56.7)		
	≥37.23	53 (32.1)	268 (41.0)			20 (29.0)	122 (43.3)		
NSE(ng/mL)				1.273	0.259			0.478	0.49
	<17.32	100 (60.6)	362 (55.4)			41 (59.4)	152 (53.9)		
	≥17.32	65 (39.4)	292 (44.6)			28 (40.6)	130 (46.1)		
Molecular driver									
EGFR mutation		21 (13.5)	115 (18.5)	1.748	0.186	9 (13.4)	48 (18.3)	0.563	0.453
KRAS mutation		13 (8.4)	34 (5.7)	1.129	0.288	5 (7.8)	16 (6.3)	–	0.585*
ROS1 rearrangement		49 (31.2)	103 (16.5)	16.459	<0.001	49 (31.2)	103 (16.5)	21.308	<0.001
ALK rearrangement		28 (17.8)	104 (16.6)	0.069	0.793	10 (14.7)	31 (11.4)	0.293	0.588
PD-L1 expression(≥50%)		39 (6.7)	19 (12.8)	5.146	0.023	24 (9.3)	9 (14.8)	1.027	0.311
Treatment history[c]									
Chemotherapy		93 (56.4)	186 (28.4)	44.504	<0.001	45 (65.2)	93 (33.0)	22.817	<0.001
Radiotherapy		54 (32.7)	125 (19.1)	13.513	<0.001	15 (21.7)	52 (18.4)	0.206	0.650
Targeted Therapy		123 (18.8)	33 (20.0)	0.057	0.812	49 (17.4)	23 (33.3)	7.707	0.006
Immunotherapy		18 (10.9)	33 (5.0)	6.785	0.009	6 (8.7)	23 (8.2)	<0.001	1
Surgery		56 (33.9)	192 (29.4)	1.102	0.294	20 (29.0)	72 (25.5)	0.187	0.666
The Khorana score				4.056	0.044			3.841	0.050
1-2 points		142 (86.1)	599 (91.6)			56 (81.2)	255 (90.4)		
≥3 points		23 (13.9)	55 (8.4)			13 (18.8)	27 (9.6)		

[a]BMI was categorized according to the Chinese population standards.
[b]According to the 8 th edition of the AJCC/UICC staging system.
[c]All the anti-cancer therapies were within 6 month before the VTE diagnosis.
*P values were derived from Fisher Exact test.
VTE, venous thromboembolism; BMI, Body Mass Index; ECOG PS, Eastern Cooperative Oncology Group performance status; CVC, central venous catheter; NLR, Neutrophil-to-Lymphocyte ratio; PLR, Platelet-to-Lymphocyte ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; Fbg, fibrinogen; ALT, alanine transaminase; AST, aspartate transaminase; LDH, Lactate dehydrogenase;TG, triglyceride; pro-BNP, Pro-Brain natriuretic peptide; CYFR121-1, cytokeratin-19-fragment; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; Pro-GRP, Gastrin releasing peptide; NSE, neuron-specific enolase.

internally using the bootstrap method with 1000-bootstrap repetitions in the development cohort, with a C-index of 0.904, which indicated that the novel proposed model achieved high prediction accuracy. Furthermore, the calibration curve of the nomogram for the prediction of the risk of VTE in patients with lung cancer demonstrated good agreement between prediction and observation in the development (Figure 4A) and validation (Figure 4B) cohorts. The findings of the Hosmer–Lemeshow goodness-of-fit test also was not significant in the development and validation sets(c^2=14.848, 4.276, P = 0.062, 0.831, respectively).

Clinical utility of the model

The DCA curves for the predictive nomogram are presented in Figure 5. The clinical utility of nomogram model was estimated using DCA by quantifying the net benefits at different threshold probabilities. The DCA displayed that the nomogram provided superior net benefit of thromboprophylaxis in patients at VTE risk than strategies of treating all and treating none, with a probability threshold interval of 2%–82% (Figure 5).

Risk of bias and applicability

An overview of the risk of bias(ROB) and the applicability for this prediction model is provided in Supplementary Table 1. The model was rated as high risk of bias in two domains: Predictors and analysis. The risk model had a high ROB and good applicability. The high risk of bias was judged according to some specific issues in the study design and statistical analysis (see the rationale of rating in Supplementary Table 1)

TABLE 2 Univariate and multivariate regression analysis of risk factors associated with VTE in lung cancer.

Variables	Univariate analysis			Multivariate analysis			Stepwise regression analysis		
	β	OR(95% CI)	*P* value	β	OR(95% CI)	*P* value	β	OR(95% CI)	*P* value
Age (years)									
<40		Ref							
40-50	0.850	2.34 (0.55-16.16)	0.301						
50-60	0.708	2.03 (0.55-13.11)	0.357						
≥60	0.798	2.22 (0.62-14.14)	0.291						
Sex, Female (vs. Male)	0.058	1.06 (0.75-1.49)	0.745						
Ethnicity									
Han	Ref	Ref							
Uygur	-0.128	0.88 (0.47-1.56)	0.68						
Others ethnic minorities	-0.288	0.75 (0.38-1.39)	0.389						
BMI[a] (kg/m2)									
Normal(<24.0)		Ref							
Overweight(24.0-28.0)	0.896	2.45 (1.73-3.51)	<0.001	0.792	2.21 (1.29-3.81)	0.004	0.643	1.90 (1.19-3.07)	0.008
Obesity (≥28.0)	0.854	2.35 (1.37-4.24)	0.003	0.779	2.18 (0.97-4.84)	0.057	0.659	1.93 (0.95-3.88)	0.066
ECOG PS, ≥2 (vs. 0-1)	-0.713	0.49 (0.18-1.08)	0.104						
Adenocarcinoma(vs. Non-Adenocarcinoma)	0.761	2.14 (1.48-3.14)	<0.001	1.273	3.57 (2.07-6.37)	<0.001	1.099	3.00 (1.88-4.87)	<0.001
cTNM stage[b]**, III-IV(vs. I-II)**	0.837	2.31 (1.49-3.72)	<0.001	1.328	3.78 (1.98-7.56)	<0.001	1.011	2.75 (1.58-4.96)	0.001
Smoke history	0.565	1.76 (1.03-2.93)	0.873						
CVC history	1.737	5.68 (3.96-8.21)	<0.001	1.610	5.00(2.92-8.72)	<0.001	1.534	4.64 (2.86-7.62)	<0.001
History of disease									
Hypertension	0.571	1.77 (1.24-2.52)	0.002	0.421	1.52 (0.9-2.58)	0.118			
Diabetes mellitus	-0.400	0.67 (0.37-1.13)	0.147						
Heart disease	-0.010	0.99 (0.53-1.75)	0.974						
Khorana score, ≥3 points(vs. 1-2 points)	-0.713	0.49 (0.18-1.07)	0.032	-0.070	0.93 (0.42-1.98)	0.858			
Blood routine									
Hemoglobin count ≥95 g/L	0.844	2.42 (1.04-7.07)	0.065						
Platelet count ≥222×109 /L	0.300	1.35 (0.96-1.9)	0.088						
NLR ≥3.42	0.565	1.76 (1.25-2.51)	0.001	0.239	1.27 (0.74-2.17)	0.381			
PLR ≥146.24	0.215	1.24 (0.87-1.78)	0.234						
Blood coagulation									
D-dimer ≥2.06 mg/L	1.765	5.84 (4.06-8.47)	<0.001	1.600	4.96 (2.86-8.74)	<0.001	1.719	5.58 (3.54-8.94)	<0.001
APTT ≥24.15 sec	-0.799	0.45 (0.28-0.73)	0.001	-0.373	0.69 (0.33-1.49)	0.337			
PT ≥11.45 sec	1.215	3.37 (2.3-5.04)	<0.001	0.716	2.05 (1.17-3.62)	0.012	0.766	2.15 (1.32-3.54)	0.002
Fbg ≥3.33 g/L	0.412	1.51 (1.07-2.15)	0.02	0.717	2.05 (1.21-3.51)	0.008	0.563	1.76 (1.12-2.78)	0.015
Biochemical routine									
Albumin ≥41 g/L	-0.562	0.57 (0.38-0.83)	0.004	0.213	1.24 (0.66-2.30)	0.501			
ALT ≥9.05 IU/L	-0.236	0.79 (0.49-1.31)	0.345						
AST≥18.05 IU/L	-0.236	0.79 (0.56-1.12)	0.188						
LDH ≥258.25 U/L	0.683	1.98 (1.35-2.89)	<0.001	-0.005	0.99 (0.55-1.77)	0.986			
TG ≥1.37 mmol/L	0.419	1.52 (1.08-2.14)	0.017	0.808	2.24 (1.33-3.83)	0.003	0.631	1.88 (1.19-2.99)	0.007
Pro-BNP ≥263.75 pg/ml	0.788	2.20 (1.49-3.23)	<0.001	0.606	1.83 (0.99-3.40)	0.054			
Tumor biomarkers									
CYFA21-1 ≥3.34ng/mL	0.344	1.41 (0.99-1.99)	0.054						
CEA ≥3.18 ug/L	0.385	1.47 (1.03-2.11)	0.033	-0.022	0.98 (0.56-1.7)	0.939			
CA125 ≥38.45 U/mL	0.588	1.80 (1.28-2.55)	0.001	-0.008	0.99 (0.57-1.71)	0.977			
Pro-GPR ≥37.23 pg/mL	-0.386	0.68 (0.47-0.97)	0.038	-0.342	0.71 (0.42-1.18)	0.193			
NSE ≥17.32 ng/mL	-0.211	0.81 (0.57-1.14)	0.224						

