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Women in cardio-oncology

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Women in cardio-oncology

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ORIGINAL RESEARCH
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Cardio-Oncology Educational Program: National Survey as the First Step to Start

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Aim: The collaboration of cardiologists, general practitioners (GPs), and oncologists is crucial in cancer patient management. We carried out a national-based survey—The Ukrainian National Survey (UkrNatSurv)—on behalf of the Cardio-Oncology (CO) Working Group (WG) of the Ukrainian Society of Cardiology to analyze the level of knowledge in cardio-oncology.

Methods: A short questionnaire was presented to specialists involved in the management of cancer patients across the country. The questionnaire was made up of eight questions concerning referred cancer patient number, CV complications of cancer therapy, diagnostic methods to detect cardiotoxicity, and drugs used for its treatment.

Results: A total of 426 questionnaires of medical specialists from different regions of Ukraine were collected and analyzed; the majority of respondents were cardiologists (190), followed by GPs (177), 40 oncologists (mainly chemotherapists and hematologists), other −19 (imaging specialists, neurologists, endocrinologists, etc.). All responders were equally involved in the management of cancer patients. However, less than half of the patients have been seen before the start of cancer therapy. GPs observe the majority of patients after the end of treatment. All doctors are sufficiently aware of cancer therapy-associated CV complications. However, the necessary diagnostic tools, mostly biomarkers, are not used widely by different specialists. The criteria for cardiotoxicity, in particular, the level of reduction of the left ventricular ejection fraction (LVEF) as a marker of LV dysfunction, are not clearly understood. The specific knowledge in the management of CV complications in cancer is required.

Conclusion: UkrNatSurv is the first survey in Ukraine to investigate the awareness of CO care provided to cancer patients with CV diseases (CVD) or developed CV complications. Providing such surveys among doctors involved in CO is an excellent tool to investigate the knowledge gaps in clinical practice. Therefore, the primary task is to develop a national educational CO program.

Keywords: cardio-oncolody, cardiotoxicity, educational cardio-oncology program, survey, cancer

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INTRODUCTION

Rapidly evolving early detection and novel cancer therapies have significantly reduced mortality. However, survival depends not only on the effective cancer treatment but also on the prevention, diagnosis, and management of complications associated with cancer therapy.

Cancer treatment can affect the CV system in many ways inducing heart failure (HF), arterial hypertension, myocardial ischemia, arrhythmias, thromboembolism, etc. (1).

The development of cancer therapy-associated cardiac complications reduces the quality of life and survival in potentially cured patients, especially in those with a history of CVD.

According to the standards of care, patients with malignancy are managed in cancer centers. However, cancer patients with comorbidities and CV complications during anticancer therapy refer to cardiologists or general practice doctors (GPs).

CV toxicity is a relevant problem among many classes of chemotherapeutic drugs. According to the ESC Position Paper on CV toxicity, nine CV complications of antitumor treatment are classified (1, 2).

However, what the range is of such CV complications in Ukraine, doctors of what specialties manage these patients, what diagnostic methods and drugs do they use in actual clinical practice, and what position statements and guidelines are they acknowledged with?

This is the first survey in Ukraine evaluating the awareness and activity of medical care providers involved in cancer patient management.

It is crucial to identify the level of knowledge of the specialists involved in cardio-oncology to get potential benefit from this service.

It is believed that the study results will figure out vital information to develop an educative CO program and to improve the level of care for cancer patients.

METHODOLOGY

The Ukrainian National Survey (UkrNatSurv) is the study that investigates how to evaluate and manage CV complications in cancer patients in the routine clinical practice setting among doctors of different specialties.

The survey was planned by CO WG of the Ukrainian Society of Cardiology and provided by CO Center of the National scientific center "The M.D.Strazhesko Institute of Cardiology."

Data were collected through the paper questionnaires provided to the doctors involved in CO across the main country regions during the years 2019–2020. The ethics committee approved the study.

The questionnaire included eight single or multiple-choice structured questions concerning the number of referred cancer patients, CV complications of cancer therapy, diagnostic methods for cardiotoxicity detection, drugs used for cardiotoxicity treatment, etc.

When filling in the answers to the questionnaire, several items were allowed to be selected.

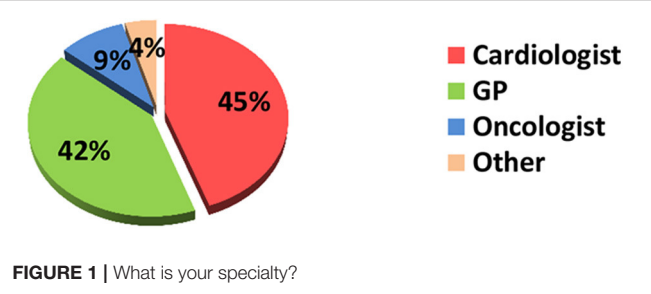

FIGURE 1 | What is your specialty?

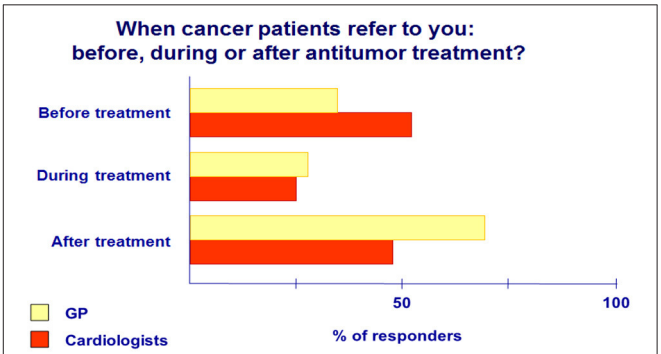

FIGURE 2 | When do cancer patients refer to you: before, during or after antitumor treatment?

The survey data were entered into a database on the RedCap platform. We used descriptive statistics to summarize these data.

RESULTS

In total, 426 responses from different regions of Ukraine were collected and analyzed.

Question 1. What is your specialty?

The majority of respondents were cardiologists (n = 190, 45%), followed by GPs (n = 177, 42%), and 40 (9%) oncologists (mainly chemotherapists and hematologists). The remaining 19 (4%) identified themselves as "others,"—neurologists, imaging specialists, endocrinologists, etc. (**Figure 1**).

Question 2. How many patients with a CV complication of cancer treatment have you managed per month?

Our findings indicate that cardiologists, oncologists, and GPs are equally involved in managing cancer patients. On average, all specialists consult from 5 to 10 patients per month.

Question 3. When do cancer patients refer to you: before, during, or after antitumor treatment?

Data analysis revealed that 52% of cancer patients are referred to cardiologists before the start of antitumor treatment; however, they observe only a quarter of these patients during cancer therapy. GPs examine 38% of cancer patients before starting antitumor therapy, less in the cancer treatment process (28%), but manage them predominately (69%) after completion of therapy (**Figure 2**).

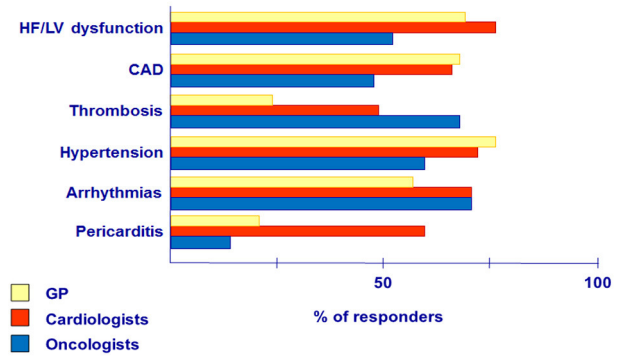

FIGURE 3 | CV complications of cancer treatment diagnosed by different specialists.

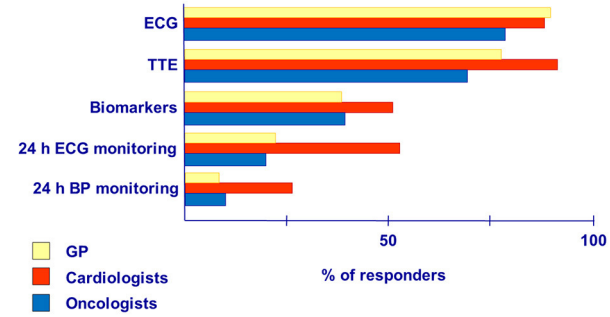

FIGURE 4 | Diagnostic methods for detection of cancer-induced CV complications.

Oncology patients may have CVD or preexisting risk factors that can lead to CV complications mainly due to cancer therapies. The role of a cardiologist or GP in cancer patient management includes prechemotherapy cardiac risk assessment, prevention, identification, and treatment of cardiotoxic complications (3).

Question 4. What is the main reason for cancer patients' referral: heart failure (HF), coronary artery disease (CAD), VTE, hypertension, arrhythmias, or pericarditis?

The main CV complications during antitumor therapy are presented in **Figure 3**.

HF—the most common complication of cancer treatment—is diagnosed mainly by cardiologists compared with GPs (80 vs. 69%) and oncologists-−55%.

Arterial hypertension and CAD in cancer patients had the highest detection rate among GPs (77 and 67%) and cardiologists (71 and 69%) compared with oncologists (60 and 47%). Hypertension is an established risk factor for cardiotoxicity (1, 2). Both cardiologists and GPs need to be informed about careful blood pressure monitoring and more aggressive antihypertensive treatment, especially in patients receiving VEGF inhibitors, due to their effect on blood pressure increase (4).

Severe complication, such as pericarditis, was detected and observed mainly through cardiologists (33%).

Oncologists often face thrombosis (70%) and prescribe anticoagulants for cancer patients, but the majority of those patients are referred then to cardiologists. In addition, both oncologists (70%) and cardiologists (70%) detected arrhythmias more often than GPs (57%).

Question 5. What diagnostic tools [ECG, transthoracic echocardiography (TTE), 24-h ECG, blood pressure monitoring, and biomarkers] do you provide in patients with cardiac complications during cancer therapy?

According to the survey data, ECG was the primary method used to diagnose CV complications of cancer therapy in the practice of cardiologists (91%), GPs (93%), and oncologists (83%).

Cardiac imaging, preferably TTE, should be performed at baseline and during therapy in recommended terms depending on the type of anticancer drugs (anthracyclines, trastuzumab, VEGF inhibitors), mainly in patients with preexisting CV diseases and risk factors (1, 5, 6).

Our data showed that TTE in cancer patients was used predominately by cardiologists (96%) than by GPs (79%) and oncologists (73%) (**Figure 4**).

Our data showed that 47% of cardiologists, 40% of oncologists, and 34% of GPs used biomarkers to detect cardiotoxicity, namely, troponins and natriuretic peptides, in their practice. However, the use of biomarkers needs to be clarified in detail among specialists, as the timing of shifts in these indicators and their detection will depend on many factors related to cancer therapy and the clinical status of the patient (7, 8).

Twenty-four-hour ECG monitoring may be helpful in patients with a history of arrhythmias or in patients in whom drugs with proarrhythmogenic effect (alkylating agents, ibrutinib, and taxanes) are prescribed in chemotherapy regimens. In our study, arrhythmias were presented in the practice of cardiologists (70%), GPs (57%), and oncologists (70%) (**Figure 3**). However, according to the survey, 24-h ECG monitoring was performed mainly by cardiologists (22%) and not widely.

Although hypertension is one of the well-known complications of cancer therapy, 24-h blood pressure monitoring has rarely been used by all groups of specialists (from 8% of GPs to 21% of cardiologists).

Question 6. What criteria of cardiotoxicity do you follow in cancer patients with LV dysfunction or HF?

Recent recommendations of the European Society of Cardiology (ESC) and the European Society of Medical Oncology (ESMO) accept cancer therapy-related cardiac dysfunction as a decline in LV EF of 10% points from baseline to an absolute value of <50% according to repeated evaluations by TTE or cardiac magnetic resonance imaging, as most previous studies were based on this EF value (1, 9). According to the survey, the awareness about the criteria for LV EF decreases because cardiotoxicity

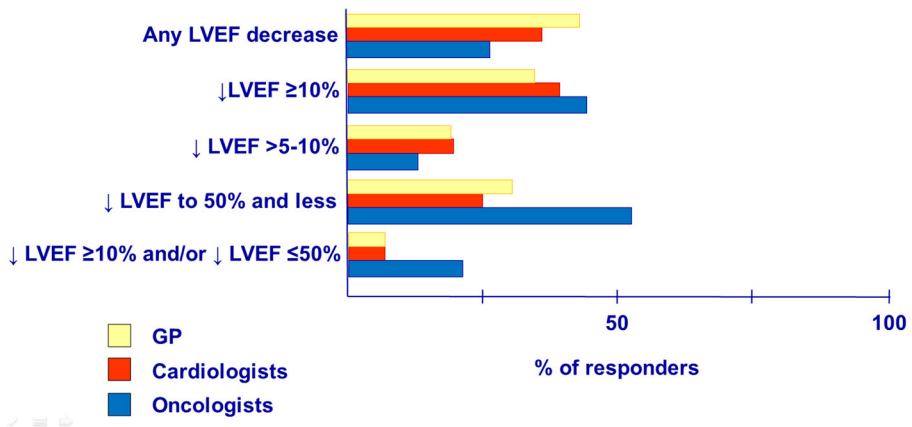

FIGURE 5 | Interpretation of cardiotoxic LV EF drop by different specialists.

in groups of cardiologists, GPs, and oncologists had differed (**Figure 5**).

Survey data indicated oncologists (and hematologists) (22%) to be more acknowledged in determining cardiotoxic cardiac dysfunction by LV EF and its reduction degree, namely, drop EF > 10 percentage points and/or drop EF to ≤50%, compared with GPs (6%) and cardiologists (6%). In contrast, the majority of GPs (39%) and cardiologists (33%) selected the answer that any LVEF decrease is a consequence of cardiotoxicity in comparison with oncologists (25%).

Question 7. What drugs do you usually prescribe to cancer patients with CVD, including those with CV complications?

Analysis of the use of the drug for CV complication treatment revealed that BB was prescribed significantly more often by cardiologists (85%) compared with GPs (58%) and oncologists (50%) (**Figure 6**).

At the same time, the use of ACE inhibitors/ARBs among cardiologists and GPs is relatively high and does not differ significantly (85 and 81%, respectively), but they are prescribed twice less by oncologists (40%).

Diuretics for the treatment of CV complications in cancer patients were prescribed by more than 50% of doctors in their practice, mostly by GPs (62%), predominately in patients with HF symptoms.

Our data showed that aspirin had been given more often by oncologists (43%) and cardiologists (37%), while GPs have prescribed aspirin significantly lower (21%). The use of aspirin in cancer patients is recommended, especially in patients with CAD and in patients with multiple myeloma during treatment with lenalidomide/thalidomide (10).

Anticoagulants are the basis of VTE pathogenic treatment (1, 10). According to the survey, oncologists (55%) and cardiologists (52%) have used anticoagulants in cancer patients more often in comparison with GPs (31%).

The issue of statins in cancer patients is controversial. However, data exist about the cardioprotective effect of statins (1).

In our study, cardiologists have prescribed statins more often (46%) compared with GPs (31%) and oncologists (25%). Today, concerning statin therapy in this cohort of patients, it is necessary to follow the general guidelines for managing patients with CV diseases, taking into account risk factors, lipid profile, liver function, etc.

Question 8. What position statements and guidelines do you follow in routine clinical practice in patients with possible CV complications of cancer treatment?

Responses to Question 8 indicated cardiologists to be guided by the recommendations of the ESC (78%) and the Ukrainian Society of Cardiology (62%) more often in their practice (**Figure 7**). GPs mainly used the recommendations of the Ukrainian Society of Cardiology (69%) and ESC (57%). However, there is low awareness of cardiologists and GPs about the recommendations of ESMO and ASCO, but oncologists predominately followed these recommendations (75 and 25%, respectively) in their routine clinical practice.

DISCUSSION

To date, the world has accumulated extensive experience in the management of cancer patients with CV complications (11, 12). The basis of effective treatment of these patients is a multidisciplinary approach: the team that, along with oncologists (chemotherapists, hematologists, and radiologists), includes cardiologists, GPs, rehabilitation specialists, psychologists, nurses, etc. (13–15).

We conducted a national survey to investigate the awareness in the management of cancer patients with CVD and CV complications among doctors of different specialties in real clinical practice and to understand the gaps in the knowledge.

After the cancer diagnosis establishment, patients should be evaluated for risk factors, CVD, and heart function (1). This will facilitate the detection of CV complications in cancer treatment by comparing the initial data and choosing appropriate monitoring and management for these patients.