(Continued)

TABLE 2 Continued

Variables	Univariate analysis			Multivariate analysis			Stepwise regression analysis		
	β	OR(95% CI)	*P* value	β	OR(95% CI)	*P* value	β	OR(95% CI)	*P* value
Molecular driver									
EGFR mutation	-0.371	0.69 (0.41-1.12)	0.151						
KRAS mutation	0.491	1.52 (0.76-2.9)	0.214						
ROS1 rearrangement	0.833	2.30 (1.54-3.41)	<0.001	0.968	2.63 (1.5-4.62)	0.001	1.055	2.87 (1.74-4.75)	<0.001
ALK rearrangement	0.086	1.09 (0.68-1.71)	0.703						
PD-L1 expression(≥50%)	0.713	2.04(1.12-3.6)	0.016	0.961	2.61 (0.8-8.05)	0.101			
Treatment history[c]									
Chemotherapy	1.179	3.25 (2.29-4.63)	<0.001	0.604	1.83 (1.04-3.22)	0.036	0.505	1.66 (1.01-2.7)	0.043
Radiotherapy	0.723	2.06 (1.40-3.00)	<0.001	0.762	2.14 (1.19-3.87)	0.011	0.674	1.96 (1.17-3.29)	0.011
Targeted Therapy	0.077	1.08 (0.69-1.64)	0.727						
Immunotherapy	0.833	2.30 (1.24-4.16)	0.007	-0.082	0.92 (0.28-3.07)	0.894			
Surgery	0.215	1.24 (0.86-1.77)	0.253						

[a]BMI was categorized according to the Chinese population standards.
[b]According to the 8 th edition of the AJCC/UICC staging system.
[c]All the anti-cancer therapies were within 6 month before the VTE diagnosis.
VTE, venous thromboembolism; BMI, Body Mass Index; ECOG PS, Eastern Cooperative Oncology Group performance status; CVC, central venous catheter; NLR, Neutrophil-to-Lymphocyte ratio; PLR, Platelet-to-Lymphocyte ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; Fbg, fibrinogen; ALT, alanine transaminase; AST, aspartate transaminase; LDH, Lactate dehydrogenase;TG, triglyceride; pro-BNP, Pro-Brain natriuretic peptide; CYFR121-1, cytokeratin-19-fragment; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; Pro-GRP, Gastrin releasing peptide; NSE, neuron-specific enolase; OR, odds ratio; CI, confidence interval.

Comparison with risk assessment models

The ROC curves of different VTE risk assessment models are shown in Figure 6. The area under the ROC curve for the specific VTE risk-stratification nomogram model (0.904; 95% CI:0.869-0.939) was significantly higher than those of the KRS, Caprini, Padua and COMPASS-CAT models(Z=12.087, 11.851, 9.442, 5.340, all P<0.001, respectively). Additionally, the risk score of 300.6 was determined as the optimal cutoff value with the maximum Youden index(OR 14.17, 95% CI 9.45-21.64, P <

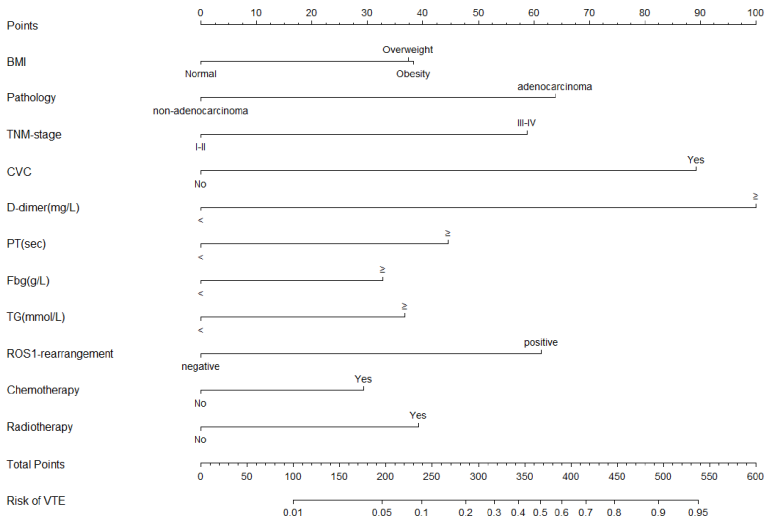

FIGURE 2
The Nomogram model for prediction of VTE in hospitalized patients with lung cancer. VTE, venous thromboembolism; BMI, Body Mass Index; CVC, central venous catheter; PT, prothrombin time; Fbg, fibrinogen; TG, triglyceride.

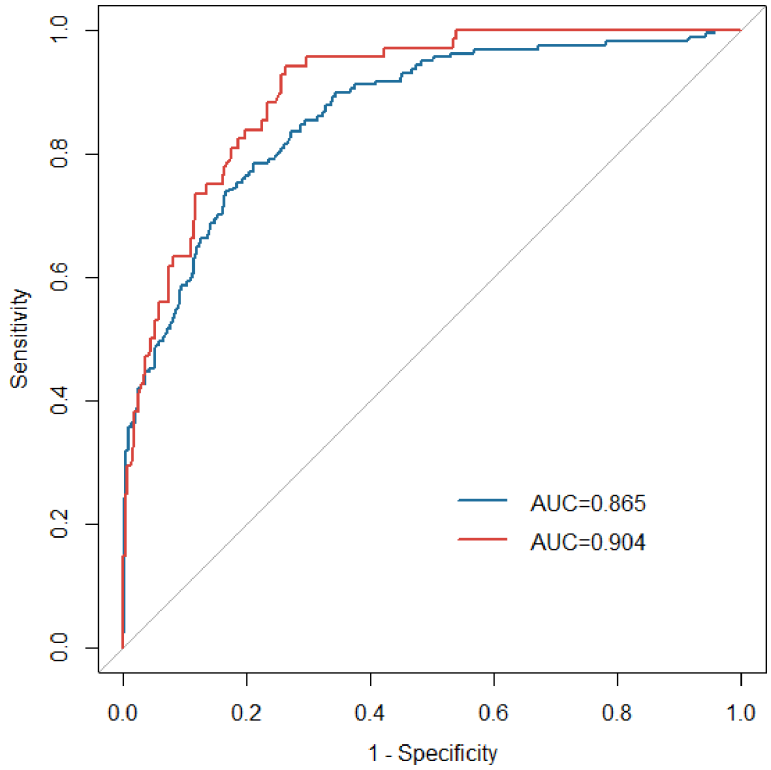

FIGURE 3
ROC curves of the nomogram in the development (blue line) and validation (red line) groups. ROC, receiver operating characteristic; AUC, area under the curve.