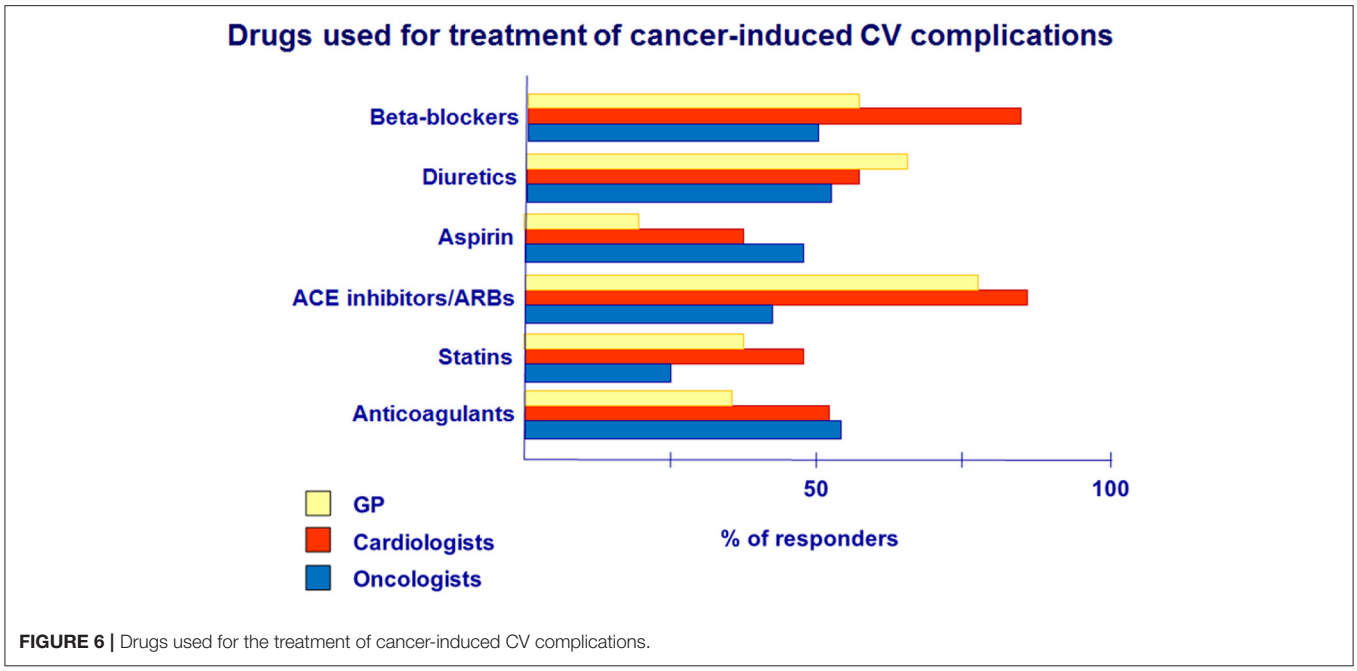

FIGURE 6 | Drugs used for the treatment of cancer-induced CV complications.

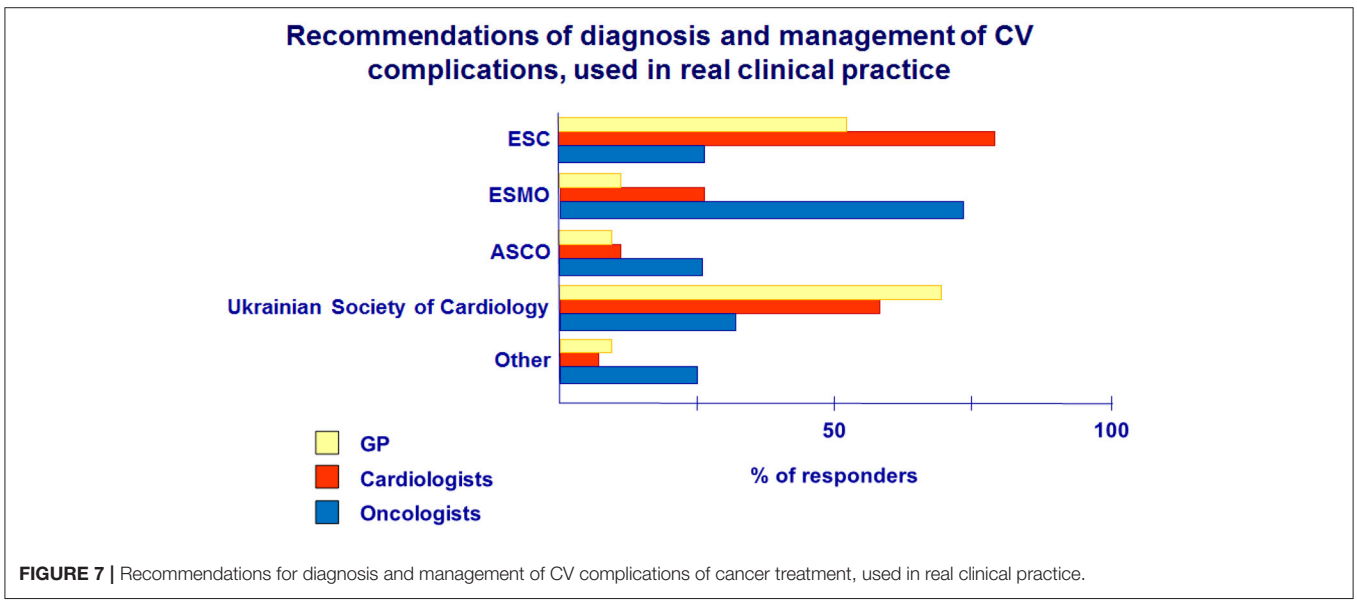

FIGURE 7 | Recommendations for diagnosis and management of CV complications of cancer treatment, used in real clinical practice.

During cancer therapy, in case of CV complications, it is necessary to follow a clear algorithm depending on the type of antitumor drug and clinical symptoms because each diagnostic method alone cannot provide complete information about the cardiac status of the patient.

According to the survey, patients are managed mostly by GPs after completion of anticancer treatment, so GPs should be aware of CV complications (HF, VTE) and, if necessary, refer those patients to cardiologists or cardio-oncology centers. Therefore, the GP is an essential member of the multidisciplinary team in the management of cancer patients.

However, follow-up strategies need to be established and adapted for different specialists for better and earlier diagnostic of CV events associated with cancer treatment in a short- or long-term perspective.

Main efforts should be directed on primary prevention strategies to reduce the risk of cardiotoxicity, identification of complications during therapy, and close monitoring after the end of cancer therapy.

LV myocardial dysfunction and HF are the most common complications of antitumor therapy, the clinical manifestations of which may occur during treatment but can develop several years later (1, 2, 5).

In our study, HF was diagnosed by cardiologists (80%), GPs (69%), and oncologists (55%). It is recommended to perform ECG and TTE in cancer patients, predominately with risk

factors and CVD, before antineoplastic treatment with potentially cardiotoxic drugs and in a monitoring setting (9).

From this perspective, the determination of LVEF before cancer treatment is crucial because the initial value of the EF will facilitate its drop assessment during cancer therapy and monitoring after treatment completion. It is vital to identify HF/LV dysfunction as early as possible and prescribe cardioprotective therapy for primary prevention or HF treatment.

Survey data indicated oncologists (and hematologists) to be more acknowledged in the determination of cardiotoxic dysfunction by LV EF and its reduction degree. However, data of LV dysfunction knowledge revealed that cardiologists and GPs should be given a more precise definition of LVEF drop criteria because the interpretation of any or slight LVEF decrease as cardiotoxicity may lead to unwarranted patient re-examinations and violation of the timing of cancer treatment, which is essential.

Once the CV complication occurs during antitumor treatment, the patient should consult the cardiologist or GP to prescribe effective cardioprotective therapy and decide on the possibility of further anticancer treatment or changes in the chemotherapy regimens. In our study, prescription of BB and ACE inhibitors by cardiologists and GPs was at high percent. The positive effects of ACE inhibitors and BB were recently evaluated in clinical trials in cancer patients (8, 16–18). It is recommended that ACE inhibitors and BB should be started as early as possible, with appropriate drug dose titration, especially in patients with LV dysfunction due to anthracycline cardiotoxicity (8, 16). As an example, the use of enalapril with carvedilol in the clinical study led to faster LV EF recovery as a response to treatment (17).

VTE occurrence can reach more than 20% in cancer patients. Anticoagulants are the basis of VTE pathogenic treatment (1, 10). Prescription of anticoagulants by GPs was low (31%); therefore, informing physicians about the risks of thrombosis associated with cancer site, the type of antitumor treatment, and personal risk factors is essential in cancer patient management. In addition, the choice of anticoagulant therapy in these patients, its duration, and bleeding control need to be explained more clearly (19).

In recent years, several guidelines and recommendations for clinical practice in cardio-oncology have been issued. Recommendations of the ESC, ESMO, the ASCO, and the European Association of Cardiovascular Imaging (EACVI) are the main documents that justify the decision on detection, monitoring, and treatment of patients during and after cancer therapy (1, 5, 6, 9). In Ukraine, the first National recommendations for managing patients with CV complications during cancer treatment were adapted and published in 2018 at the initiative of the CO Center and the support of the National Cancer Institute. In our study, cardiologists and GPs were guided mainly by the recommendations of the Ukrainian Society of Cardiology and ESC; however, the awareness of ESMO and ASCO recommendations is low, but they are followed mainly by oncologists.

The need for specialists in CO is growing rapidly. Thus, CO requires special knowledge, experience, and dedicated training. In 2020, the CO Leadership Council published a document about education and training in CO that may serve as a roadmap toward CO as a new discipline (20). The authors proposed a three-level CO training.

Based on this approach and the results of UkrNatSurv, we have started the implementation of the first-level CO training program for cardiologists, GPs, and oncologists, which includes basic knowledge on the assessment and management of cancer patients.

However, government support is needed to make this training program available for doctors involved in cardio-oncology across the country.

Additionally, the development of local clinical protocols, recommendations for cancer patient management, and their implementation in real clinical practice should be provided. The Ukrainian CO WG has published recommendations on VTE in cancer, CV complications in breast cancer treatment, and HF in cancer.

Such initiative as providing surveys will give understanding about how to provide optimal care for the cancer patient population.

LIMITATIONS

Survey results and implications of findings are discussed.

The data of this study are not directly representative of the whole country. It was not possible also to assess regional differences.

We suppose that the survey had higher uptake by specialists who were interested and experienced in cardio-oncology. GPs were less likely to participate if they did not have confidence in their knowledge of this field.

These limitations should inform clinicians on the importance of ongoing educational activity and updated guidelines to assist in clinical decision making.

CONCLUSION

UkrNatSurv is the first survey in Ukraine to investigate the awareness of cardio-oncology care provided to cancer patients. The study results indicated cardiologists and GPs to be equal players in the cardio-oncology team. However, more clear recommendations for managing cancer patients with CV complications should be published and implemented among these specialists.

Therefore, the priority is to develop a national CO educational program in accordance with the statement of the American College of Cardiology CO Council.

Results of the survey underlined that it is crucial to identify the level of knowledge of the specialists involved in cardio-oncology to get benefit from this service. Different grades of training program will be proposed for the specialists in order to upgrade their experience.

A multidisciplinary approach to cancer patient management, stratification of CV complications before cancer treatment, careful monitoring during treatment, and subsequent long-term monitoring are the key points to improving the survival, quality, and life expectancy of cancer patients.

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 SI National Scientific Center The M.D.Strazhesko Institute of Cardiology. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SK and ND conceptualized and designed the study, collected, analyzed, and interpreted the data. ND drafted the article. SK made critical revisions to the article and approved the final version to be published. All authors contributed to the article and approved the submitted version.

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REVIEW
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Immune Checkpoint Inhibitors and the Heart

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Immune checkpoint inhibitors (ICIs) represent a break-through treatment for a large number of cancer types. This treatment is increasingly being recommended. ICIs are prescribed for primary tumours and for metastases, adjuvant/neo-adjuvant therapy. Thus, there is an increased need for expertise in the field, including the ways of response and toxicities related to them. ICIs become toxic because of the removal of self-tolerance, which in turn induces autoimmune processes that affect every organ. However, when relating to the heart, it has been noticed to be leading to acute heart failure and even death caused by various mechanisms, such as: myocarditis, pericarditis, arrhythmia, and Takotsubo cardiomyopathy. This review aims to address the above issues by focusing on the latest findings on the topic, by adding some insights on the mechanism of action of ICIs with a special focus on the myocardial tissue, by providing information on clinical manifestations, diagnosis and (wherever possible) treatment of the cardiotoxic events related to this therapy. The information is expanding and in many cases, the articles we found refer mainly to case-presentations and studies conducted on small populations. However, we consider that it is worthwhile to raise awareness of this new treatment, especially since it is widely now and it provides a significant increase in the survival rate in patients who receive it.

Keywords: immune checkpoint inhibitors, chemotherapy, cardiotoxicity, immune-related adverse events, cancer, CTLA-4, PD-L1

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INTRODUCTION

The immune system plays a paramount role in maintaining the balance between self and non-self cells, but it might have a serious problem when having to make a distinction between malignant and benign cells. To be able to do this, it needs to have the ability to eliminate the tumour cells, which in turn always try to evade the immune system and proliferate. These mechanisms are known as "immune editing" (1). As we can easily conclude, cancer develops secondary to the toleration of the malignant cells because tumour cells are able to cause an overexpression of the checkpoint proteins that protect them from being destroyed by the immune system. Thus, in order to be able to maintain the balance, this system needs both inhibitory and stimulating signals. First of all, it needs a stimulator in order for the system to start producing immune factors and then it needs inhibitors so that the system does not start overreacting and hence self-tissue destroying (1).

Over the last years a large variety of cancer types were targeted through checkpoint inhibition: melanoma, lung, head and neck, renal cell, urothelial, Hodgkin's lymphoma, etc. However, the problem with this type of immunological treatment is the adverse reactions that can occur on different levels: brain, skin, gastrointestinal system, liver, pancreas, lungs, kidneys, endocrine system, neurologic system, haematologic system, ophtalmologic level, cardiac system and musculoskeletal level as well (1). These effects range from minor to major.

Recently, several authors have reported cases of severe cardiotoxicity in patients treated with immunotherapy, but their incidence is still low maybe because, until now there have not been conducted large populational studies on these effects. Given that cases of severe heart failure and death are reported, cardiologists and oncologists give special consideration to this therapy.

TYPES OF CHECKPOINT INHIBITORS

One of the pivotal modulators and effectors of the immune system are T cell lymphocytes. Antigen presenting cells (APCs) activate naïve T cells through the interaction between MHC (major histocompatibility complex) expressed on the APCs and the T cell receptor (TCR). Furthermore, there are several other stimulatory signals, namely: CD28, CD80 (B7-1) or CD86 (B7-2), which are also essential for the activation of T cell lymphocytes. But to prevent the hyperactivation of the immune system, they need to be regulated by immune checkpoints (2).

Several major classes of ICIs have been used up until now, namely:

a. monoclonal antibodies against PD-1–programmed cell death protein-1 (pembrolizumab, nivolumab, cemiplimab, dostralimab) and its ligand PD-L1 (atezolizumab, avelumab, durvalumab)
b. monoclonal antibodies against cytotoxic T lymphocyte-associated antigen 4 (CTLA-4): ipilimumab, tremelimumab, quavonlimab
c. combination of CTLA-4 and PD-1: ipilimumab and cemiplimab; Ipilimumab + pembrolizumab, Tremelimumab + durvalumab.
d. novel checkpoint inhibitors targeting: lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), B and T cell lymphocyte attenuator (BTLA), T cell immunoglobulin and ITIM domain (TGIT), V domain Ig suppressor of T cell activation (VISTA) and B7 homologue 3 protein (B7-H3) (3).

MECHANISM OF ACTION

The main goal of the checkpoint inhibitors is to decrease autoimmunity by activating more non-T cells as opposed to T regulatory cells, thus targeting tumour cells (2). There are many types of tumour, that can benefit from treatment with ICI, as shown in **Table 1**.

Several events allow the immune system to target tumour cells, as follows (6):

TABLE 1 | Types of checkpoint inhibitors and targeted cancers.

Class of ICI	Drug	Types of targeted cancers
CTLA-4-i	Ipilimumab	Melanoma
PD1-i	Nivolumab	Melanoma, NSCLC, SLCL, RCC, HCC, Hodgkin's lymphoma, head and neck cancer, metastatic colorectal cancer, urothelial carcinoma
	Pembrolizumab	Melanoma, NSCLC, Hodgkin's lymphoma, urothelial carcinoma, gastric cancer, large B cell lymphoma primarily mediastinal location, cervical cancer
	Cemiplimab	Metastatic cutaneous squamous cell carcinoma
PD-L1-i	Atezolizumab	NSCL, urothelial carcinoma
	Avelumab	Meckel cell carcinoma, urothelial carcinoma
	Durvalumab	Urothelial carcinoma, NSCLC
Combination of PD1-i and CTLA-4 i	Ipilimumab+ Nivolumab	Colorectal cancer (some subtypes), melanoma and RCC

PD-1-I, Programmed cell death ligand 1 inhibitor; CTLA-4 I, cytotoxic T lymphocyte antigen 4 inhibitor; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; SCLC, small cell lung cancer [adapted after Zhou et al. (4) and Tajiri et al. (5)].

1 the priming phase consists in the amplification of the T cell response. This cycle begins when the dendritic cells recognise cancer cell antigens via a major histocompatibility complex, thus priming the activation of effector T cells onto cancer cells.
2 The effector phase: activated effector T cells travel and infiltrate the tumour starting destruction of cancer cells. This activity is made possible through the interaction between the T cell receptor (TCR) and cognate antigen bound to MHC. Subsequently, more cancer cell antigens are released and a mechanism of positive feedback expands the immunity of T cells to tumour cells.

The main goal of checkpoint inhibitors is to decrease autoimmunity/autoimmune activity by activating more non-T cells as opposed to T regulatory cells, thus targeting tumour cells (2) Numerous types of tumour can benefit from treatment with ICI, as shown in **Table 1**.

Some of the mechanisms of adaptive immune resistance include:

- down-regulation of major histocompatibility complex antigen expression,
- secretion of immunosuppressive cytokines,
- negative regulation of cytotoxic CD8+ T cells through checkpoint inhibition (7).

PD-L1

PD-L1 is expressed on the B lymphocyte membrane and other antigen presenting cells (APCs) such as macrophages and dendritic cells. PD-L1 is the programmed cell death ligand expressed in tumour cells. PD-1 action revolves around the tumour environment and it prevents T cells from expressing

their function (8). They act mostly in the effector phase, and the blockade occurs mainly at the tissue level and in the microenvironment of the tumour (9).

The PD-1/PD-L1 duo reduces the cytokine production and the T lymphocyte proliferation and survival. These actions help blocking the negative regulatory signalling pathway, thus enhancing the actions of the immune system against tumours. They do this by activating earlier primed T cells, which have lost previous effector and proliferative functions (4, 10, 11). After activation, T cells, B cells, natural killer cells, natural killer T cells, macrophages and dendritic cells express PD-1 on their surface (2). Several types of cells express PD-L1, namely: the haematopoietic and non-haematopoietic cells such as hepatocytes, astrocytes, epithelial cells, muscle cells (including cardiomyocytes), vascular endothelial cells and pancreatic cells (2). Many authors have also concluded that the tumour expression of PD-L1 is associated with a poor prognosis.

CTLA-4

CTLA-4 is found in the intracellular vesicles only on activated T cells and is responsible for the amplitude of T cell activation (4). It belongs to the B7/CD28 family and acts by indirectly lowering signalling through the co-stimulatory receptor CD28, which also restores T cell-three-signal activation in the tumour, draining lymph nodes (9). It is translocated to the cell surface in response to T-cell receptor (TCR) activation. CD28 and/or IL2 co-stimulate their upregulation. It competes with CD28 for binding with B7 ligands (CD80, CD86), for which it also has higher affinity (10, 11). This leads to the suppression of the priming phase. CTLA-4 also suppresses regulatory T cells (9).

Naturally, cancer cells start expressing PD-1/PD-L2 as they try to protect themselves and survive. It is understandable that targeting PD-1, PD-L1, PD-L2, CTLA-4 leads to an enhanced immunological response against tumour cells.

RISK FACTORS FOR CARDIAC ADVERSE EVENTS ASSOCIATED WITH ICIS

Authors have not concluded yet on the risk factors that predispose to important cardiac toxicity, in patients treated with ICIs. However, some specialists have pointed out some elements of predisposition (**Table 2**) but they have not been confirmed yet on large cohorts. **Table 2** shows a list of possible risk factors identified more frequently in patients who have developed immune-related adverse events (IRAEs). Therefore, we believe that cardio-oncology specialists should give special attention and perform frequent follow-up examination during treatment with ICIs.

ADVERSE EVENTS ASSOCIATED WITH THE TREATMENT WITH IMMUNE CHECKPOINT INHIBITORS

The current literature has shown that the treatment with ICIs, used as standard therapy for cancer patients, is often accompanied by multiple immune-related adverse events (IRAEs) such as colitis, thyroid hormones imbalance, dermatological, musculoskeletal, gastrointestinal and cardiovascular events. These seem to be correlated with the number of drugs prescribed and used (single class or combination) and occur more frequently during the first 3 months of treatment. They are usually induced by erratically autoreactive T cell activation (12–14). Most of the IRAEs can be antagonised with anti-inflammatory agents such as glucocorticoids and in some cases more potent therapies such as infliximab (an anti-TNF alpha receptor agent) or mycophenolate (an inhibitor of purine synthesis in T and B-cells) (15). However, some IRAEs do not respond to any of these treatments.

TABLE 2 | Risk factors for developing cardiac IRAEs [adapted after Varricchi et al. (2) and Zhou et al. (4)].

Therapy with combination of ICIs
Detection of skeletal myositis (usually precedes myocarditis)
Lung cancer (combination of radiotherapy and ICIs)
Autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis)
Male gender
Concomitant use of anthracyclines, anti-ErbB2 drugs, Raf and MEK inhibitors, VEGF tyrosine kinase inhibitors
Genetic polymorphism of CTLA-4, PD-1, PD-L1; activation of T-cell clones against cardiac antigens
Cardiotoxic therapies
Decreased global longitudinal strain - GLS (hypertension, coronary artery disease, heart failure, myocardial infarction, myocarditis, diabetes mellitus, dyslipidemia)
ECG conduction disease
Flu vaccination – protection from IRAEs

The exact mechanisms of cardiac involvement still require clarification. Some cardiac pathologies might just be coincidental with the malignancy in a patient, and therefore it is rather difficult to identify cardiac adverse events associated with the ICIs therapy but this is of paramount importance however, as such a condition can be profoundly serious and even life-threatening, having the potential to lead to death.

Elosta et al. demonstrated in a meta-analysis, which included 28 clinical trials, that IRAEs occur more frequently in patients treated with CTLA-4 inhibitors as compared to PD-1 and PD-L1 blockers (53.8, 26.5, and 17.1%, respectively) (1). Consequently, they have concluded that targeting immune and regulatory T cells is accompanied by a higher incidence of adverse events.

MECHANISM OF IMMUNE CARDIOTOXICITY

A 2018 paper by Xiaoxiao et al. showed that during a period of 10 years the total number of cardiac IRAEs declared in the WHO global database counted 31,321 (16). The autopsy and histological specimens from patients or animal models treated with ICIs have shown that myocarditis is a major cardiac lesion.

Other types of cardiovascular adverse events also exist, namely: pericardial effusion, arrhythmias (out of which supraventricular tachycardia is more commonly encountered), acute coronary syndrome, vasculitis (e.g., temporal arteritis or rheumatic polymyalgia) (17).

In healthy individuals, the thymus regulates the number of autoreactive T cells that are released in the periphery. According to this "central tolerance" some of them are deleted and others are distributed in the periphery according to "peripheral tolerance." The "immunotolerance" results from the downregulation of T cell activation by means of the competition between CTLA-4 and CD28 (2). Once this tolerance is removed however, the immune system develops a state of hyperactivity with subsequent macrophage-mediated toxicity and production of antibodies from activated B cells alongside a low level of T reactive cells (18).

Moreover, the interval of time required for toxicity to occur has not been exactly established yet; besides, it seems not to follow any pattern driven either by type or by target. In addition, mechanisms differ even in patients treated with the same agent.

Types of Immune Checkpoint Inhibitors-Related Cardiac Events

Main clinical cardiotoxic events are shown in **Figure 1**.

Myocarditis

The predominant histopathological trait in myocarditis is lymphocytic infiltrates in the myocardium and the conduction system. They are mostly represented by CD3, CD4+/CD8+ lymphocytes and by some CD68 cells (macrophages) and multinuclear giant cellular infiltration (16, 19–21). This finding was also demonstrated in murine models. The development of severe myocarditis was observed in CTLA-4 -deficient mice. They also proved to have massive T cell infiltration (22). Compared to them, another type of behaviour was found in PD-1 -deficient mice. Thus, those with BALB/c background developed autoimmune dilated cardiomyopathy (23), whereas PD-1 -deficient autoimmune-prone MRL mice showed an important CD4+ and CD8+ T cell infiltration (24). Similar findings of severe myocarditis were reported in PD-L1 -deficient MRL mice (22). All things considered, the severity of the clinical manifestation of this autoimmune disease relates to the disruption in the PD-1/PD-L1 pathway and may be attributed to polymorphism in specific genes as highlighted on the murine model of the PD-L1/MRL mice. Authors state that similar assumptions can also be made in human subjects (22).

Myocarditis was observed at a median of 27 days (range 5–155) from the initial dose of ICI therapy but apparently most cases emerge during the first 6 weeks (25). Unfortunately, there is limited information about the exact onset time of the disease as the number of cases included in the studies so far is limited, so the data is uncertain.

The severity of the myocardial disease was positively correlated with the number of doses of anti-CTLA-1 but not with that of anti PD-1/antiPD-L1 antibodies (26, 27). However, there were also reports of cases, in which patients developed this condition after they were administered only one dose of anti-CTLA-1 antibodies.

Some patients may be asymptomatic but some develop signs and symptoms of heart failure. The conduction system may also be involved and therefore patients can present with conduction abnormalities, such as block of different types and degrees. Moreover, malignant arrhythmias can occur (2). Hence, sudden cardiac death is also possible. Unfortunately, no algorithms have been found so far that allow identification of patients at risk. Because of the heterogeneity of the clinical picture and time of onset, it is extremely important to develop tools for the early detection of ICI-related myocarditis so that patients can receive the proper treatment. Therefore, the introduction of biomarkers related to myocyte damage would be a promising step forward. Some authors suggest the measuring of troponin levels, while others consider NT-proBNP to be helpful (28). One should however, also carefully assess whether the elevation of these biomarkers cloud also be caused by other concomitant cardiac conditions. Therefore, perhaps a dynamic assessment, which includes a series of periodical clinical evaluation combined with an EKG, biomarkers and echocardiography, might be helpful to allow the patient to be properly referred to the cardio-oncology team for assessing whether further investigations and/or treatment are required (MRI, PET-scan) [**Figure 2**; (29)].

Chen et al. reported that the degree of troponin elevation could predict cardiovascular death, cardiogenic shock and cardiac arrest while persistent troponin elevation was a significant predictor of a 4-fold increased risk for major adverse cardiaovascular events (MACE) (30).

ECG abnormalities in cancer patients treated with ICIs include sinus tachycardia, ventricular and supraventricular arrhythmias, bundle branch block, complete AV block and ventricular tachycardia, therefore basic ECG is also important to be performed baseline and during treatment. Unfortunately, all of these are non-specific and the ECG examination is often times normal in myocarditis (19, 31).

The study of Mahmood et al. on patients with ICI-related myocarditis found abnormal ECG in 89% of the patients, NT-proBNP elevation in 66% of them, while the left ventricular ejection fraction (LVEF) only in 49% and concluded that LVEF is not a suitable diagnostic item for these patients (32). Similar results where reported by Awadalla et al., who showed that 60% of the patients presenting with myocarditis following ICIs had preserved EF in spite of a large amount of affected myocardium (33).

In contrast, the study published by Escudier et al. found LV systolic dysfunction was found in 79% of the patients (34). These conflicting results suggest that LVEF alone might not be a reliable tool to assess myocarditis (30). Thus, in order to identify the myocardial involvement and to establish risk criteria, the global longitudinal strain (GLS) was proposed for monitoring cancer patients who receive chemotherapy (35, 36) because it was shown that GLS could identify myocardial involvement even in the context of preserved EF. In patients with myocarditis after ICIs, Awadalla et al. have reported that GLS is reduced in all myocarditis patients regardless of decreased or preserved EF at baseline. GLS decreases during hospitalisation and also proved to have predictive power because every decrease in GLS was

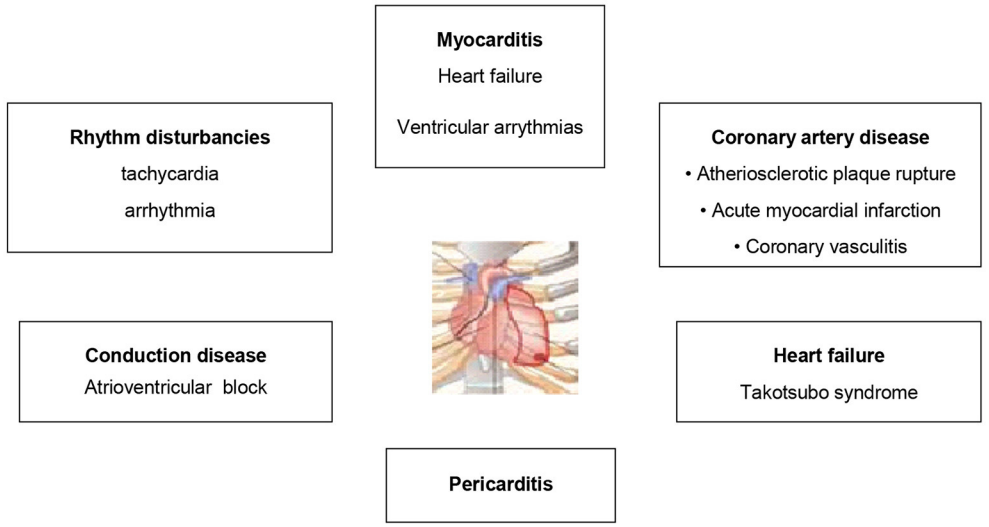

FIGURE 1 | Main clinical types of cardiac involvement during treatment with immune checkpoint inhibitors.

associated with an increase in MACE (1.5-fold in patients with decreased EF and 4-fold in patients with preserved EF) (33).

Mincu et al. showed that monitoring GLS in melanoma patients could identify ICIs- induced subclinical left ventricular dysfunction (in the absence of myocarditis) and extracardiac adverse events during the first month of treatment, whereas ejection fraction monitoring could only identify the radial strain but not the circumferential strain (37).

Kasner et al. showed that patients with chronic myocarditis have reduced GLS even with preserved EF (38).

Further, GLS was found to have superior diagnostic performance (sensitivity, specificity, and accuracy of 82, 70, and 76%, respectively) when compared to cMRI based on the Lake Louise criteria (sensitivity, specificity, and accuracy of 54, 71, and 67%, respectively), while their combination further increased the diagnostic performance (sensitivity, specificity, and accuracy of 96, 55, and 75%, respectively) (38).

Cardiac magnetic resonance is the non-invasive technique commonly used in myocarditis, being also helpful (76% sensitivity and 96% specificity) (39). The features of ICIs induced myocarditis are slightly different from those usually found in other types of myocarditis. In some cases, no inflammation, no fibrosis or scarring can be found (32, 40–42).

Escoudier et al. reported myocardial ischemia in 33% of the patients and late gadolinium enhancement (LGE) in 23%, but the number of subjects included in the study was only 15 (39). Mahmood et al. studied 35 cases and found LGE in 74% of them. The discrepancies are high and consequently there is a need to evaluate them on larger cohort studies (34).

The gold-standard in the diagnosis of myocarditis remains the endomyocardial biopsy because it can provide evidence of the lymphocytic infiltrate, CD4 and CD* T cells, CD68 macrophages, rare CD20 cells and plasmocytes with no evidence of eosinophilic granulomas or giant cells (40, 41, 43, 44) because the mechanism is a direct injury by hiperactivated T cells (30).

Recently, Finke et al. have used FAPI PET/CT in patients treated with ICIs and showed that it can be useful for the early detection of myocarditis and cardiac risk stratification (in combination with biomarkers, ECG and echocardiography (45).

With regard to management, the treatment usually consists of corticosteroids and immunotherapy (immunosuppressive agents, high-dose intravenous immunoglobulin, immunoabsorption, and plasmapheresis) for non-responders to steroids (22, 46).

Immunotherapies for cancer are relatively new, and therefore long-term data regarding prognosis in patients with cardiotoxicity following ICIs treatment is scarce. However, this issue has been addressed in some studies that have found and reported high fatality rates. For example, a systematic review that included 99 patients has found a fatality rate of 35%. Other observational studies have concluded that there is a 50% risk of major cardiac adverse events in ICIs related myocarditis in comparison to non-ICIs related myocarditis (47–49).