0.001) and was divided into a low-risk group (562 patients with risk score ≤ 300.6) and high-risk group (220 patients with risk score>300.6), respectively. The nomogram model presentedwith a sensitivity (Se) of 73.9%, specificity (Sp) of 83.4%, positive predictive value (PPV) of 52.7% and negative predictive value (NPV) of 92.7%.

Discussion

Currently, early detection of high-risk factors for cancer patients combined with VTE should be paid for particular attention. Previous studies mainly focused on the analysis of risk factors of lung cancer-associated VTE to establish the risk

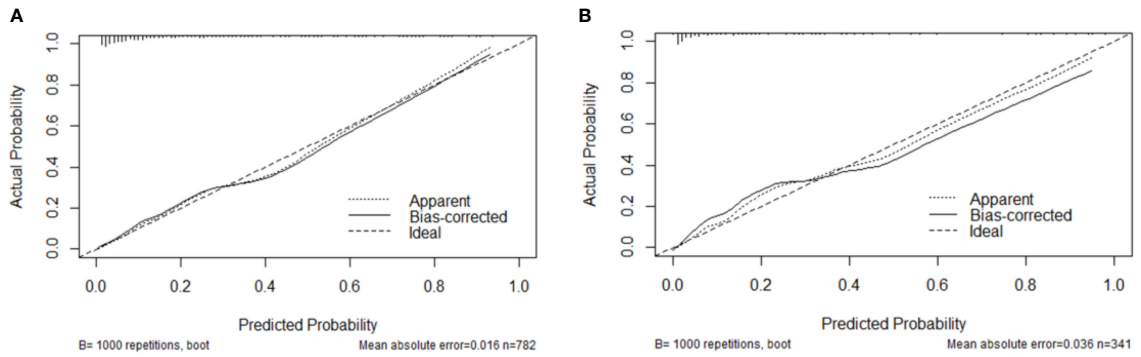

FIGURE 4
Calibration plots of the nomogram in the development **(A)** and validation **(B)** cohorts.

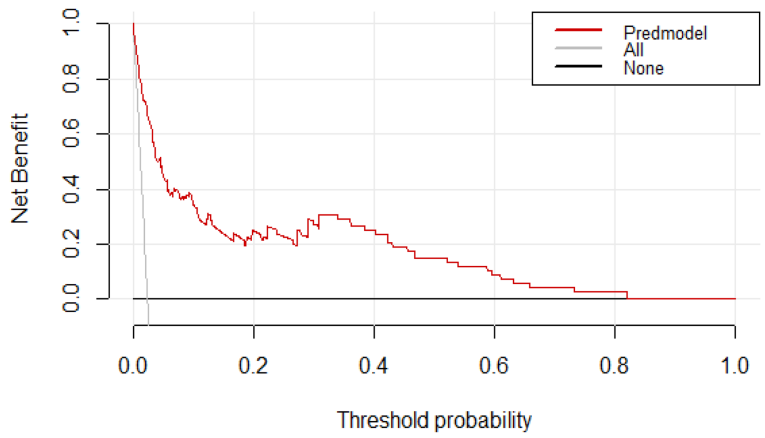

FIGURE 5
Decision curve analysis (DCA) for assessment of the clinical utility for thromboprophylaxis. The threshold probability represents the predicted risk of VTE for recommending primary thromboprophylaxis.The net benefit balances the risk of VTE with the potential harms of unnecessary thromboprophylaxis and is equal to the true-positive rate minus the weighted false-positive rate.

score system (19–21). However, few studies have been performed for the development of novel nomograms for the prediction of VTE in lung cancer patients, particularly given other factors influencing cancer-associated VTE such as genetic and therapeutic factors (31).Therefore, with a specific focus on both genetic and therapeutic factors, we developed and validated a simple yet highly discriminating, well-calibrated, and parsimonious nomogram prediction model for the occurrence of VTE in hospitalized lung cancer patients in this study, which can provide a theoretical basis for clinical decision-making on

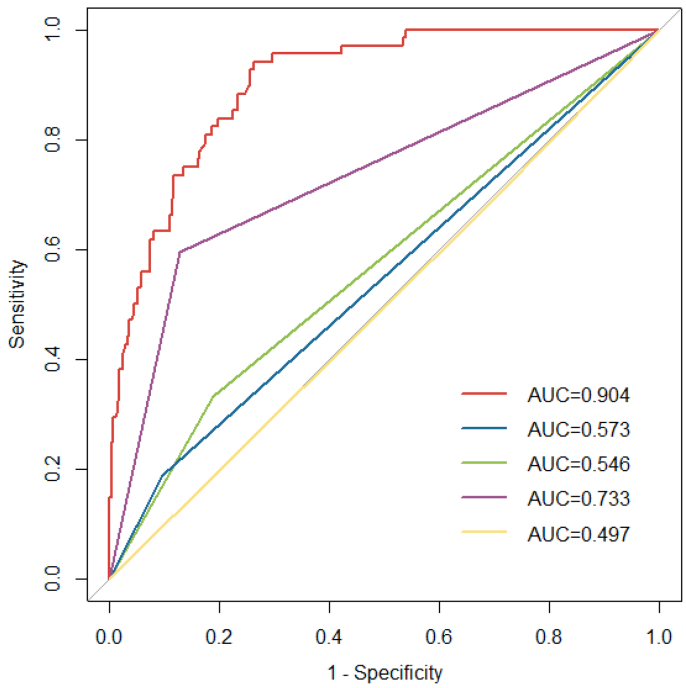

FIGURE 6
ROC curves for the existing risk assessment models [Khorana score(green line), Caprini risk assessment model(yellow line), Padua score(blue line) and COMPASS-CAT model(purple line)] and nomogram model (red line). ROC, receiver operating characteristic; AUC, area under the curve.

thromboprophylaxis on the basis of VTE risk levels. To our knowledge, this is the first to integrate readily obtainable clinical parameters, genetic and therapeutic factors into a modeling of the nomogram for the prediction of VTE in lung cancer. Furthermore, the nomogram prediction model proposed in this study was superior to other established scoring models in risk stratification of VTE patients.

According to the results of the logistic regression analyses, we established a simplified eleven-variables nomogram model, which contains four clinical variables(BMI, histology, clinical stage and CVC history), four biomarkers(D-dimer, PT, Fbg and TG), oncogenic abnormalities(ROS-1 rearrangement) and history of previous radiotherapy and chemotherapy treatment. Many of the risk factors of cancer-associated VTE identified in this analysis, particularly in lung cancer, were mostly consistent with those of the previous literatures (18–21). Among the numerous risk factors, it is widely accepted that higher BMI were patient-related risk factors related to VTE. Several studies have revealed that being obesity or overweight was associated with higher risk of VTE (8, 18, 32), while being underweight was associated with a lower risk of VTE (33). This study confirmed the previous data with a calculated OR of 1.90(95%CI: 1.19-3.07) for overweight. Furthermore, an OR value of 1.93(95%CI: 0.95-3.88) for obesity was observed but was not significant. Considering the body structure, dietary habits and ethnic differences exist between Chinese and Caucasian populations, the Chinese criteria for BMI were used instead of the World Health Organization(WHO) standard for classification in this study. Moreover most studies noted that the modified version of Khorana scale setting the cut-off points of BMI at 24kg/m^2 for Chinese population could improve the risk stratification and identification of patients with VTE (34). Therefore, we have adjusted the cut-off points of BMI according to the Chinese population standards in our models, for a better evaluation of association between BMI and VTE risk in a Chinese population.

With respect to cancer-related factors, adenocarcinoma was shown to be one of the most powerful predictors for VTE development in our nomogram model. Based on our analysis, the results showed that patients with adenocarcinoma have an approximately 3-fold higher risk of developing VTE than that of non-adenocarcinom apopulation. This result is consistent with the findings of previous studies (19, 21, 35). There is also clear evidence that patients with adenocarcinoma was associated with a markedly higher risk of VTE compared with other pathological types (8, 35). Another recent studies by Tsubata et al. (19) and Li et al. (21) has demonstrated that adenocarcinoma was one of the most powerful predictors for VTE development in the predictive scoring system. These studies led to widespread belief that adenocarcinoma plays a critical role in activating pro-coagulants factors by secreting mucin that may result in thrombus formation. Additionally, we found that patients with advanced stage have a risk of VTE that is 2- to 3-fold higher than those from patients with early stages, which is in accordance with the findings of more recent studies (8, 19, 21, 36, 37). These results suggest that patients with advanced and metastatic cancer has been linked to an increased risk of VTE as compared to tumors that are localized. It is now well acknowledged that high prevalence of VTE occurrence in advanced stage patients may likely be related to the synchronicity of tumor progression and increased coagulation activity (38).