Pericarditis

Pericarditis is another possible complication of ICIs therapy; it can occur even after the first dose but usually it occurs 6–11 months after the initial dose of the ICI treatment. Patients can develop either tamponade, or effusive-constrictive pericarditis. The exact mechanism that leads to the pericardial effusion has not been fully explained yet; it might be inflammation. In a systematic review paper that included 705 cases of ICI-associated pericardial disease, the authors have stated that this condition is not as rare as initially believed but they have mentioned that there might be some biases coming from the fact that some malignancies complicate with pericardial effusion even in the

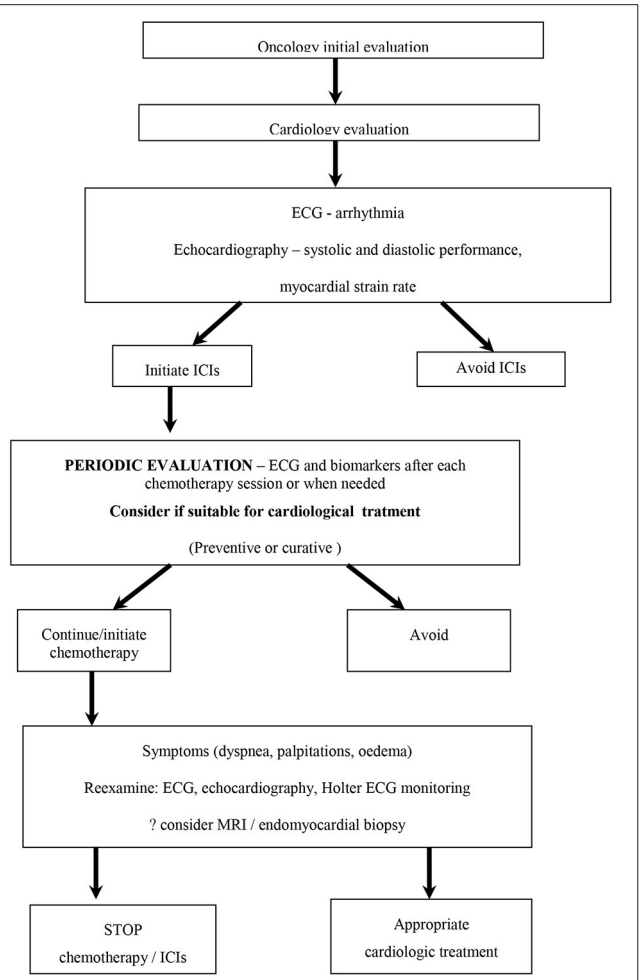

FIGURE 2 | Algorithm for evaluating the patient who will be treated with immune checkpoint inhibitors [adapted after Liu et al. (28)].

absence of immunotherapy. Hence, the diagnosis of ICIs-related pericardial effusion is challenging (50).

ICIs- associated pericardial disease mainly affects men (60%) it was more frequently associated with anti-PD-1/PD-L1 regimens and combination therapy did not increase this. Moreover, the various types of cancer and the different ICIs approved for these specific tumours might influence the occurrence of the pericardial disease. Another confounding factor that might alter the percentage of pericarditis is the use of radiation in conjunction with immunotherapy. It appears that radiation primes an endogenous antigen specific immune response (17, 50). They expose potential shared antigens to T cell recognition, and this in turn might contribute to the development of pericarditis (30). Some studies have attributed this adverse event to nivolumab therapy for lung cancer. Some patients with previous tuberculosis have experienced a reactivation of this condition apparently because of host induced hypersensitivity response (51–53).

Clinically, these patients present similar symptoms to those described in pericarditis of other causes: chest pain, shortness of breath, etc., though in some cases, it might rapidly develop into respiratory failure. The ECG shows low QRS voltage, PR segment depression, and inversion of T waves. The echocardiography is a useful tool to detect the pericardial effusion, but in some cases CT and MRI were used. Troponin was usually elevated when pericarditis was accompanied by myocarditis (30, 34, 52–55).

We have found several articles, consisting of case-reports and studies conducted on small cohorts. In all cases, pericardiocentesis was the treatment of choice; the pericardial fluid analysis showed leukocytes with lymphocyte predominance and no signs of malignant cells (51–55).

Arrhythmias

As hypothesised before, MRI tests conducted in patients with myocarditis showed signs of inflammation. This in turn contributes to a significant heterogeneity in the myocardium, which can lead to a multitude of rhythm and conduction disturbances. Escoudier et al. reported atrial fibrillation in 30% of patients, ventricular arrhythmias in 27% and conduction disturbances in 17% of the patients in their study (34). Some authors mention that the presence of atrial fibrillation should be regarded with caution as it might be due to the ICI treatment itself. However, arrhythmias are more likely to coexist with other conditions such as myocarditis rather than be caused by the ICI treatment itself. We also need to mention that ventricular tachycardia and ventricular fibrillation cause sudden death, and therefore extra care should be given to any of the above signs (56, 57).

Inflammatory T cells infiltrate the conduction system so the ICI- mediated conduction disease is very serious and can be fatal. Puzanov et al. in their article, suggested that all patients receiving ICIs should be regularly screened at baseline and every 1–2 weeks for 6 weeks using an ECG. These patients should be taken into consideration for early pacing, even more so if they also have myocarditis because the progression towards complete AV block is frequent, and there is increased risk for sudden death (11). We conclude that whenever bradycardia or heart block is found, the patient should be referred for Holter ECG monitoring, echo and even an MRI so that the physician can obtain more information about subclinical inflammation or myocarditis allowing an oncology-cardiology team to make the appropriate decision.

It appears that the inflammation secondary to T lymphocytes patchy infiltration in the sinoatrial and atrioventricular nodes is also responsible for atrial fibrillation. In conclusion, the development of atrial fibrillation is directly connected to the treatment with ICIs (49, 58).

An evaluation report made public by the European Medicine Agency revealed the fact that the authors reported 1.3% incidence of tachycardia, 0.4% incidence of arrhythmia and 0.2% incidence of atrial fibrillation in the patients treated with nivolumab in combination with ipilimumab (59).

Takotsubo Syndrome

Also known as "the broken heart syndrome," this condition consists of left ventricular dysfunction accompanied by wall motion abnormalities, which usually involves the apical

and mid-myocardium portion of the left ventricle. This is transient and it occurs in the absence of a significant atherosclerotic disease. The mechanism underlying this condition is unknown but there are several suppositions: one is the direct action of ICIs on coronary arteries, which leads to coronary vasospasm in multiple areas (probably affecting not only large epicardial arteries but also the microvasculature).

Other authors have proposed an interesting mechanism mainly concentrated around a myocardial response to an increased release of catecholamines from the adrenal gland and postganglionic sympathetic nerves (28, 60). The exact mechanism is unclear yet.

Some studies have mentioned a high prevalence (up to 28.5%) of cancer in TTS patients and this subgroup has also been reported to have high mortality rates (61, 62). Data shows that in most of these cases the contractility of the left ventricle is especially poor at the level of the apex, which is also ectatic. This feature is similarly found in non-cancer patients with TTS so it might is not necessarily be related to ICIs. A significant number of patients have been reported to develop the "inverted TTS," which is basal and mid segment akinesia and minimal/moderate LV systolic dysfunction (63, 64). Apparently, these patients develop TTS later in the course of immunotherapy (15 weeks−8 months) but the alterations are reversible with conventional treatment such as beta-blockers, ACEIs, corticotherapy in conjunction with heart failure treatment (30).

Myocardial Infarction

Ischaemic heart disease is a condition accompanied by chronic inflammation. This substantially accelerates plaque rupture, which is the fundamental event that leads to myocardial infarction and stroke. When using ICIs, there are at least 2 mechanisms that have been postulated as being involved in the acute myocardial infarction:

1. The activation of inflammation in preexisting plaques which triggers fibrous cap rupture and therefore acute coronary thrombosis
2. The direct activation of T cell-mediated coronary vasculitis in the absence of atherosclerosis.

The latter mechanism still needs to confirmation. The exact sequence of events is difficult to fully establish as patients with cancer are usually older and with concomitant/associated cardiovascular disease. Numerous questions still require answers, namely: whether immunotherapy could increase long-term cardiovascular inflammation; whether immunotherapy transiently increases plaque inflammatory activity, which in turn would trigger future acute coronary events. Another question also needs an answer on how acute inflammatory reactions to tumours trigger other events such as activation of platelets and coagulation cascade, which in turn contribute to cardiovascular toxic events (28).

MANAGEMENT

The management of immunotherapy-related complications requires multiple approaches and depends on the severity of the cardiotoxicity. In a position paper, the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group, highlitghts 4 degrees of severity:

1. Abnormal cardiac biomarker testing, including abnormal ECG: it does not require discontinuation or immunotherapy.
2. Abnormal screening tests with mild symptoms: requires management of additional cardiac disease and risk factors.
3. Moderately abnormal testing or symptoms with mild activity: withdrawal of the ICIs therapy; initiation of high-dose prednisolone (1–2 mg/kg).
4. Moderate to severe decompensation that requires intravenous medication or intervention or life-threatening conditions: consider high-dose corticosteroid therapy. Also consider immunoglobulins, infliximab, or anti-thymocyte globulin as second-line therapy (11).

Discontinuation of the Treatment With Immune Checkpoint Inhibitors

The decision of discontinuation of the ICIs treatment requires a multidisciplinary cardio-oncology approach. We should keep in mind that ICIs have long half-life and cessation of the treatment at one point would not correct the adverse effects at once. This decision also requires certainty that the clinical cardiac complication is related to the treatment with ICIs. In mild ICIs cardiotoxic events, authors conclude that restarting treatment is reasonable after the resolution of cardiotoxicity, but with close surveillance regarding the recurrence of such events. Nevertheless, these decisions are difficult to make and close monitoring by the/a cardio-oncology specialist is mandatory (28).

Consider Conventional Therapies for Cardiac Events

Whenever necessary, specialists must use other conventional cardiac treatments to manage complications like overt pulmonary oedema (use of diuretics, nitrates), complete AV blocks (use of temporary/permanent pacemakers), ventricular tachyarrhythmias (use of beta-blockers, amiodarone or electrical cardioversion/defibrillation). In extreme cases of cardiogenic shock, the use of inotropic support, extracorporeal membrane oxygenation or a left ventricular assist device should be taken into consideration, depending on the clinical context, comorbidities, prognosis of cardiac and non-cardiac complications alongside the cancer type/stage.

In cases of pericarditis, the guideline recommendations, that should be applied, include pericardiocentesis of large effusions and tamponade.

Patients with suspected acute coronary syndrome, who are also receiving ICIs therapy, should be admitted to a coronary unit for continuous EKG monitoring, surveillance of cardiac biomarkers and of the left ventricular function including measurement of the left ventricular ejection fraction

and strain. Beta-blockers and angiotensin-converting-enzyme-inhibitors have not been directly correlated to the inhibition of the emergence of adverse cardiac events related to ICIs. However, all this medication should be administered in cases of left ventricular dysfunction. Moreover, in compliance with the recommendations made in the current ESC guidelines on the management of acute coronary syndromes, a coronary angiography should be performed when an acute coronary syndrome is suspected (28).

Immunosuppression

The intensity of immunosuppression therapy depends on the severity of the adverse cardiac event, as described above. High intensity corticotherapy should be considered for severe cases of myocarditis, symptomatic heart failure, complete A-V block, ventricular arrhythmias (e.g., 500–1,000 mg/day i.v. methylprednisolone until the patient is clinically stable, followed by 1 mg/kg/day oral prednisolone with weaning, depending on the clinical course of the complication and periodical evaluation of troponin, inflammation on MRI, left ventricular dysfunction on echocardiogram, EKG).

If cortisteroid therapy is not sufficient, second line treatment with infliximab or mofetil should be considered. Immunoglobulin or anti-thymocyte globulin might be considered in extreme cases too (11, 28).

CONCLUSION

Having all these considered, it becomes clear that before initiating ICIs treatment in a cancer patient, a baseline accurate cardiac examination is required. This examination should include clinical workup combined with a serum biomarker report, an ECG and an echocardiogram that will provide information on the LV ejection fraction and strain measurements as well. The appropriate time between tests remains unclear, but currently we have an ongoing project, in which we are testing a set of biomarkers in conjunction with some echo parameters in order to be able to assess cardiac toxicity related to ICIs before it is too late for the patient's well-being. As literature confirms, cardiac troponin and NT-proBNP can be chronically elevated in some subsets of patients. Therefore, we have chosen other types of biomarkers in order to be able to detect LV dysfunction before the onset of decreased EF. Much is still unknown about the ICIs-related cardiotoxicity and therefore, further research is required.

AUTHOR CONTRIBUTIONS

DM-H, AF, RM-H, AP, and AB contributed equally to this work. DM-H, AF, and ST research the literature. RM-H, AP, AF, and AB studied and analysed the articles. DM-H, AF, RM-H, ST, AP, and AB wrote the paper. All authors contributed to the article and approved the submitted version.

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frontiers in Cardiovascular Medicine

CASE REPORT
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Case Report: A Rare Case of a Ventricular Perivascular Epithelioid Cell Tumor With Histologic Characteristics That Resembled a Primary Cardiac Rhabdomyoma

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We present the case of a young male patient with an initial diagnosis of a rhabdomyoma that was surgically treated at a different hospital when he was 17. After a 2-year disease-free period, the patient presented another intra-cardiac mass. He refused surgical treatment and died 5 years later. Post-mortem immunochemistry studies of both tumors led to the diagnosis of a primary malignant cardiac PEComa with histopathologic characteristics that resembled a rhabdomyoma with abundant "spider cells."

Keywords: cardiac tumor, PEComa, perivascular epithelioid cell neoplasm, rhabdomyoma of heart, arrhythmia, cardiooncology

Learning Points

- Cardiac PEComas are very infrequent, and the histological similarities with rhabdomyomas make them a diagnostic challenge.
- It is imperative to correctly identify these tumors since the expectant management that is usually used in rhabdomyomas could lead to the death of these patients.
- There are very few cases of cardiac PEComas in the literature. We encourage clinicians to report their cases so more can be known about this tumor.

INTRODUCTION

Perivascular cell tumors, or PEComas, are mesenchymal neoplasms that are immunoreactive to both smooth muscle and melanocytic markers (1). The PEComa family includes angiomyolipoma, pulmonary clear cell "sugar" tumor, and lymphangioleiomyomatosis. Other tumors with similar features are simply termed PEComas (2). We present a case of a malignant primary PEComa of the heart that was initially confused with a rhabdomyoma.

CASE HISTORY

A 19-year-old male presented with sustained ventricular tachycardia (SVT). There was a family history of gastric cancer of unknown type. At the age of 16, the patient had myocarditis that presented with SVT and a mass in the left ventricle that was interpreted as a thrombus. He was treated with immunosuppressants with an adequate clinical response. However, 6 months later, he

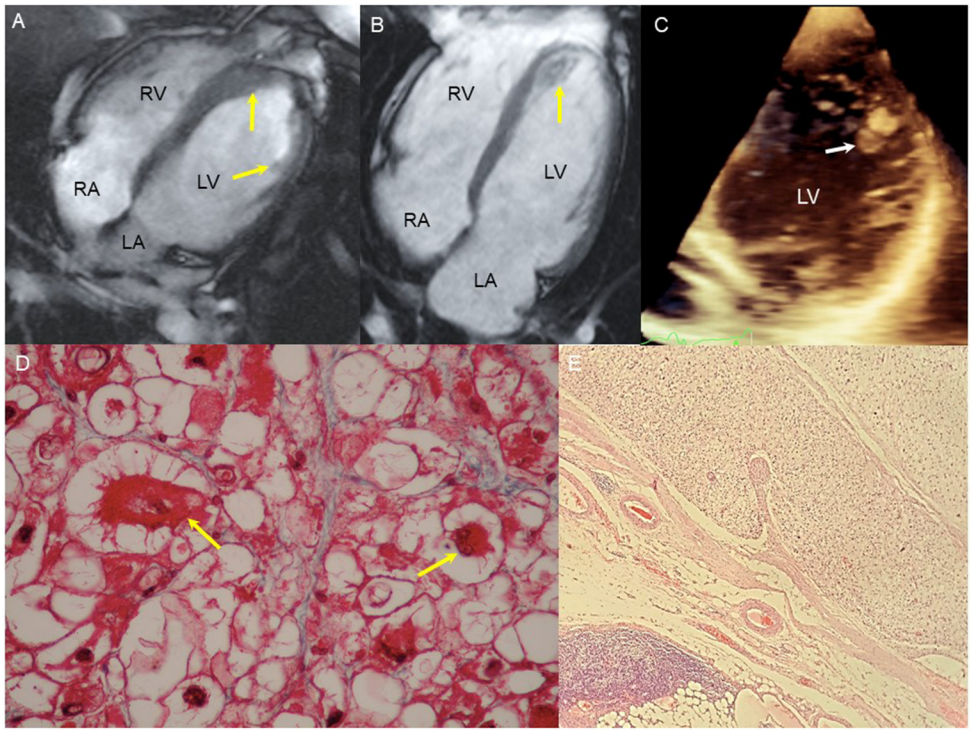

FIGURE 1 | Image and tissue findings. **(A)** CMR 4 chamber image revealing an 8 × 6 mm mass in the lateral LV wall on the papillary muscle insertion and septal thickening (arrows). **(B)** CMR 4 chambers, 36 × 35 mm mass with diffuse enhancement occupying the totality of the LV apex (arrow). **(C)** Four-chamber echocardiogram, 8-mm hyperechoic, homogeneous, rounded mass on the lateral wall of the LV (arrow). **(D)** Micrograph of the first heart tumor. Some nuclear atypia can be seen and two "spider" cells (yellow arrows). Masson trichomic. 40×. **(E)** Micrograph of lymph node with tumoral cells in an alveolar pattern. On the lower left side of the image, the residual lymphatic tissue can be seen. H/E. 4×.

presented another SVT event and growth of the left ventricular mass to 50 × 30 mm visualized on a transthoracic echocardiogram (TTE). The mass adhered to the interventricular septum, and it was surgically removed when the patient was 17 years old. The histopathologic study performed at the hospital that attended him reported a cardiac rhabdomyoma. After surgery, he was asymptomatic for 2 years.

On admission to our institution, a TTE was performed and revealed an 8-mm rounded hyperechoic apical mass that was corroborated by computed tomography (CT) (**Figure 1**). The patient rejected surgical intervention, and over the following 2 years, sporadic SVT episodes were reported on the 24-h Holter monitoring. On the last consult, the patient described shortness of breath and presented adenopathy in the right axillary region.

Differential diagnosis included rhabdomyosarcoma, PEComa, and primary malignant tumor of unknown origin with metastases.

The final diagnosis of PEComa was reached based on the immunohistochemistry findings (**Table 1**). Due to the aggressiveness of this tumor, the initial diagnosis of cardiac rhabdomyoma was abandoned, and the possibility of a rhabdomyosarcoma was considered. However, since the first tumor had no characteristics that suggested a rhabdomyosarcoma, and the possibility of a malignant transformation from a rhabdomyoma to a rhabdomyosarcoma had not been reported before, this diagnosis was thoroughly questioned.

DIAGNOSTIC IMAGING AND HISTOPATHOLOGY

The TEE with 3D remodeling showed an increase in the dimensions of the tumor with occupation of the apex, global hypokinesia, and reduced left ventricular ejection fraction (35%). The CT scan revealed an isodense nodular image of 44 × 26 × 27 mm that was fixed to the deep planes of the internal wall of the anterior thorax along the trajectory of the right internal mammary artery, compatible with regional lymphatic metastasis. In addition, an isodense nodular image of 25 × 20 × 30 mm was observed on the right hepatic lobe with fat interface. The histopathologic study of the axillary nodule showed clear cells with eosinophilic cytoplasm, positive for HMB 45 and SMA and negative for vimentin and desmin (**Figure 2**).

MANAGEMENT

The patient declined surgical treatment due to his past surgical failure, and before starting the chemotherapy, he suffered sudden cardiac death.

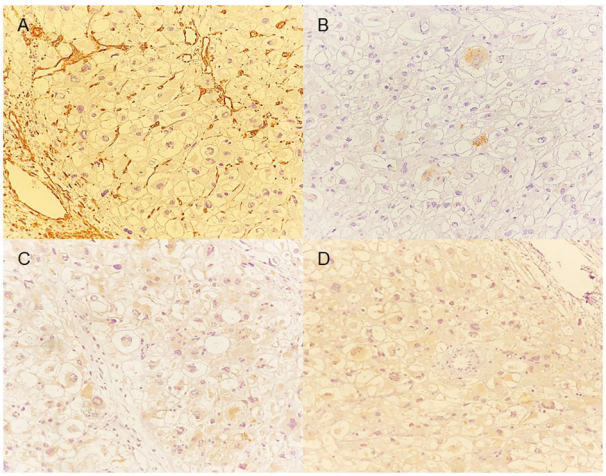

FIGURE 2 | Immunohistochemistry. **(A)** Micrograph of the heart tumor with vimentin that shows negative staining (–): there is no evidence of vimentin in any of the cells. The brown staining in the vessels serves as a control. **(B)** Micrograph of the heart tumor with the HMN-45 marker that shows mild reactivity (+). <25% of the cells show staining. **(C)** Micrograph of the heart tumor that shows moderate reactivity (++) with synaptophysin staining. **(D)** Micrograph of the heart tumor that shows intense positivity (+ + +) for the MyoD1 marker.

TABLE 1 | Immunohistochemistry findings.

Immunohistochemistry Marker	First tumor	Second tumor
Vimentin	–	–
HMB-45	+	+
MyoD1	+ + +	+ + +
PS-100	+ + +	+ + +
Synaptophysin	++	++
Smooth muscle actin	+ + +	+ + +
Muscle specific actin	+ + +	+ + +
Desmin	–	–
Chromogranin	++	++

–negative; +mildly positive; ++moderately positive; + + +strongly positive.

DISCUSSION

Due to the tumor's affinity for the vascular walls and the fact that it was immunoreactive for both smooth muscle and melanocytic markers, the definitive diagnosis was established to be an epithelial perivascular cell tumor, or PEComa. To the best of our knowledge, this type of tumor has been reported in other tissues, but only three cases in the heart: two in the ventricles and one in the pericardium (3–5). Since rhabdomyomas are the most frequent primary heart tumors in children (6), and given the morphologic and epidemiologic circumstances, it is understandable that this was the final diagnosis of the other hospital.

PEComas are a group of tumors with a variety of histopathological presentations. Four main groups are described: angiomyolipoma, clear cell "sugar" tumor of the lung, lymphangioleiomyomatosis, and other tumors with similar features that are simply termed PEComa (7). Some PEComas are associated with tuberous sclerosis complexes. The most frequent sites are the gastrointestinal tract, uterus, retroperitoneum, and sometimes soft tissue (3). The cases reported in the literature of malignant PEComas had similar prognoses to our patient with an aggressive behavior and metastatic spread of the tumor.

This case presents an interesting situation involving the previous resection of a heart tumor confirmed by histopathology to be a rhabdomyoma due to its characteristics: ovoid and vacuolated big cells, "spider" cells and positive immunohistochemistry for myoglobin and smooth muscle actin, and negative for desmin and vimentin (**Table 2**). The immunohistochemistry of this "first" tumor was also positive for SP-100 and negative for vimentin, which should have steered the clinician away from the diagnosis of rhabdomyoma (4). Spider cells are considered a pathognomonic finding of rhabdomyomas (7); this case demonstrates that pathognomonic findings rarely exist. The patient had a 2-year disease-free period, and a subsequent recurrence was documented with metastases to the diaphragm, liver, and regional ganglia. These new findings ruled out the possibility of the primary tumor being a rhabdomyoma, and in conjunction with the immunohistochemical findings, the definitive diagnosis of PEComa was reached.

The immunohistochemical findings of our patient correlate with those reported in the literature for PEComas. S100 protein was positive in our patient; in our review, we found that one-third of patients present this finding (8).

Biopsy of the axillary ganglia showed characteristics of a malignant alveolar PEComa, with spider cells and a great degree of nuclear atypia. The evidence of nodular involvement diminishes the possibility of a rhabdomyosarcoma, since sarcomas in general rarely involve lymph nodes, and the possibility of a malignant transformation from a rhabdomyoma to a rhabdomyosarcoma has never been reported before to the best of our knowledge.

Cardiac tumors may be symptomatic or found incidentally during evaluation for a seemingly unrelated problem. Symptoms are usually related to their cardiac location, although some can produce systemic symptoms (9). "Malignant" arrhythmias, such as SVT, have been associated with cardiac tumors. In a study of 173 pediatric patients (10) with diverse primary cardiac tumors, SVT was the most prevalent arrhythmia, occurring in 64%. The presence of malignant arrhythmias without an apparent cause should oblige the clinician to investigate the possibility of an undiagnosed cardiac tumor.

CONCLUSION

Cardiac primary PEComas are extremely rare, and their histological similarities to rhabdomyomas make them a diagnostic challenge. Although we lack the information to make definitive statements about the prognosis, due to the experience

TABLE 2 | Timeline.

Day 0
- *16-year-old male that presents to the emergency department due to a chief complaint of chest pain, dyspnea, and palpitations.*
- *Electrocardiogram*
  - → Supraventricular tachycardia.
- *Trans-thoracic echocardiogram*
  - → Hyperechoic mass in the left ventricle (25 x 21 mm).
- *Cardiac computed tomography*
  - → Hyperdense left intraventricular mass vs. intracavitary thrombus, and findings suggesting myocarditis.
- He was scheduled to a heart biopsy due to the high suspicion of a primary heart tumor.

Month 3
- *Heart tumoral biopsy*
  - → Hypertrophy, incipient myocyte degeneration in patch like fashion with fibrosis.
- Initiation of immunosuppressive therapy with methylprednisolone and azathioprine due to the diagnosis of myocarditis.

Year 1
- *17-year-old male that presents to the emergency department with severe dyspnea.*
- *Electrocardiogram*
  - → Supraventricular tachycardia.
- Reinstitution of the immunosuppressive therapy suspended shortly after due to intercostal herpes zoster.
- *Trans-thoracic echocardiogram*
  - → Same findings but the mass seems vascularized in the Doppler study.
- Surgical resection of the ventricular mass (5x3 cm), histologic report concluded the presence of a rhabdomyoma (debated diagnosis, see text).

Year 3
- *19-year-old male admitted to the emergency department due to palpitations and chest pain.*
- *Electrocardiogram*
  - → Sustained ventricular tachycardia.
- Successful catheter ablation of the arrhythmia.
- *Trans-esophageal echocardiogram*
  - → Left ventricular rounded, hyperechoic, homogenic 8 mm mass with apical implantation.
- *Cardiac Computed Tomography*
  - → Corroborated the echocardiogram findings with the additional presence of a rounded isodense mass on the anterior papillary muscle.
- The patient and its family denied surgical intervention.

Year 5
- *The patient seeks medical attention because he palpated an axillar mass.*
- *Biopsy of the axillary ganglionic mass*
  - → Leiomyosarcoma with low grade of malignancy vs. PEComa.
- *Trans-thoracic echocardiogram*
  - → Growth of the previous mass.
- *Cardiac magnetic resonance*
  - → Apical mass with diffuse re-enhancement, 36 x 35 mm, occupying the totality of the left ventricular apex. Same size of the anterior papillary muscle mass. Nodular images on the pectoral muscle and right lobe of the liver.
- *PET*
  - → Confirmed the metastatic lesions on the anterior wall of the thorax and the liver.
- The patient is referred to oncology.
- Patient presents sudden cardiac death before the start of the chemotherapy.

with our patient, we can presume that these are aggressive tumors with a malignant potential. It is essential to report these types of cases to raise awareness that spider cells in the histologic report of a cardiac tumor do not necessarily establish the diagnosis of a cardiac rhabdomyoma.

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

Written informed consent was obtained from the relevant individual's legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JC-A: Conceptualization (equal), Data curation (equal), Investigation (lead), Methodology (equal), and Writing (equal). AA-F: Conceptualization (equal), Data curation (equal), Investigation (lead), Methodology (equal), and Writing (equal).

JB: Conceptualization (lead), Data curation (lead), Investigation (lead), Methodology (equal), and Writing (lead). NE-Z: Conceptualization (equal), Data curation (equal), Investigation (lead), Methodology (equal), and Writing (equal). LG-M: Resources (equal) and Supervision (equal). CV-A: Resources (equal) and Supervision (equal). All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Trpc6 Promotes Doxorubicin-Induced Cardiomyopathy in Male Mice With Pleiotropic Differences Between Males and Females

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Background: Doxorubicin is a widely used and effective chemotherapy, but the major limiting side effect is cardiomyopathy which in some patients leads to congestive heart failure. Genetic variants in *TRPC6* have been associated with the development of doxorubicin-induced cardiotoxicity, suggesting that TRPC6 may be a therapeutic target for cardioprotection in cancer patients.

Methods: Assessment of *Trpc6* deficiency to prevent doxorubicin-induced cardiac damage and function was conducted in male and female B6.129 and Trpc6 knock-out mice. Mice were treated with doxorubicin intraperitoneally every other day for a total of 6 injections (4 mg/kg/dose, cumulative dose 24 mg/kg). Cardiac damage was measured in heart sections by quantification of vacuolation and fibrosis, and in heart tissue by gene expression of *Tnni3* and *Myh7*. Cardiac function was determined by echocardiography.

Results: When treated with doxorubicin, male *Trpc6*-deficient mice showed improvement in markers of cardiac damage with significantly reduced vacuolation, fibrosis and *Myh7* expression and increased *Tnni3* expression in the heart compared to wild-type controls. Similarly, male *Trpc6*-deficient mice treated with doxorubicin had improved LVEF, fractional shortening, cardiac output and stroke volume. Female mice were less susceptible to doxorubicin-induced cardiac damage and functional changes than males, but *Trpc6*-deficient females had improved vacuolation with doxorubicin treatment. Sex differences were observed in wild-type and *Trpc6*-deficient mice in body-weight and expression of *Trpc1*, *Trpc3* and *Rcan1* in response to doxorubicin.

Conclusions: Trpc6 promotes cardiac damage following treatment with doxorubicin resulting in cardiomyopathy in male mice. Female mice are less susceptible to cardiotoxicity with more robust ability to modulate other Trpc channels and Rcan1 expression.

Keywords: cardiotoxicity, anthracycline, heart failure, sex differences, ion channel, TRPC

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INTRODUCTION

Doxorubicin is a widely used and effective chemotherapy agent for multiple adult and pediatric cancers. However, a potential side effect is cumulative, dose-related, progressive myocardial damage that can lead to congestive heart failure (CHF), even several years after completion of treatment (1–6). The mechanisms of cardiotoxicity leading to cardiomyopathy are likely complex including generation of reactive oxygen species (ROS) and iron (7), doxorubicin binding to topoisomerases (8), impaired mitochondrial function (9), disruption of calcium homeostasis (10–12), up-regulation of death receptors (13), and up-regulation of the potent vasoconstrictor endothelin 1 which causes fibrosis and the generation of ROS (14).

ASCO guidelines for monitoring and preventing cardiac dysfunction after doxorubicin therapy state that currently there is not sufficient evidence to recommend any single heart failure medication such as angiotensin-converting enzyme (ACE) inhibitors or beta blockers to improve function (15). To date, the only FDA-approved cardioprotective drug for doxorubicin-induced cardiomyopathy is the iron chelating agent, Dexrazoxane, which is thought to deplete topoisomerase IIb (16, 17) and prevent mitochondrial iron-catalyzed ROS damage (7). However, for a therapy to be useful in mediating cardioprotection it is important that it does not counteract the anti-tumor effect of the chemotherapy agent, and there are concerns that Dexrazoxane may interfere with the antitumor efficacy of doxorubicin (18). To date, alternative iron chelators have yielded negative or mixed outcomes (7) indicating that there is a need to find alternative strategies for cardioprotection from anthracyclines.

Given the multiple, complex mechanisms of doxorubicin-induced cardiotoxicity and the variability in patient cardiovascular outcome, we previously used a genome-wide approach to identify genetic variants that were associated with doxorubicin-induced decline in left ventricular ejection fraction (LVEF) (19). That study identified transient receptor potential cation channel subunit 6 (*TRPC6)* as a potential risk locus for doxorubicin-induced cardiomyopathy in patients with breast cancer (19). In a follow-up study from our group using 984 patients from the Mayo Clinic Biobank, we replicated the association of toxicity, specifically with the outcome of doxorubicin-induced congestive heart failure (CHF) (20).

TRPC1-7 channels are an important group of calcium permeable ion channels that induce changes in cardiac function in response to cardiac strain and/or disease (21). Different missense mutations in *TRPC6* have been shown to result in excess calcium influx, largely by gain-of-function mutations (22), leading to the hypothesis that individuals carrying *TRPC6* variants could be at increased risk of doxorubicin-induced cardiotoxicity and cardiomyopathy and perhaps be candidates for TRPC inhibition as a cardioprotective strategy. A number of studies have demonstrated the potential of TRPC1, 3 and/or 6 channels as therapeutic targets for heart failure, predominantly using *in vivo* models of pressure overload in male mice (23–25). We previously published that pre-treatment of male mice with a TRPC6 inhibitor GsMTx-4 significantly reduced fibrosis and improved LVEF and cardiac strain in mice given doxorubicin (20). In this study, we hypothesized that genetic deficiency of *Trpc6* would decrease cardiotoxicity and cardiomyopathy in male and female mice given doxorubicin.

Abbreviations: CHF, congestive heart failure; ROS, reactive oxygen species; LVEF, left ventricular ejection fraction; TRPC6, transient receptor potential cation channel subunit 6; CHF, congestive heart failure; WT, wild-type; KO, knock-out; ip, intraperitoneally; qPCR, quantitative reverse transcriptase-mediated real-time PCR; (qRT)-PCR, quantitative real time; pi, post inoculation.