Moreover, most previous studies noted that the use of long-term central venous catheters(CVCs) especially peripheral insertion central catheter (PICC) was strongly associated with increased risk of upper-extremity DVT (39, 40). This study shows that a prior history of CVCs was an independent risk factor for VTE in patients with lung cancer, with an OR of 4.64. This is in agreement with previous studies, which showed CVCs indwelling might be the main cause of upper extremity DVT among cancer patients (8). Furthermore, it should be noted that the incidence of catheter-related thrombosis (CRT) is closely related to the type of CVC, the thickness of the Catheter and the position of the CVC tip (39–41). This phenomenon might be correlated with vascular endothelial injury and slow blood flow caused by the deep venous indwelling catheter, or compression of the cervical lymph nodes metastasis.

D-dimer is the specific degradation products of crosslinked fibrin and is used as sensitive marker of hypercoagulability status and of endogenous fibrinolysis. It has been established by several studies that D-dimer levels was an independent risk predictor of VTE in various types of cancer (21, 42–44). There is even some evidence suggesting that incorporating D-dimer into the VTE risk score significantly improves VTE risk discrimination and reclassification (45). These results are highly consistent with those from our study, which suggested that D-dimer≥2.06 mg/L can be considered independent risk factors of VTE and can be useful in stratifying risk for VTE in lung cancer. However, it should be noted that the exact D-dimer threshold for the diagnosis of VTE in the models is still controversial. We applied a highly sensitive cut-off of 2.06 mg/L that adapted from the cut-off given by the Youden index, which is similar or higher than these previous studies (20, 42, 43). This may be due to the fact that the tumors of the enrolled patients were mostly at advanced stage, which might resulted in higher baseline D-dimer levels.

Moreover, we have incorporated other noninvasive and obtained easily in clinic indicators into our analyses. The present study found that PT levels≥11.45sec, Fbg levels≥3.33 g/L and TG levels≥1.37mmol/L were an independent risk factor for VTE in patients with lung cancer, with an OR of 2.15, 1.76 and 1.88, respectively. Currently, the association of inflammatory parameters(PLR and NLR) and the risk of thromboembolism has been attracted increasing interest in recent years (46, 47). Based on the logistic regression analysis, our results showed that NLR at baseline were statistically significant in the univariate analysis, whereas elevated NLR were not associated with an increased risk of VTE in the

multivariate analysis(P=0.381), which is partly compatible with the findings of more recent studies (46, 48). The reason might be related to the effects of neutrophil extracellular traps(NETs) released by tumor-activated neutrophils.

Currently, there is a growing interest in exploring the potential correlation between driver genes and VTE risk in lung cancer (49–51). Clearly, there is a critical need to incorporating positive driver genes into a risk assessment model for lung cancer to improve performance. One of the strengths of our study is the inclusion of molecular drivers in the construction of this model. Interestingly, our results showed that the risk of VTE in ROS1 rearrangements(ROS1+) patients is 2.63-fold greater than that in ROS1- patients, and the odds of VTE in ROS1+ lung cancer were higher than ALK+, EGFR+ and KRAS+ cohorts in the univariate analysis. Similar to our findings, more recent studies by Zhu et al. (50) and Ng et al. (51) have also found that the risk of VTE is significantly increased in patients with ROS+ NSCLC compared to EGFR+ and KRAS+ cases. Although the mechanism is not clear yet, The mucus produced in ROS1 fusions lung adenocarcinoma probably contributes to further platelet recruitment and, consequently, to the thrombus development.

Considering the the treatment-related factors suggested in the literatures including history of chemo-, radiation- and immuno-therapies, several studies have confirmed that chemotherapy, widely used in more than half of the patients, has been reported to be associated with a 2- to 6-fold increased VTE risk (8, 15). By multivariate logistic regression analysis our study showed that having previously undergone radiation or chemotherapy within 6 months before VTE diagnosis was independently associated with an increased risk of VTE for patients with lung cancer, which is in accordance with the findings of Li et al. (21). Plausible explanations for this could be attributed to vascular endothelium damage, reduced endogenous anticoagulant factors(antithrombin, protein C, protein S) and platelet activation (15). Nonetheless, there is less evidence available on the influence of RT on outcome in cancer patients with VTE. Still, a few studies have displayed that there remained a significant correlation between RT and VTE in patients with cancer (16, 52). This may be in part due to the endothelial damage to veins caused by radiation exposure.

To date, many randomized controlled trials(RCTs) have developed several risk assessment models suitable for cancer patients, such as KRS, Caprini, Padua and COMPASS-CAT score. Concerning the comparison of the current model with other existing models, our current data suggest that the area under the ROC curve for the specific VTE risk-stratification nomogram model(0.904; 95% CI:0.869-0.939) was significantly higher than those of the KRS, Caprini, Padua and COMPASS-CAT models. The reason for this differences may have been due to the scale discrimination in the applicable population. KS scale was originally designed for cancer outpatients, which did not include some therapeutic factors (e.g. chemotherapy) occurred during hospitalization. Therefore, the proportion of patients who were at a high risk of VTE may be underestimated. Additionally, Caprini scale has a good predictive value in cancer inpatients, particularly among patients undergoing surgery, which can easily lead to pharmacologic overprophylaxis, and thus result in an inherent risk of bleeding (53). Consequently, it is necessary to establish a meticulous benefit and risk assessment model for VTE in patients with malignancies to balance of the risk of bleeding against the risk of thromboembolism. Based on our results, DCA revealed that the nomogram provided superior net benefit of thromboprophylaxis in patients with high VTE risk for threshold probabilities between 2% and 82%. Further large randomised-controlled trials(RCTs) are needed to evaluate the benefits of thromboprophylaxis in patients at high risk of VTE.

Some limitations of the current study must be considered. First, selection bias could not be avoided due to the single-center retrospective study design. Thus, this model needs to be further validated with larger sample sizes, and/or performed in other centers and other geographic regions to determine its generalizability and efficiency. Second, it is noted that we did not performroutine VTE screening for enrolled patients. This study only identified patients with symptomatic or incidental VTE, which may result in a bias underestimating of the prevalence of VTE in enrolled patients. Moreover, local compression of vascular structures *via* mass lesion and presence of genetic mutations associated with increased thrombosis are risk factors for developing VTEs that cannot be ignored.As a result, limitations of the absence of the above datas must be considered in the current study. Finally, further multi-center prospective clinical trials and community-based prospective studies are needed to validate and refine the model.

Conclusions

In conclusion, this study systematically developed and validated a novel VTE risk prediction nomogram model for patients with newly diagnosed lung cancer, which incorporated available clinical parameters, genetic and therapeutic factors into the assessment system. Notably, the novel nomogram model was significantly more effective than the existing well-accepted models in routine clinical practice in stratifying the risk of VTE in those patients. We have provided evidence to support that high-performance nomogram model can be reliably used to identify hospitalized patients with lung cancer at a high risk of VTE and to guide individualized decision-making on thromboprophylaxis. However, the model needs external validation in other clinical centers, and should be extended to other care settings (e.g. community-based ambulatory 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.

Ethics statement

Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

HL contributed to the study design, statistical analyses, and manuscript writing. YT, HN, LH, GC, CZ and KK performed data collection. HL and YT analyzed and interpreted the data. QL designed and supervised the research and revised the manuscript. All authors read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.988287/full#supplementary-material

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High cardiovascular disease mortality after penile squamous cell carcinomas diagnosis: Results from the United States SEER population, 2005-2016

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Background: Cancer survivorship care is an emerging and necessary component of oncology management. To explore cardiovascular disease (CVD)-specific mortality and prognostic factors among patients with penile squamous cell carcinomas (PSCC). These results aid clinicians in furtherly understand this disease's prognosis.