MATERIALS AND METHODS

Chemotherapy Agent

Doxorubicin was purchased from Selleckchem (Houston, TX) in powder form (25 mg) and dissolved in sterile water as 1.25 mL aliquots to a concentration of 20 mg/mL and stored at 4°C. For injections, the 20 mg/mL stock solution was diluted in sterile saline to a final concentration of 1 mg/mL.

Animal Model

Animal protocols were performed according to NIH guidelines with approval from the Institutional Animal Care and Use Committee, Environmental Health and Occupational Safety Committee and the Biosafety Committee at Mayo Clinic. Mice were bred and maintained under pathogen-free conditions in the animal facility at the Mayo Clinic, fed standard chow and water *ad libitum*, and housed in animal rooms where the temperature was monitored. Breeding pairs of B6.129 wild-type (WT) (Cat#101045) and B6.129 Trpc6 whole body knock-out (KO) mice (26) (Cat#37345) were obtained from the Jackson Laboratory (Bar Harbor, ME). Male and female WT and Trpc6 KO mice (8–10 weeks old), ten mice per group, received either 100 μL intraperitoneally (ip) of control sterile saline or 4 mg/kg/dose doxorubicin for a cumulative dose of 24 mg/kg on days 1, 3, 5, 7, 9, 11 according to (20). Results were confirmed by repeating each experiment. Hearts were evaluated for cardiac function using echocardiography and tissues collected on day 14 and 21.

Echocardiography

Cardiac function was performed by transthoracic echocardiogram using a Visual Sonic Vevo 2100 with a 55-megahertz (MHz) transducer (Bothell, WA). Echocardiography was performed on living male and female animals under isoflurane inhalation at day 14 and 21 as per our previous publications (20, 27–30).

Histology

Mouse hearts were cut longitudinally, fixed in 10% phosphate-buffered formalin, and embedded in paraffin for histological analysis. Five-micron-thick sections were stained with hematoxylin and eosin to detect vacuolation or trichrome blue to detect fibrosis. Vacuolation and fibrosis were calculated as the number of grids with vacuoles or fibrosis, respectively, compared to the total number of grids in the heart section using an eyepiece grid with a 2x objective lens (20x magnification) and

converted to a percentage, as previously (31, 32). Sections were scored by two individuals blinded to experimental group.

RNA Extraction

At harvest, half of the heart was collected and stored at −80°C for RNA isolation. Hearts were homogenized and lysed using Tissuelyser (Qiagen) with 7 mm stainless steel beads in RTL buffer with 0.5% DX buffer to reduce foam (Hilden, Germany). The homogenate was then placed in an automated RNA isolation and purification instrument, QIAcube, with reagents for RNase Easy Fibrous Mini Kit including a DNase and Proteinase K step (Qiagen #74704). RNA was eluted into 30 μL. If the heart had been divided in the earlier step, the eluted RNA was pooled prior to being aliquoted. RNA quantification was determined in μg/μL using NanoDrop (Thermo Scientific, Waltham, MA).

Quantitative PCR

Two-step quantitative reverse transcriptase-mediated real-time PCR (qPCR) was used to measure abundance of individual mRNAs. Total RNA from mouse hearts was assessed by quantitative real time (qRT)-PCR using Assay-on-Demand primers and probe sets and the ABI 7000 Taqman System from Applied Biosystems (Foster City, CA) after RNA was converted to cDNA using a High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems), and qPCR reactions were performed in triplicate with 100 ng of cDNA and the TaqMan Universal PCR master mix (Applied Biosystems), as previously described (28, 29). The following primer/probe sets were purchased from Applied Biosystems: Trpc1 (Mm00441975_m1), Trpc3 (Mm00444690_m1), Trpc6 (Mm01176083_m1), Myh7 (Mm00600555_m1), Tnni3 (Mm00437164_m1) and Rcan1 (Mm01213406_m1). Amplification data were collected with an Applied Biosystems ViiA7 detector and analyzed with ViiA7 v 1.2.4 software (Life Technologies). Data were normalized to the endogenous control *Polr2a* (Mm00839502_m1) (33) and mRNA abundance was calculated using the ΔΔCT method and displayed as fold change (FC) (34).

TUNEL Assay

Hearts were fixed in 10% buffered formalin for 48 h and transferred to containers of PBS prior to paraffin embedding and mounting on slides. TUNEL Assay was performed using the Click-iT Plus TUNEL Assay for *in situ* Apoptosis Detection on the Alexa 647 (ThermoFisher, Cat: C10619). Slides were deparaffinized per manufacturer recommendations and steamed for 30 min prior to permeabilizing with Proteinase K. Tissue autofluorescence was quenched with Vector TrueVIEW Autofluorescence Quenching Kit (Vector Laboratories, Cat: SP-8400-15). Heart sections were incubated with TdT Reaction Buffer for 20 min at 37°C prior to performing the TdT Reaction for 60 min at 37°C. TUNEL reaction was performed for 45 min at 37°C. Nuclei were counter-stained with Hoechst 33342 (ThermoFisher, Cat: H21492) and then mounted with Vectashield Antifade Mounting Medium (Vector Labs, Cat: H-1000-10). After drying for 48 h, slides were scanned with a Panoramic 250 fluorescent slide scanner (3DHISTECH). The ventricles of heart sections were selected and annotated in CaseViewer (3DHISTECH). TUNEL positivity was determined in QuantCenter (3DHISTECH) using cell quant with the following parameters: Channel Matching – default; Detection – nuclei selected for both the DAPI and Cy5 channels; Nuclei – contrast set to 35, other settings were default; Cytoplasm – n/a; Membrane – n/a; Scoring – object selected was nuclei and channel selected was Cy5. These parameters allowed for identification of all nuclei and then determination of the frequency of TUNEL/Cy5 positivity where the aggregate score of Medium and Strong Positive Nuclei = TUNEL positive.

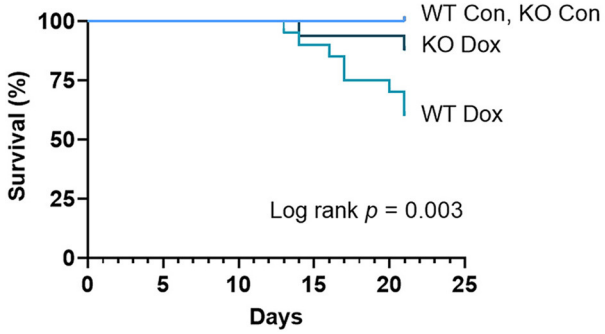

FIGURE 1 | Trpc6 deficiency improves survival in male mice treated with doxorubicin. Male wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. Two separate experiments of 10 mice/group were combined ($n = 20$/group) and analyzed by log-rank (Mantel-Cox) test.

Statistical Analysis

Statistical analyses were performed in GraphPad Prism 9.0.1. Differences between two groups were tested by unpaired 2-tailed Student's *t*-test. Differences between more than two groups were tested by one-way ANOVA followed by Tukey's or Holm-Sidak's multiple comparison tests. Differences between groups over time were compared by two-way ANOVA. Survival curves were analyzed by log-rank (Mantel-Cox) test. Data are expressed as mean ±SEM. A value of $p < 0.05$ was considered significant.

RESULTS

Trpc6 Deficiency Improves Survival in Male Mice Following Doxorubicin Treatment

Shortly after the accumulative dose of doxorubicin was achieved at day 11 post inoculation, male wild-type and *Trpc6*-deficient mice began to die, although the specific cause of death was not ascertained (**Figure 1**). Deficiency in *Trpc6* improved survival after doxorubicin treatment in males, ($p = 0.003$, **Figure 1**). These findings suggest that *Trpc6* contributes to mortality following doxorubicin therapy in male mice.

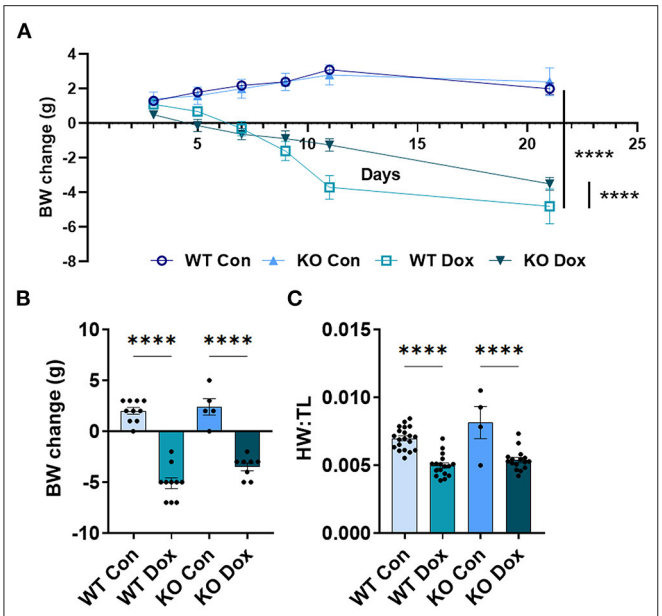

FIGURE 2 | *Trpc6*-deficiency reduces doxorubicin-induced body weight loss in males. Male wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. **(A)** Change in body-weight (BW) from baseline in grams (g) over time. Data were analyzed by two-way ANOVA. **(B)** Change in body weight (BW) comparing baseline to day 21. **(C)** Ratio of heart weight to tibia length (HW:TL) at day 21. ****$p < 0.0001$. Data shown as mean +/- SEM using one-way ANOVA with Tukey's multiple comparisons test with 4–20 mice/ group.

Trpc6 Deficiency Improves Doxorubicin-Induced Body Weight Loss in Males

Mice were weighed immediately prior to each injection of doxorubicin to ensure the correct dose was used (approximately 4mg/kg per dose). As expected, both wild-type and *Trpc6*-deficient males treated with doxorubicin progressively lost body-weight relative to control mice ($p < 0.0001$, **Figure 2A**), while wild-type and *Trpc6*-deficient control males maintained their weight over the duration of the experiment ($p = 0.724$, **Figure 2A**). The loss in weight for wild-type and Trpc6-deficient mice treated with doxorubicin was observed at day 21 ($p < 0.0001$, **Figure 2B**). However, *Trpc6*-deficient mice treated with doxorubicin lost less weight than wild-type mice treated with doxorubicin over the duration of the experiment ($p < 0.0001$, **Figure 2A**), suggesting that *Trpc6* worsens the effects of doxorubicin.

In mice that survived to day 21, we also determined the heart-weight to tibia length (HW:TL) ratio. An elevated HW:TL indicates cardiac hypertrophy. Instead, we found that doxorubicin treatment caused a reduction in HW:TL in wild-type and *Trpc6*-deficient males ($p < 0.001$, **Figure 2C**), indicating cardiac damage, that was not recovered by *Trpc6* deficiency ($p = 0.64$, **Figure 2C**). Thus, *Trpc6* contributes to loss of body weight due to doxorubicin treatment but does not alter heart weight in male mice.

Trpc6 Deficiency Improves Cardiac Damage and Function at Day 21 in Male Mice Treated With Doxorubicin

We next examined gene expression of two known biomarkers of heart damage, cardiac troponin (*Tnni3*) and myosin heavy chain 7 (*Myh7*, also known as myosin heavy chain beta), in male mice at day 21. Both *Tnni3* and *Myh7* gene expression was significantly different between groups by ANOVA ($p < 0.0001$ and $p < 0.0001$, respectively, **Figures 3A,B**). *Tnni3* expression in the heart of wild-type mice was significantly reduced by doxorubicin treatment compared to saline controls, $p < 0.0001$, and the reduction was almost completely reversed by *Trpc6* deficiency, ($p < 0.0001$, **Figure 3A**), indicating that *Trpc6* promotes cardiac damage. *Myh7* expression, which is known to increase in failing human (35, 36) and mouse hearts (37, 38), increased significantly in male wild-type mice treated with doxorubicin, $p < 0.0001$, and was also reversed by *Trpc6* deficiency, ($p < 0.0001$, **Figure 3B**), indicating that *Trpc6* promotes cardiac damage. The gene expression levels of *Tnni3* and *Myh7* were very similar between wild-type and *Trpc6*-deficient saline control males indicating that there was no apparent underlying difference in cardiac damage between the two mouse strains. Together, these data show that *Trpc6* worsens cardiac damage in response to doxorubicin.

TUNEL Assay was performed to determine whether cardiac apoptosis was present 21 days after treatment with doxorubicin. We did not observe significant changes in apoptosis at day 21 after doxorubicin exposure between groups (**Figure 3C**). Fibrosis was found to be present in the heart at day 21 (**Figure 4**) and apoptosis is a process that primarily occurs prior to remodeling and fibrosis.

Vacuolation, a known effect of doxorubicin-induced cardiac damage in humans, was observed in male mice treated with doxorubicin ($p < 0.0001$, **Figures 3D–H**). *Trpc6*-deficiency significantly reduced cardiac vacuolation compared to wild-type controls following treatment with doxorubicin ($p < 0.0001$, **Figures 3D–H**), further demonstrating that *Trpc6* promotes cardiac damage following doxorubicin treatment.

Cardiac fibrosis is well known to cause cardiomyopathy/dilated cardiomyopathy that can be detected by echocardiography in conditions such as viral myocarditis (31, 32). Cardiac fibrosis was assessed at day 21. Wild-type mice treated with doxorubicin showed a significant increase in fibrosis in the heart ($p = 0.010$, **Figure 4A**) while *Trpc6*-deficiency significantly decreased fibrosis ($p = 0.028$, **Figure 4A**).

Cardiac function was measured in male mice at day 14 and 21 by echocardiography (**Figure 4**). No significant changes were observed for any group at day 14 (data not shown). At day 21, wild-type mice treated with doxorubicin showed a significant decrease in heart rate, ($p = 0.029$, **Figure 4B**), LVEF, ($p = 0.042$, **Figure 4C**), fractional shortening, ($p = 0.037$, **Figure 4D**), cardiac output, ($p < 0.0001$, **Figure 4E**) and stroke volume, ($p = 0.0001$, **Figure 4F**) compared to wild-type control males. *Trpc6*-deficiency significantly improved cardiac function compared to

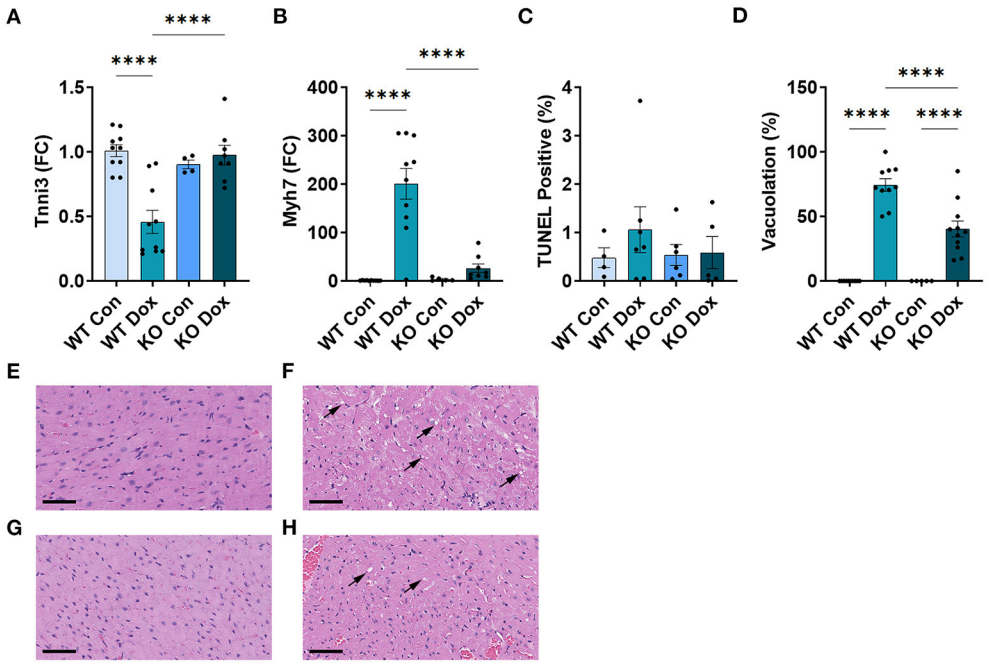

FIGURE 3 | *Trpc6*-deficiency reduces cardiac damage following doxorubicin in males. Male wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. **(A)** Cardiac troponin (*Tnni3*) or **(B)** myosin heavy chain 7 (*Myh7*) gene expression shown as a fold change (FC) relative to WT Control. **(C)** TUNEL Positive (%). **(D)** Percent vacuolation. ****$p < 0.0001$. Data shown as mean +/- SEM using one-way ANOVA with Tukey's multiple comparisons test with 4–10 mice/ group. Hematoxylin and eosin **(**H&E) staining of representative heart sections from **(E)** male wild-type (WT) mice treated with saline (Con), **(F)** male wild-type (WT) mice treated with doxorubicin (Dox), **(G)** *Trpc6*-deficient (KO) mice treated with saline, or **(H)** *Trpc6*-deficient (KO) mice treated with Dox showing vacuoles in black arrows. Magnification 400x. Scale bars are 60 μm.

wild-type males treated with doxorubicin for heart rate $p = 0.022$, LVEF $p = 0.048$, fractional shortening $p = 0.043$, cardiac output $p = 0.002$, and stroke volume $p = 0.048$, respectively (**Figures 4B–F**). Measures of left ventricular end diastolic and left ventricular end systolic diameters (LVEDD, LVESD) used to determine cardiac dilatation showed that neither doxorubicin nor *Trpc6* deficiency led to dilated cardiomyopathy at this time point in males (**Figures 4G,H**). Thus, these data indicate that *Trpc6* promotes cardiac damage that leads to cardiomyopathy following doxorubicin treatment in males.

In Male Mice, *Trpc6* Deficiency Improved *Trpc*-Related Gene Expression in the Heart Following Treatment With Doxorubicin

The TRPC family of proteins (TRPC1-7) function as both homo- and hetero-tetramers, and both *Trpc1* and *Trpc3* as well as *Trpc6* have been implicated in heart failure induced by pressure overload (23–25, 39). Another study reported that *Trpc6* is a positive regulator of calcineurin-NFAT signaling through the regulator of calcineurin (*Rcan1*) (40). Therefore, we sought to characterize the changes in cardiac gene expression of *Trpc6* in response to doxorubicin and *Trpc1*, *3* and *Rcan1* in *Trpc6*-deficient mice after doxorubicin treatment.

In the hearts of male wild-type mice, we observed decreases in *Trpc6*, *Trpc1* and *Trpc3* gene expression in response to doxorubicin compared to saline controls, $p = 0.0087$, $p = 0.032$ and $p < 0.0001$, respectively (**Figures 5A–C**), but no significant change was observed in the expression of *Rcan1* (**Figure 5D**). In *Trpc6* deficient mice, the doxorubicin-induced changes in expression of *Trpc1* and *Trcp3* were reversed (**Figures 5B,C**). However, the expression of *Trpc3* in the hearts of *Trpc6* deficient control mice was significantly lower than that of wild-type control mice, ($p = 0.004$, **Figure 5C**), indicating that *Trpc6*-deficiency alters cardiac *Trpc3* expression regardless of doxorubicin treatment.

Female Mice Are Less Susceptible to Doxorubicin-Induced Cardiac Damage, Cardiomyopathy and Death Compared to Males

Given that women with breast cancer are commonly treated with doxorubicin and that our initial genetic studies identified *TRPC6* genetic variants as associated with a decline in LVEF in women with breast cancer (19), in this study we also assessed female mice treated with the same dose of doxorubicin as the dose given to males. In female wild-type mice, all wild-type and *Trpc6*-deficient mice survived treatment with doxorubicin (data not shown). In contrast to males, only *Trpc6*-deficient female mice treated with doxorubicin lost body weight over the duration of the experiment (**Figure 6A**). At day 21 (**Figure 6B**) wild-type female mice treated with doxorubicin maintained their weight, and no changes were observed in HW:TL in females for any group (**Figure 6C**).

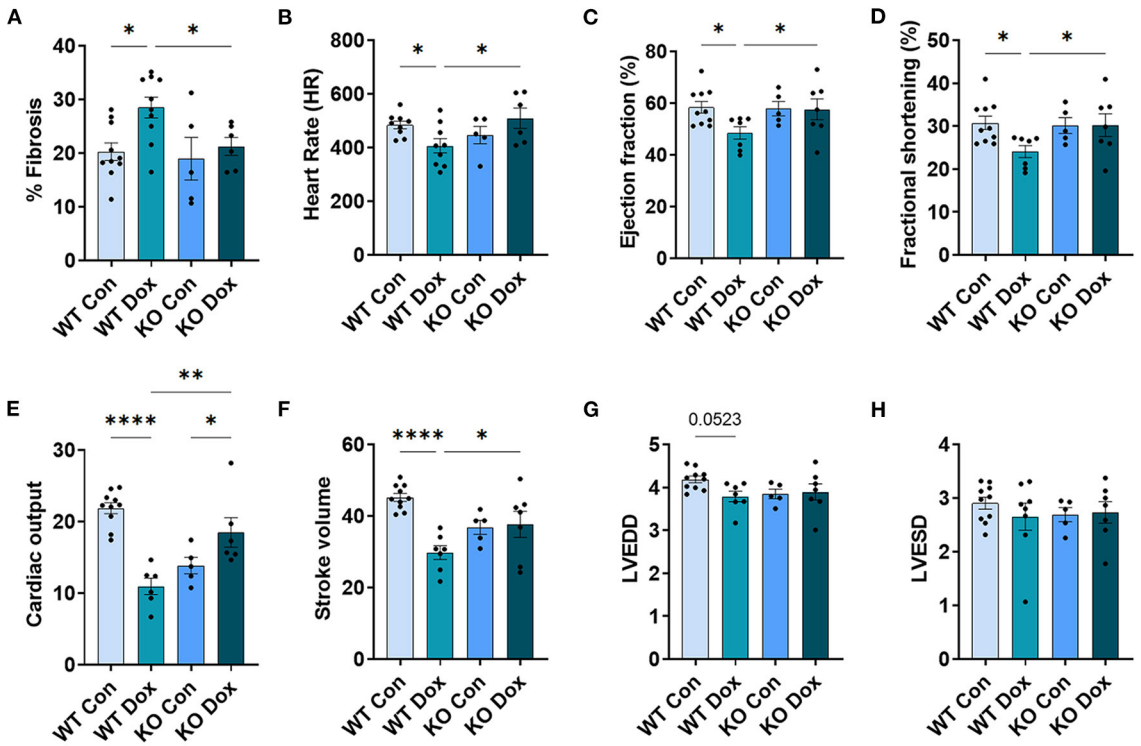

FIGURE 4 | *Trpc6*-deficiency improves cardiac function assessed using echocardiography in males treated with doxorubicin. Male wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. Trichrome blue staining was performed at day 21 to assess **(A)** % fibrosis. Echocardiogram was performed at day 21 to assess **(B)** heart rate (HR), **(C)** % left ventricular (LV) ejection fraction, **(D)** % fractional shortening, **(E)** cardiac output, **(F)** stroke volume, **(G)** LV end diastolic diameter (LVEDD) or **(H)** LV end systolic diameter (LVESD). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$. Data shown as mean +/- SEM using one-way ANOVA with Holm-Šídák's multiple comparisons test with 5–10 mice/ group.

As observed in male mice, *Tnni3* cardiac gene expression was significantly reduced in wild-type females treated with doxorubicin ($p = 0.050$, **Figure 7A**), but unlike males, gene expression of *Myh7* in wild-type females was not significantly altered by doxorubicin, ($p > 0.999$, **Figure 7B**). Similar to males, female mice developed vacuolation following treatment with doxorubicin, ($p < 0.0001$, **Figure 7D**) that was less severe than males (mean vacuolation in wild-type females treated with doxorubicin = 18.24% vs. 74.44% in males) (**Figure 7D**). And as with males, *Trpc6*-deficiency significantly reduced vacuolation in females treated with doxorubicin ($p = 0.049$, **Figure 7D**). Finally, we did not observe any significant change in cardiac fibrosis or echocardiographic parameters in female mice at day 21 in response to doxorubicin or *Trpc6*-deficiency (**Figure 8**). Thus, cardiac damage caused by doxorubicin was far less in females and did not lead to cardiomyopathy at day 21.

Pleiotropic Effects of Trpc6 Deficiency in Female Mice Following Doxorubicin Treatment

Although female wild-type and *Trpc6*-deficient mice were less susceptible to doxorubicin-induced cardiac damage and cardiomyopathy, we did observe other significant effects of *Trpc6* deficiency in female mice compared to males.

In contrast to males (**Figure 2A**), female wild-type mice treated with doxorubicin did not lose weight (**Figure 6A**). The reason for this is not clear. Rather than wild-type mice being worse in males, *Trpc6*-deficient females treated with doxorubicin had a greater loss in body weight over time and at day 21 compared to wild-type mice treated with doxorubicin ($p < 0.0001$, **Figures 6A,B**). Although doxorubicin significantly decreased HW:TL (caused heart damage) in males (**Figure 2C**), there was no change in heart weight (no cardiac damage) in females (**Figure 6C**).

In contrast to males (**Figure 3A**), *Tnni3* cardiac gene expression was significantly lower in *Trpc6*-deficient compared to wild-type saline control females ($p < 0.0001$, **Figure 7A**). In contrast to males, *Tnni3* gene expression was significantly decreased in control and doxorubicin treated *Trpc6*-deficient females (**Figure 7A**), suggesting that *Trpc6* deficiency altered *Tnni3* levels in females. Pleiotropic effects of *Trpc6* deficiency in response to doxorubicin were also observed for *Myh7* gene expression in the hearts of female (**Figure 7B**) vs. male (**Figure 3B**) mice. In female mice, *Myh7* levels remained low in all groups except for *Trpc6*-deficient mice treated with

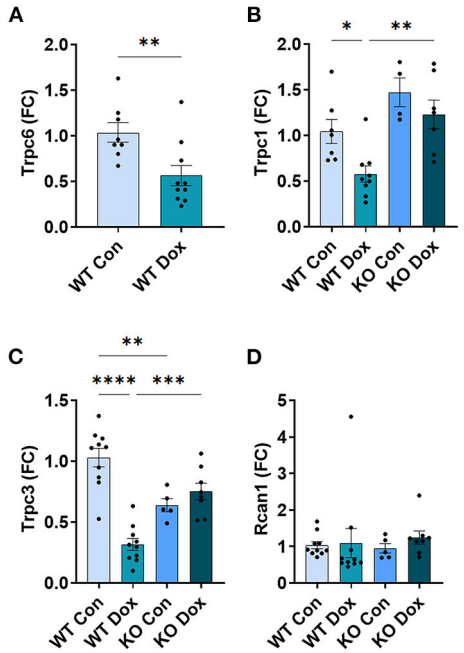

FIGURE 5 | Other *Trpc* gene expression is not altered in *Trpc6*-deficient males after doxorubicin treatment. Male wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. Cardiac gene expression shown as a fold change (FC) relative to WT control for **(A)** *Trpc6*, **(B)** *Trpc1*, **(C)** *Trpc3* or **(D)** *Rcan1* at day 21. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. **(A)** Data shown as mean ± SEM using 2 tailed Student's *t* test with 10 mice/ group. **(B–D)** Data shown as mean ± SEM using one-way ANOVA with Tukey's multiple comparisons test with 4–10 mice/ group.

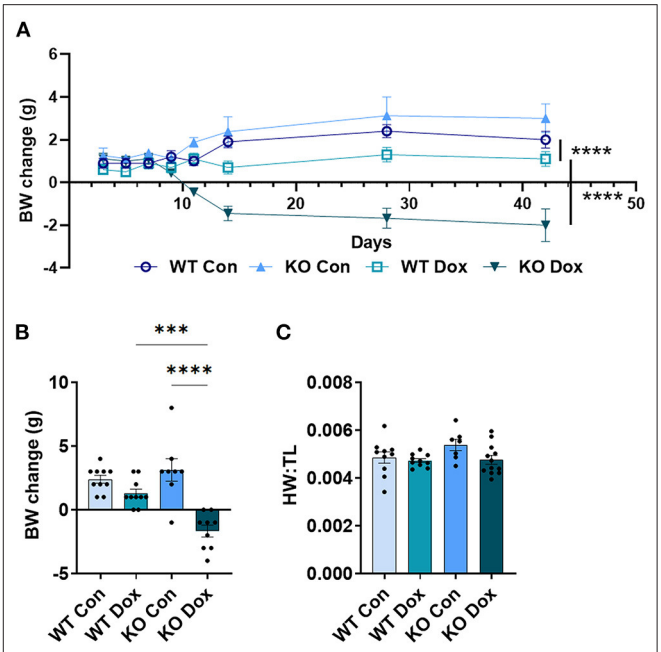

FIGURE 6 | *Trpc6*-deficienct females treated with doxorubicin lose body weight, with no effect on heart weight. Female wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. **(A)** Change in body-weight (BW) from baseline in grams (g) over time. Data were analyzed by two-way ANOVA. **(B)** Change in body weight (BW) from baseline to day 21. **(C)** Ratio of heart weight to tibia length (HW:TL) at day 21. $^{***}p < 0.001$, $^{****}p < 0.0001$. Data shown as mean +/- SEM using one-way ANOVA with Tukey's multiple comparisons test with 7–12 mice/ group.

doxorubicin, where there was a significant increase relative to wild-type controls, ($p = 0.049$, **Figure 7B**).

In the hearts of female wild-type mice, doxorubicin induced a significant reduction in *Trpc6* gene expression compared to wildtype controls ($p = 0.039$, **Figure 9A**) similar to the decrease observed in male mice (**Figure 5A**), but did not induce changes in *Trpc1*, *Trpc3* or *Rcan1* in wild-type mice (**Figures 9B–D**). A direct comparison of Trpc6 expression levels in the heart of male and female wild-type mice revealed that there were no significant differences in its expression before or after treatment with doxorubicin by sex (**Figure 10**). Interestingly, female *Trpc6*-deficient mice treated with saline had significantly lower expression of *Trpc1*, ($p = 0.002$, **Figure 9B**), *Trpc3* ($p < 0.0001$, **Figure 9C**) and *Rcan1* ($p = 0.009$, **Figure 9D**) than wild-type control mice. Thus overall, *Trpc6* appears to increase cardiac damage in response to doxorubicin in females but not severely enough to lead to cardiomyopathy at the dose used in these experiments.

DISCSUSSION

In breast cancer patients, genetic variants at *TRPC6* have been associated with doxorubicin-induced cardiomyopathy and congestive heart failure (19, 20). *In vitro* and *in vivo*, *Trpc6* inhibition with the peptide GsMTx4 reduced doxorubicin-induced cardiotoxicity and cardiomyopathy in male mice (20). However, GsMTx4 also inhibits Piezo 1 and Piezo 2 (41) and TRPC1 (42) such that its cardioprotective effects could be mediated through mechanosensitive ion channels other than Trpc6 or the combination of multiple ion channels.

In this study, we specifically tested the role of *Trpc6* in doxorubicin-induced cardiotoxicity and cardiomyopathy using male and female *Trpc6* whole body knockout mice. In male mice in this study, we found that *Trpc6* deficiency improved doxorubicin-induced cardiac damage (vacuolation, fibrosis, *Tnni3* and *Myh7*) and cardiomyopathy indicating that Trpc6 promotes cardiac damage associated with doxorubicin therapy. To our knowledge, our study is the first to examine the effect of *Trpc6* in doxorubicin-induced cardiomyopathy. Seo et al. (24) found that *Trpc6* deficiency in male mice had no significant effect on hypertrophy induced using a pressure overload model by transverse aortic constriction (TAC). Improvement in hypertrophy following TAC required combined *Trpc3* and *Trpc6* deficiency (24). There are several reasons for differences between our study and Seo *et al.* Firstly, the genes and mechanisms involved in doxorubicin-induced heart failure are different than those in pressure overload models, and mice treated with doxorubicin in this study did not show signs of hypertrophic

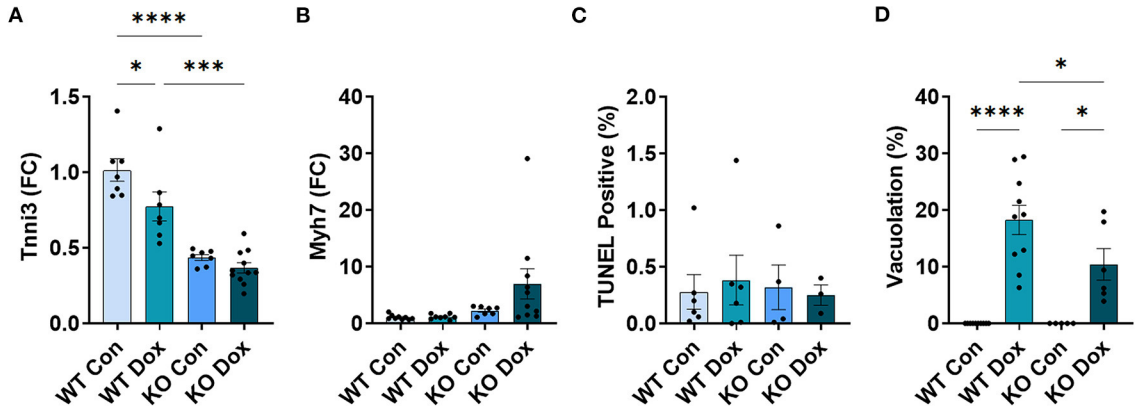

FIGURE 7 | Trpc6-deficiency reduces cardiac damage in females. Female wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. **(A)** Cardiac troponin (*Tnni3*) or **(B)** myosin heavy chain 7 (*Myh7*) gene expression shown as a fold change (FC) relative to WT control. **(C)** TUNEL Positive (%) **(D)** Percent vacuolation. $^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Data shown as mean +/- SEM using one-way ANOVA with Tukey's multiple comparisons test with 4–12 mice/ group.