Method: We analyzed Surveillance, Epidemiology and End Results Program data for 2668 PSCC cases diagnosed between 2005 to 2016. We calculated standardized mortality ratios (SMRs) of CVD and all-cause mortality, comparing PSCC patients with general population men. A cumulative mortality curve and competitive risk regression model were utilized to evaluate the prognostic factors of CVD-specific death.

Results: Death distribution is as follows: PSCC (42.4%), other causes (21.3%) CVD (19%), and other cancers (17.3%). PSCC patients are more like to die from CVD (SMR=3.2, 95%CI: 3.1-3.3) and all-cause death compared with the general population. Meanwhile, patients undergoing surgery show a relatively higher CVD-specific mortality than the general population (SMR=2.7, 95%CI: 2.4-3.2). In the competitive risk model, higher CVD mortality is associated with age, region, year of diagnosis, stage, and marital status (all P<0.05). Patients with the localized stage show a higher risk of CVD-specific death than those with regional or distant stage.

Conclusion: Our study mainly reveals that cardiovascular disease was the important cause of death and higher CVD-specific mortality among PSCC

patients. Several associated factors related to CVD-specific death are also identified. In the future, more work in educating health care professionals on the components of survivorship care is needed to meet the long-term and late effects cancer patients experience.

KEYWORDS

penile squamous cell carcinomas, cardiovascular disease, Surveillance, Epidemiology and End Results (SEER), cause of death, prognosis, mortality

Introduction

Penis cancer is a rare disease, accounting for 0.4% to 0.6% of all malignancies in men in the United States and Europe (1). About 95% penis cancer derives from the squamous cells of glandular and preputial skin and is diagnosed with penile squamous cell carcinomas (PSCC) (2). PSCC presented great regional heterogeneity. For example, the incidence rate is 0.1-1 per 100,000 men in high-income countries. At the same time, it accounts for 10% of the total number of male malignancies in some African, Asia, and South American countries. The primary reason for this heterogeneity may be related to confirmed risk factors, including circumcision practices, infection with human papillomavirus (HPV), smoking, obesity, chronic inflammation, and so on (3–5).

PSCC patients diagnosed with the localized stage (about 40%) showed relatively good survival outcomes and reported data suggested that the overall survival rate of these patients was as high as 90% (5). However, once the tumor metastases, the prognosis will deteriorate sharply. The management of PSCC has always been a comprehensive and complex challenge, considering that cancer has a far-reaching physiological and psychological impact on the quality of life of patients and survivors by altering sexual and urinary functions (2). There is increasing evidence that the management of co-morbidities, especially cardiovascular disease (CVD), should receive more attention, considering that the survival of penile squamous cell carcinomas has not significantly improved since 1990 in the United States (6). The main reason for this consideration is that cardiovascular disease has become the leading cause of death for cancer patients in recent years (7, 8). Meanwhile, patients with PSCC are diagnosed at a relatively high age (range 50 to70 years), the age group with a high incidence of cardiovascular disease (1). Moreover, effective lifestyle interventions and other strategies to prevent CVD complications exist but may not be fully utilized in this population (9, 10). Therefore, strengthening the understanding and management of CVD may effectively improve survival in patients with PSCC.

Up to our knowledge, there is still a lack of studies focusing on CVD in PSCC patients considering the study population is relatively small. Therefore, we have explored the U.S. Surveillance, Epidemiology, and End Results Program (SEER) database to obtain patients with PSCC. The primary purpose of this study is to explore the distribution of CVD-specific death among PSCC patients stratified by characteristics. In addition, the risk factors of CVD-specific death were investigated by utilizing the Fine-Gray multivariable-adjusted competing risks model.

Methods

Study population and variables description

The study involved a secondary analysis of the National Cancer Institute's SEER project database. We identified PSCC patients who were histologically diagnosed with penile squamous cell carcinoma based on the list of '*Site Recode ICD-O-3/WHO 2008 classification*' and ' *Histology recode - broad groupings*' between 2005 and 2016 from the SEER 18 registries research database, covering approximately 28% of the U.S. population (based on the 2010 census). We excluded the following patients: age at diagnosis <15 years, unknown survival months, and unknown cause of death.

We used the SEER * stat (version 8.2.1) to generate a list of cases and incorporated the following variables: age at diagnosis, race (white, black, and other, unknown), year of diagnosis, marital status (married, separated, or divorced or widowed (SDW), single, unknown), PRCDA region (east, northern plains, pacific coast, southwest), SEER historic stage (Localized, Regional, Distant, unknown), surgery record, survival month, and cause of death (COD) to site recode. We divided the causes of death into four categories based on the International Classification of Diseases, tenth Edition (ICD-10): PSCC, cardiovascular diseases, other cancers, and other causes.

Statistical analysis

We described the distribution of causes of death in PSCC patients by the index of the proportional mortality ratio (PMR), which was calculated by dividing the number of patients who

died from special causes (PSCC, cardiovascular diseases, other cancers, and other causes) by the total number of PSCC patients. In addition, the study population was stratified by age, race, year of diagnosis, region, tumour stage, operation records, and the cause of death distribution in the subgroups were further examined. We compared CVD and all-cause mortality between PSCC patients in the SEER cohort and general men in the American population and expressed it as standardized mortality (SMR). The age-specific risks of CVD and all-cause death were obtained from the Centers for Disease Control and Prevention Wide-ranging Online Data for Epidemiologic Research (WONDER) Tool (11). The expected number of deaths in each age group was calculated by multiplying the number of PSCC patients in each age group by the age-specific crude risk in the general population. The SMRs were calculated according to the number of observed PSCC deaths divided by the expected number of deaths. If SMRs were more significant than one and the P-value< 0.05, it indicates that the cardiovascular mortality rate of patients with PSCC was higher than that of the general population in the United States, and the results were statistically significant. The Fine-Gray multivariable-adjusted competing risks model was performed to adjust confounding factors (including age, race, marital status, region, year of diagnosis, grade, and stage) and evaluate the risk factors of CVD-specific mortality and PSCC-specific mortality. We used the Cox proportional hazards regression model to calculate the risk of all-cause mortality. All results were expressed as hazard ratios (HR) and 95% CI. The cumulative incidence function curves of PSCC and CVD mortality were described using parameter estimates and the baseline survivor function from the Fine-Gray model (11).

The R version 3.6.3 was used for all statistical analysis, and a P <.050 was recognized as significant.

Results

Patients characteristics

Finally, we enrolled 2668 cases diagnosed with penile squamous cell carcinoma from 2005 to 2016. The mean age at diagnosis was 64.91 (standard deviation =13.72). 2226 (83.4%) patients were white, and 1452 (54.4%) cases were married. There were 1173(44.0%) patients on the pacific coast. The localized stage was the most common (51.8%). There were 2410(90.3%) cases undergoing surgery (Table1).

Proportional mortality ratios among PSCC patients

Proportional mortality ratios of all study populations were as follows: PSCC (42.4%), other causes (21.3%) CVD (19%), and other cancers (17.3%). (Figure 1A). In the subgroup analysis, PSCC was still the four most common causes of death among patients in all subgroups. Patients with age over 70 years (25.1%), race of other (21.6%), diagnosed between 2005-2010(19.9%), in the northern plains (20.6%) and with localized stage tumours (27.7%), the PMR of CVD death was relatively higher compared to the other cases in the same subgroup. (Figure 1).

Cardiovascular diseases and all-cause mortality compared to the general population in the USA

The age-specific risk of CVD death and all-cause mortality were compared between patients with PSCC and general men in the USA (Table 2). For all study populations, patients with PSCC were observed to have a 3.2(3.1-3.3) times higher risk of cardiovascular death than the general men population, and this value was 6.1(6-6.3) times in terms of all-cause death. Similar results were observed for patients with different ages. Notably, this higher risk of CVD-specific death than the general men population was more significant in PSCC patients with younger age and localized stage (Maximum SMR). In addition, patients receiving surgery had a relatively higher SMR of CVD compared to those without.

Cumulative CVD-specific mortality and Relative risk model

Figure 2 presented cumulative incidence function curves for CVD mortality stratified by population characteristics (We had hidden the lines of ends of PSCC and other causes of death). We observed a relatively higher CVD-specific mortality rate in patients over 70 years and status of SDW compared with other cases in the same cohort (Figures 2A, C). Meanwhile, patients diagnosed in the northern plains had a relatively higher incidence of CVD-specific death than other regions (Figure 2D). Patients with localized stage showed a remarkably higher cumulative incidence of CVD-specific deaths than those diagnosed with the regional or distant stage (Figure 2E).

The Fine–gray model of competitive risk was utilized to explore the associations between prognostic factors and CVD-specific death or PSCC-specific death in PSCC patients (Table 3). We found that age was the most significant prognostic factor affecting CVD-specific death, and the risk of CVD-specific death had gradually increased with each 10-year advance in age [from SHR (sub-distribution hazard ratio) = 1.35; 95%CI (1.01- 1.8) in patients aged 50-59 to SHR=3.04; 95%CI (2.36- 3.9) in patients aged over 70]. Compared with those in the region east, patients in the northern plains, pacific coast, or

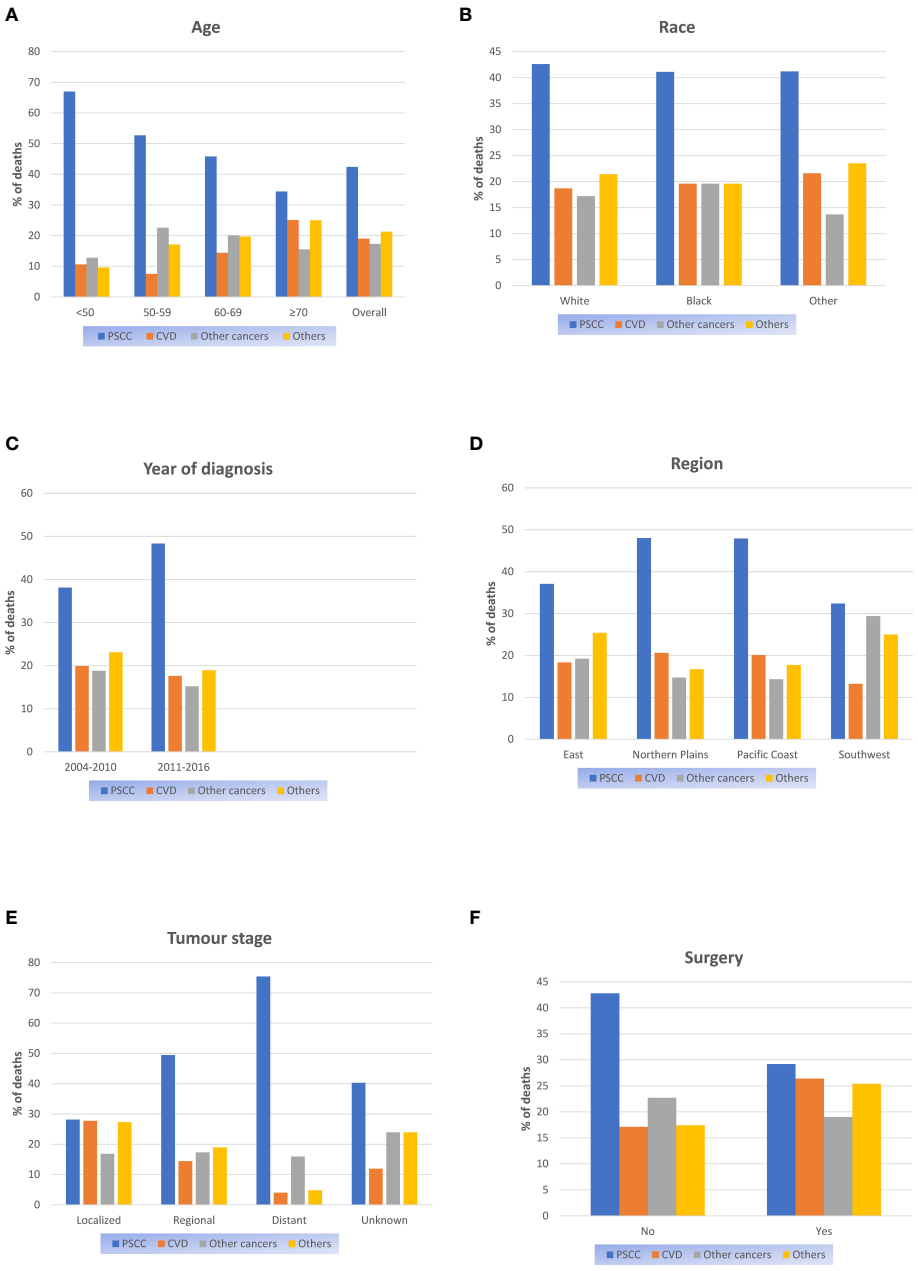

FIGURE 1
Proportional mortality ratios according to **(A)** Age; **(B)** Race; **(C)** Year of diagnosis; **(D)** Region; **(E)** Tumour stage; **(F)** Surgery.

southwest showed a higher risk of CVD-specific death (all SHR>1, P<0.05). Compared with cases diagnosed from 2005 to 2010, patients in 2011-2016 had a relatively lower risk of CVD-specific death (all SHR<1, P<0.001). The risk of CVD-specific death in patients with the regional or distant stage was lower relative to those diagnosed with the localized stage(P<0.001). Married patients presented a relatively lower risk of CVD-specific death than those with SDW and single status.

The tumour stage was the most significant prognostic factor associated with PSCC-specific death. Patients with regional or distant stage had a significantly higher risk of PSCC-specific death than those with the localized stage (SHR=1.93, 95%CI: 1.43-2.59, P<0.001 for the regional stage; SHR=2.33, 95%CI: 1.69-3.21, P<0.001 for distant stage). Meanwhile, the status of SDW of single, age, and higher tumour grade were all proved to associate with PSCC-specific death.

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TABLE 1 Description of population-based cohort of men with PSCC.

Characteristic	n	%
Overall	2668	100%
Age at diagnosis, mean (SD)	64.91(13.72)	
<50 years	391	14.7%
50-59 years	497	18.6%
60-69 years	736	27.6%
≥70 years	1044	39.1%
Race		
White	2226	83.4%
Black	261	9.8%
Other[a]	145	5.4%
Unknown	36	1.3%
Marital status		
Married	1452	54.4%
SDW	489	18.3%
Single	500	18.7%
Unknown	227	8.5%
Region		
East	1115	41.8%
Northern Plains	228	8.5%
Pacific Coast	1173	44.0%
Southwest	152	5.7%
Year of diagnosis		
2005-2010	1212	45.4%
2011-2016	1456	54.6%
Summary stage		
Localized	1383	51.8%
Regional	789	29.6%
Distant	153	5.7%
Unknown	343	12.9%
Undergoing surgery		
No/unknown	258	9.7%
Yes	2410	90.3%
Cause of death		
Alive	1576	59.1%
PSCC	463	17.4%
CVD	208	7.8%
Other cancers	189	7.1%
Other cause	232	8.7%

a: American/Indian/Alaska/Native and Asian/Pacific Islander.
SDW, Separated, Divorced, Widowed; SD, standard deviation; PSCC, penile squamous cell carcinoma; CVD, cardiovascular diseases.

Discussion

This study found that cardiovascular disease was the important cause of death after penile squamous cell carcinomas diagnosis based on a large study population. PSCC patients showed a significantly higher CVD-specific mortality rate than the general men population. We also identified associated factors including age, region, year of diagnosis, stage, and marital status associated with CVD-specific death from the competing risks model.

PSCC was uncommon, especially in developed countries, and it was also an aggressive disease with a relatively high mortality rate (12). Patients with the localized disease were generally effectively treated with topical therapy, surgery, or radiotherapy (2). For patients with metastatic disease, multimodal management was required considering that there

TABLE 2 The CVD-specific and all-cause mortality rates of patients with PSCC were standardized by age, surgery and cancer stage relative to the general population of the United States.

	CVD mortality			All-cause mortality		
Category	**Observed deaths**	**Expected deaths**	**SMR (95%CI)**	**Observed deaths**	**Expected deaths**	**SMR (95%CI)**
Overall	208	66	3.2 (3.1-3.3)	1092	181	6.1 (6-6.3)
Age						
<55	15	2	15 (6.7-23)	149	25	12.1 (7.9-16.3)
55–64	22	5	8.8 (5.4-12.1)	207	26	8.1 (5.5-10.7)
65–74	53	12	4.4 (3.9-5.1)	285	38	6.8 (6.6-7.1)
≥75	118	47	2.5 (2.3-2.7)	451	102	4.5 (4.3-4.7)
Stage						
Localized	135	10	13.5 (11.7-15.4)	486	276	1.8 (1.5-2.1)
Regional	60	47	1.3 (0.9-2.3)	417	157	2.7 (2.4-2.9)
Distant	5	9	0.6 (0.3-2.9)	124	31	4.1 (3.8-4.4)
Undergoing surgery						
No/Unknown	15	14	1.1 (0.7-2.6)	150	51	2.9 (2.5-3.5)
Yes	193	72	2.7 (2.4-3.2)	942	482	1.9 (1.6-2.3)

PSCC, penile squamous cell carcinoma; CVD, cardiovascular diseases; SMR, standardized mortality ratio; CI, confidence interval.

was still no strong evidence to confirm the best optimal sequencing of treatments and patient selection (2). Cisplatin-based chemotherapy regimens were commonly used in these patients, but high rates of toxic effects had always been a problem that cannot be ignored (13, 14). In this study, we observed higher CVD-specific mortality in patients undergoing surgery compared with the general population. Although organ preservation technology has been developed for patients with cT0-2 disease in order to maximize the protection of penile tissue and function, the impact of surgery on the quality of life of patients was still profound (15). Penile amputation could lead to the loss of sexual and urinary function. Patients after surgery often experience mental illness, avoidance behavior, health damage, and post-traumatic stress disorder based on questionnaire investigation (16, 17). This effect might be an important cause contributing to the higher CVD-specific mortality. It might be that the survival time of patients with metastatic diseases was too short of supporting this observation, considering that the late effects of cancer treatment might become clinically obvious years or decades after the treatment was completed (18).

Cardiotoxicity was a potential complication in the treatment of various cancers. The new ESMO Clinical Practice Guidelines are based on the multidisciplinary cardio-oncology review of current evidence intended to provide strict standards-based advice on cardiovascular risk prevention, assessment, monitoring, and management during antineoplastic therapy (19). In general, the risk-benefit ratio of drugs needed more consideration in terms of the nature and severity of cancer.

In this study, age was the most significant factor associated with CVD-specific death in PSCC patients (maximal SHR in age over 70years). Meanwhile, we performed a comparison in the risk of CVD-specific death between PSCC patients and the general population and observed a significantly higher risk in PSCC patients. Data derived from childhood cancer proved that the cardiac mortality rate of child cancer survivors was 8.2 times higher than that of the age-matched and gender-matched populations (20). Similarly, the risk of cardiac death was significantly higher in younger patients than in older patients when compared with the general population in our study. This cardiovascular death from cancer or cancer treatment might be more noteworthy in young patients. Another remarkable result was that patients diagnosed with localized stage had a significantly higher risk of CVD-specific death than those diagnosed with regional or distant stage. One explanation for this phenomenon was that patients diagnosed with the localized stage survive significantly longer than those with metastatic diseases (2, 21). Therefore, it seemed no surprise that the probability of cardiovascular events increased in patients with localized stages. Moreover, the "discrepancy" in risk of CVD-specific death was likely explained by the competing death events (PSCC-specific death) incorporated in the Fine-Gray model. Patients with regional or distant stages normally were more likely to die from PSCC but not cardiovascular diseases, which influenced the cumulative incidence of CVD mortality. It was hard to make an explanation for the association between factors (marital status and region) and CVD-specific death. One possible explanation was that some important risk factors for cardiovascular death, such as smoking, alcohol consumption, and high-fat diet, were distributed differently in the study population. Meanwhile, there were great differences in the treatment of PSCC in different regions, and some radiotherapy

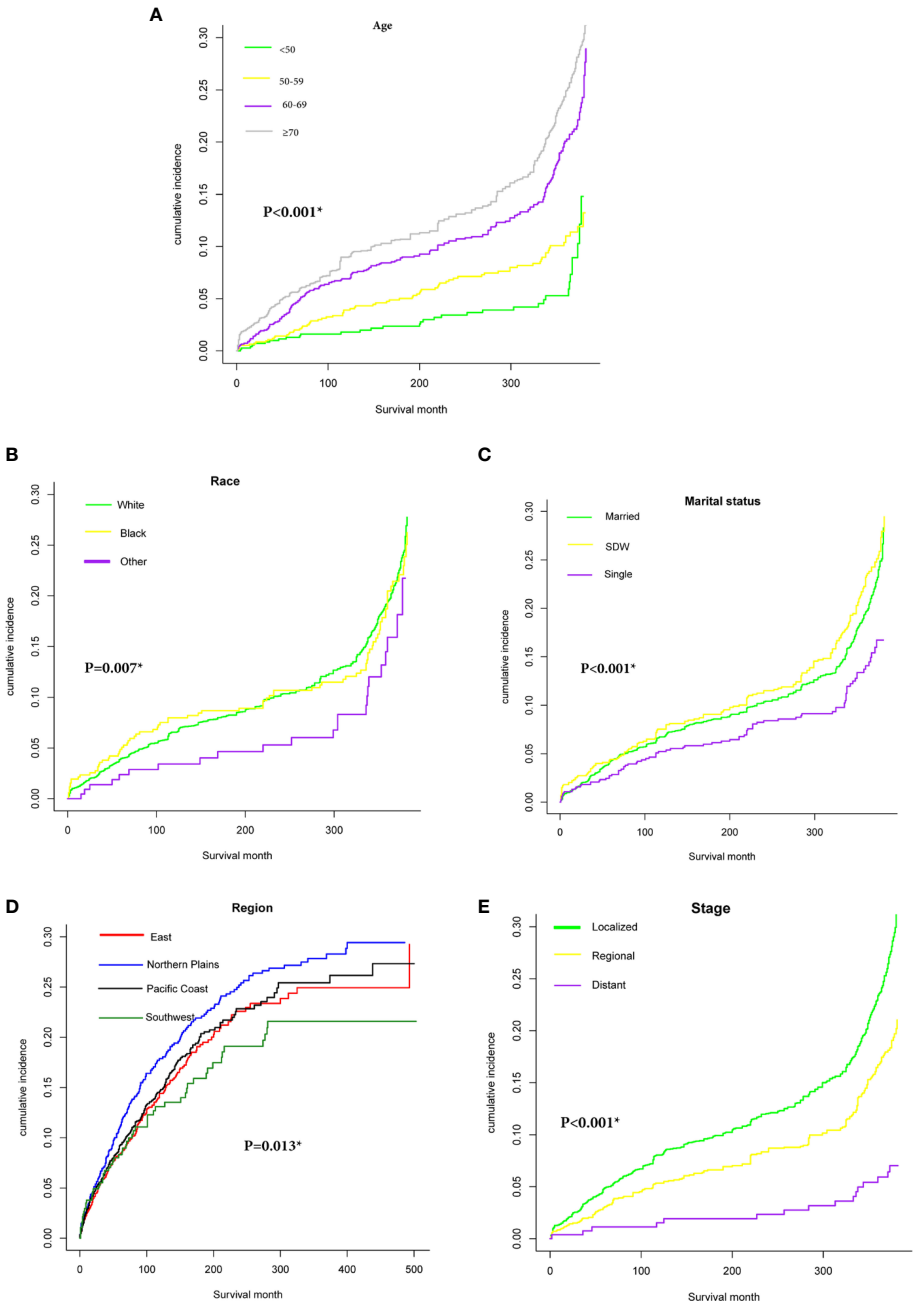

FIGURE 2
Cumulative incidence function curve of PSCC patients after adjust for multivariate. Cardiovascular diseases (CVD)-specific death: **(A)** age (≤50, 50-59, 60-69, ≥70); **(B)** Race (white, black, other); **(C)** Marital status (Married, SDW, Single); **(D)**: Region (East, Northern Plains, Pacific Coast, Southwest); **(E)** Stage (Localized, Regional, Distant).

or chemotherapy techniques might be an important cause of high cardiovascular death in different regions.

Cancer and heart disease are the main causes of morbidity and mortality in industrialized countries (18). With the development of modern treatment strategies, the survival of cancer patients has been significantly improved. However, some studies have pointed out that the overall survival of PSCC patients has not effectively improved since the 1990s in the United States (6). This might be due to the diagnostic delay in the advanced stage and limited nursing and disease management improvements. On the contrary, the overall survival of penile cancer had improved significantly after establishing centralized health care in the U.K (22). This was the

TABLE 3 All-cause, CVD-related, and PSCC-related mortality of patients by demographic and clinical characteristics at diagnosis.

	All causes		CVD-specific death		PSCC-specific death	
Age	HR (95%CI)	P-value	SHR (95%CI)	P-value	SHR (95%CI)	P-value
<50	Ref.		Ref.		Ref.	
50-59	1.17 (0.89- 1.52)	0.24	1.35 (1.01- 1.8)	<0.001*	0.89 (0.63-1.25)	0.51
60-69	1.55 (1.22-1.98)	<0.001*	1.72 (1.31- 2.24)	<0.001*	1.04 (0.75-1.43)	0.81
≥70	3.18 (2.54-4.1)	<0.001*	3.04 (2.36- 3.9)	<0.001*	1.58 (1.17-2.14)	<0.001*
Race						
White	Ref.		Ref.		Ref.	
Black	1.18 (0.96-1.45)	0.1	0.91 (0.87-1.37)	0.46	1.1 (0.79-1.5)	0.58
Others	0.77 (0.58-1.04)	0.08	1.18 (0.61-1.17)	0.16	0.73 (0.46-1.15)	0.68
Unknown	0.09 (0.01-0.64)	0.016*	0.28 (0.04-1.59)	0.36	0.24 (0.03-1.78)	0.16
Marital status						
Married	Ref.		Ref.		Ref.	
SDW	1.33 (1.15-1.55)	<0.001*	1.36 (1.15-1.61)	<0.001*	1.2 (1.02-1.42)	0.02*
Single	1.42 (1.2-1.67)	<0.001*	1.43 (1.19-1.73)	<0.001*	1.33 (1.04-1.7)	0.022*
Unknown	1.11 (0.86-1.42)	0.42	0.88 (0.86-1.48)	0.38	0.836 (0.53-1.31)	0.43
Region						
East	Ref.		Ref.		Ref.	
Northern Plains	0.88 (0.71-1.09)	0.24	1.13 (1.1-1.51)	<0.001*	1.23 (0.89-1.71)	0.19
Pacific Coast	0.88 (0.77-1.01)	0.075	1.9 (1.71-2.13)	<0.001*	1.12 (0.91-1.37)	0.27
Southwest	1.11 (0.86-1.44)	0.43	2.7 (2.04-3.57)	<0.001*	0.93 (0.59-1.47)	0.78
Year of diagnosis						
2005-2010	Ref.		Ref.		Ref.	
2011-2016	0.99 (0.87-1.13)	0.95	0.57 (0.49-0.66)	<0.001*	0.96 (0.79-1.17)	0.74
Grade						
Grade I	Ref.		Ref.		Ref.	
Grade II	1.39 (1.18-1.65)	<0.001*	0.88 (0.68-1.1)	0.06	2.001 (1.64-2.46)	<0.001*
Grade III-IV	1.62 (1.34-1.96)	<0.001*	0.98 (0.71-1.09)	0.44	2.12 (1.7-2.66)	<0.001*
Unknown	0.96 (0.77-1.21)	0.77	1.08 (0.88-1.32)	0.68	1.33 (1.03-1.71)	0.026*
Stage						
Localized	Ref.		Ref.		Ref.	
Regional	1.7 (1.48-1.95)	<0.001*	0.54 (0.41-0.67)	<0.001*	1.93 (1.43-2.59)	<0.001*
Distant	5.42 (4.37-6.72)	<0.001*	0.41 (0.25-0.69)	<0.001*	2.33 (1.69-3.21)	<0.001*
Unknown	1.71 (1.29-2.25)	<0.001*	0.68 (0.46-0.96)	0.03*	1.23 (0.83-1.83)	0.29

SDW, Separated, Divorced, Widowed; SHR, sub-distribution hazard ratio; HR, hazard ratio; CI, confidence interval; Ref, Reference.
*: Statistical significance.

result of more appropriate management for penile cancer. In the future, co-morbidities, especially cardiovascular disease, might be an important aspect of improving the survival of PSCC patients, and a more appropriate medical management and nursing system should also be established for those with high-risk cardiovascular diseases.

This study has several limitations to note. First, this study was a retrospective study, and the selection bias caused by itself was inevitable. Then, the SEER program recorded the cause of death from the death certificate information, and this mode tended to overestimate the frequency of cardiovascular events like coronary heart disease (23, 24). In particular, reported data showed that the death certificate accuracy in the SEER database was not relatively satisfactory regarding some cancers. (87% for pancreatic cancer, but there lacked data on PSCC). We expect the impact on the study results was minimal. Furthermore, we used SMR to present the discrepancy in the CVD mortality rates between PSCC patients and the general population. In this case, we ignored PSCC patients in the general male population. Compared with the general population, the low incidence of this disease made our SMR estimation less likely to be biased (2). In addition, due to the limitations of the SEER database, we were unable to obtain more detailed data on PSCC patients like HPV infection, BMI, comorbidity, and smoking behavior, which were all associated with PSCC and cardiovascular diseases.

Conclusion

Our study mainly reveals that cardiovascular disease was the important cause of death among PSCC patients. PSCC patients showed a significantly higher CVD-specific mortality rate than the general men population. We also identified several associated factors including age, region, year of diagnosis, stage, and marital status associated with CVD-specific death among PSCC patients. Notably, patients diagnosed with localized stage showed a significantly higher cardiovascular disease mortality than those with regional stage or distant stage. Cancer survivorship care is an emerging and necessary component of oncology management. Considering that the survival of patients with PSCC has not improved significantly in recent years, this may prompt the cancer management transition from active cancer treatment to survivorship care, although it is not easy. In the future, more work in educating health care professionals on the components of survivorship care is needed to meet the long-term and late effects cancer patients experience.

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

Ethical review and approval were not required for the study of human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

XZ was responsible for data collection and analysis. The manuscript was written by MJ and BF. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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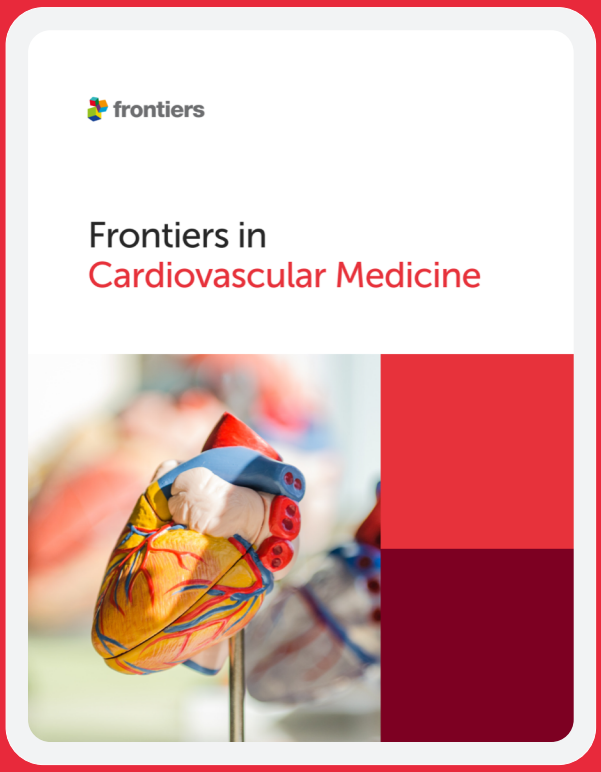

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