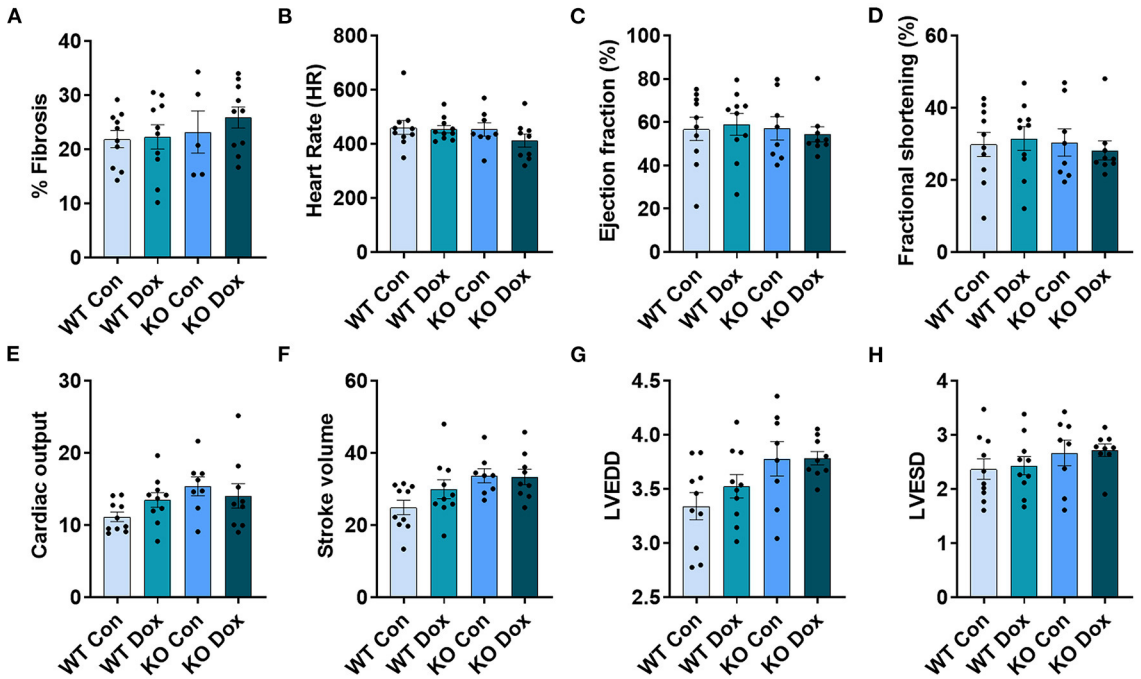

FIGURE 8 | In female mice, cardiac function measured by echocardiography was not impacted by doxorubicin or Trpc6 deficiency. Female wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. Trichrome blue staining was performed at day 21 to assess **(A)** % fibrosis. Echocardiogram was performed at day 21 to assess **(B)** heart rate (HR), **(C)** % left ventricular (LV) ejection fraction, **(D)** % fractional shortening, **(E)** cardiac output, **(F)** stroke volume, **(G)** LV end diastolic diameter (LVEDD) or **(H)** LV end systolic diameter (LVESD). Data shown as mean ± SEM using one-way ANOVA with Tukey's multiple comparisons with 5–10 mice/ group.

cardiomyopathy according to measurement of heart weight to tibia length. Secondly, some mouse strains are more sensitive to heart failure than others (both our study and Seo *et al.* used *Trpc6* whole body knockout mice (26), but in the Seo study Trpc6 KO mice were backcrossed onto a C57BL/6J background and the mice in this study were on a B6.129 background). Thirdly, it is possible that combined *Trpc3* and *Trpc6* deficiency could improve cardioprotection even further in mice treated with doxorubicin.

In the KO male and female mice in this study, we also observed that *Trpc3* expression was significantly lower in *Trpc6* KO controls relative to wild-type controls raising the possibility

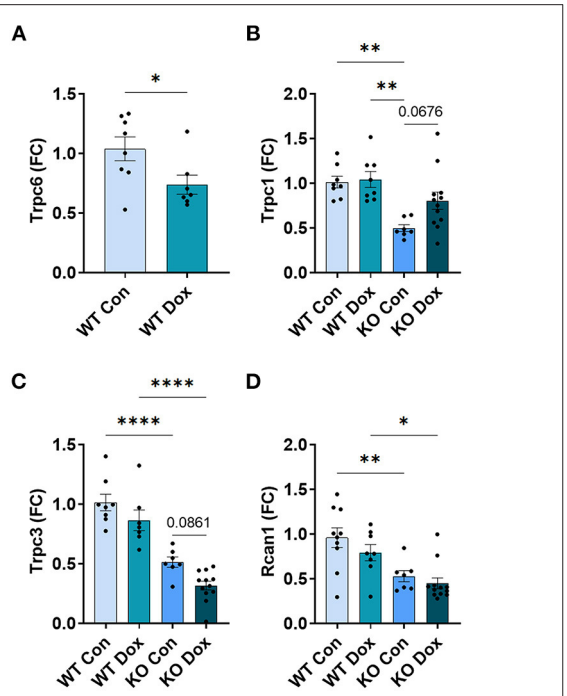

FIGURE 9 | *Trpc6* expression is reduced by doxorubicin in female wild-type mice while *Trpc1*, *Trpc3* and *Rcan1* expression is reduced by *Trpc6* deficiency. Female wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. Cardiac gene expression shown as a fold change (FC) relative to WT control **(A)** *Trpc6*, **(B)** *Trpc1*, **(C)** *Trpc3* and **(D)** *Rcan1* at day 21. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$. Data shown as mean ± SEM using one-way ANOVA with Tukey's multiple comparisons with 7–12 mice/ group.

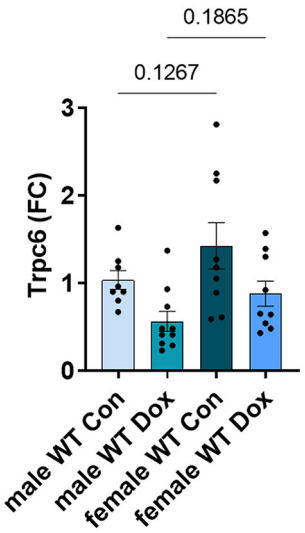

FIGURE 10 | *Trpc6* expression was not significantly different between male and female wild-type control mice. Female and male wild-type (WT) and *Trpc6*-deficient (KO) mice were treated with 100 mL saline (CON) or 4 mg/kg doxorubicin (DOX) on days 1, 3, 5, 7, 9, 11 for a cumulative dose of 24 mg/kg. Cardiac gene expression of *Trpc6* shown as a fold change (FC) relative to male WT control at day 21. Data shown as mean ± SEM using one-way ANOVA with Fisher's LSD multiple comparisons test with 8–10 mice/ group.

that at least some of the protective effect of *Trpc6* deficiency could be mediated by decreased expression of *Trpc3*. Indeed, the work of others in pressure overload models of heart failure demonstrated that Pyr3- specific inhibition of *Trpc3* attenuated pressure overload-induced heart failure in male mice (43), and the same group demonstrated that inhibition of the *Trcp3-Nox2* complex suppressed doxorubicin-induced myocardial atrophy (44). However, an independent group demonstrated that both deletion and inhibition of *Trpc6* reduced pressure overload-induced fibrosis, but did not reduce pressure overload-induced cardiac dysfunction or ROS production (45), and a recent *in vitro* study found that doxorubicin-induced cell death was independent of TRPC6 channel up-regulation but involved mitochondrial activation of ROS (46). Taken together, our data and that of others suggest that cardioprotection through Trpc6 deficiency may be mediated by reduction of fibrosis (our previous work showed that doxorubicin-induced fibrosis was reduced in mice that were pre-treated with the Trpc6 inhibitor GsMTx4, which does not inhibit Trpc3) as well as by reduction in *Trpc3* expression.

We are also the first study to our knowledge to examine whether sex differences exist in the effect of *Trpc6* on cardiomyopathy following doxorubicin therapy. We found that female B6.129 wild-type and *Trpc6*-deficient mice were far less susceptible to doxorubicin-induced cardiac damage and cardiomyopathy than males. This is consistent with the known sex differences in cardiomyopathy and heart failure where male mice and men develop worse cardiac function than females (47, 48). In animal models of heart disease, estrogen has been found to improve cardiac function in females (30, 49). Specifically, estrogen has been found to prevent cardiac hypertrophy by reducing calcineurin activity (50). Age also influences sex differences, with cardiovascular disease increasing in women after menopause and with increasing age (after 70 years of age) (51, 52). Additionally, older women (age >65 years, which accounts for 50% of breast cancer cases) are at higher risk of chemotherapy-related heart failure compared to younger women (53, 54) and the mice used in this study were 8-10 week old young adults. Furthermore, other female rodent models have shown that ovariectomy is necessary for the development of heart failure (55). Future studies should examine whether ovariectomized female mice treated with doxorubicin develop more severe cardiac damage and cardiomyopathy.

In this study, we found that doxorubicin reduced *Trpc6* gene expression in both male and female wild-type mice, suggesting a homeostatic response to buffer Trpc6-induced damage. We observed that *Trpc1* and *Trpc3* expression were also significantly reduced in male wild-type mice in response to doxorubicin, but this did not occur in females. As female mice were much less susceptible to doxorubicin-induced cardiac damage, they may have greater ability to buffer Trpc6-induced changes in calcium levels. Sex hormones have been found to influence

calcium channels (56, 57), and 17b-estradiol to upregulate canonical transient receptor potential channels (TRPC) in particular (58). This could explain the reduction in cardiac *Trpc1*, *Trpc3* and *Rcan1* gene expression in *Trpc6*-deficient saline control and doxorubicin-treated female mice that was not observed in males. *Rcan1* is involved in both development and maintenance of the cardiovascular system, and reduction in *Rcan1* prevents pathological cardiac remodeling (59, 60). In this study, *Rcan1* expression was unchanged in male mice in response to doxorubicin or *Trpc6*-deficiency, but in *Trpc6*-deficient female mice, *Rcan1* expression was significantly reduced in females and the pattern of expression closely followed that of *Trpc3* in each group of mice by treatment and *Trpc6* status.

The observed sex differences in *Trpc* channel expression in response to doxorubicin and *Trpc6*-deficiency suggest that estrogen is not only cardioprotective, but perhaps the mechanism of estrogen-related cardioprotection is mediated through TRPC-related calcium signaling in the heart. Regulation of TRPC gene expression by estrogen was first reported in 1997 (61) and both E_2 and the G-protein estrogen receptor (GPER) act to moderate calcium-activities in the cardiovascular system by lowering the peaks and raising the troughs, thus refining calcium levels to a more narrow and sustained operating range [reviewed in (62)]. Taken together, these data suggest that TRPC6 inhibition may serve as a potential cardioprotective therapy for male and post-menopausal female cancer patients that require doxorubicin.

Additionally, other work from our group identified genetic variants that were associated with both chemotherapy-induced heart failure and a decline in LVEF, and the same variants were also associated with increased TRPC6 expression in the heart, and in one case we identified a TRPC6 gain-of-function variant in a 32 year old women with breast cancer who developed heart failure following doxorubicin and trastuzumab treatment (20) and (63) suggesting that TRPC6 inhibition may be particularly appropriate as a cardioprotection strategy for men and women who carry *TRPC6* risk variants.

Our use of both female and male mice also demonstrated a significant interaction in female mice between doxorubicin and *Trpc6* that may be clinically relevant to the management of patients receiving anthracyclines. The most striking sex difference was the loss of body-weight over time, in which male wild-type mice treated with doxorubicin lost a significant amount of body-weight whereas female wild-type mice were almost completely unaffected. In male mice, *Trpc6*-deficiency did not prevent doxorubicin-induced weight loss, but in female mice *Trpc6*-deficiency had a dramatic effect on weight gain in mice that received only saline control and a dramatic effect on weight loss in mice that were treated with doxorubicin, demonstrating genetic pleiotropy of *Trpc6* deficiency in response to doxorubicin, specifically in female mice. These changes in body-weight appeared independent to changes in heart weight or cardiac function. However, we note that TRPC channels are often overexpressed in tumors, are a hallmark of metastasis (64) and *in vitro*, TRPC channel knock down or inhibition reduces tumor cell growth and migration (65, 66). Therefore, our observations of the pleiotropic effects of *Trpc6*-deficiency in female mice in relation to doxorubicin treatment may be of clinical relevance to the efficacy of doxorubicin in breast cancer patients. For example, would *Trpc6* inhibition make breast tumors more sensitive to doxorubicin or prevent resistance to doxorubicin?

In summary, we demonstrated that *Trpc6* deficiency alone is sufficient to protect male mice from doxorubicin-induced cardiac damage and decline in cardiac function, suggesting that TRPC6 may be a valuable therapeutic target for cancer patients who require doxorubicin. We further showed that wild-type female mice are less susceptible to doxorubicin-induced damage, with low levels of cardiomyocyte vacuolation and no progression to cardiomyopathy at doses used in this study. Finally, we demonstrated significant sex differences in the gene expression of *Trpc1*, *Trpc3* and *Rcan1* in wild-type and *Trpc6*-deficient mice that may be due to the reciprocal relationship between estrogen and TRPC activity, which is of relevance to cardioprotection strategies for women with breast cancer.

CONCLUSIONS

Trpc6 induces cardiac damage and cardiomyopathy following treatment with doxorubicin in male mice and may be a therapeutic target for cardioprotection in patients. Female mice are less susceptible to cardiotoxicity but showed cardioprotection in *Trpc6*-deficient mice.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by Mayo Clinic, American Association for Laboratory Animal Science.

AUTHOR CONTRIBUTIONS

NN, KB, and DF designed the animal experiments. KB, DD, EW, AH, AM-L, AM, JS, JM, LD, GS, ZA, LM, SK, MM, PG, AK, DB, and DF performed animal experiments and animal maintenance. NN, KB, DD, EW, MM, AM-L, and AM performed doxorubicin dosing, weight tracking and collected survival data. KB, DD, EW, and AH performed echocardiography. KB, DD, EW, JS, and CL analyzed echocardiography data. KB and SK performed tibia length measurements. KB, LM, and DF performed histological analysis. NN, KB, AH, JM, LD, GS, ZA, BN, RW, and DF performed RT-PCR experiments. DD, LM, and SK performed TUNEL Assay. NN, KB, and DF interpreted

the data and wrote the manuscript. All authors critically revised the manuscript.

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BRIEF RESEARCH REPORT
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Myocardial PD-L1 Expression in Patients With Ischemic and Non-ischemic Heart Failure

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Objective: Immune checkpoints inhibitors are promising and wide-spread agents in anti-cancer therapy. However, despite their efficacy, these agents could cause cardiotoxicity, a rare but life-threatening event. In addition, there are still no well-described predictive factors for the development of immune-related adverse events and information on high risk groups. According to known experimental studies we hypothesized that cardiovascular diseases may increase myocardial PD-L1 expression, which could be an extra target for Checkpoint inhibitors and a potential basis for complications development.

Methods: We studied patterns of myocardial PD-L1 expression in non-cancer-related cardiovascular diseases, particularly ischemic heart disease ($n = 12$) and dilated cardiomyopathy ($n = 7$), compared to patients without known cardiovascular diseases ($n = 10$) using mouse monoclonal anti-PD-L1 antibody (clone 22C3, 1:50, Dako). Correlation between immunohistochemical data and echocardiographic parameters was assessed. Statistical analyses were performed using R Statistical Software—R studio version 1.3.1093.

Results: In the myocardium of cardiac patients, we found membranous, cytoplasmic, and endothelial expression of PD-L1 compared to control group. In samples from patients with a history of myocardial infarction, PD-L1 membrane and endothelial expression was more prominent and frequent, and cytoplasmic and intercalated discs staining was more localized. In contrast, samples from patients with dilated cardiomyopathy displayed very faint endothelial staining, negative membrane staining, and more diffuse PD-L1 expression in the cytoplasm and intercalated discs. In samples from the non-cardiac patients, no convincing PD-L1 expression was observed. Moreover, we discovered a significant negative correlation between PD-L1 expression level and left ventricular ejection fraction and a positive correlation between PD-L1 expression level and left ventricular end-diastolic volume.

Conclusions: The present findings lay the groundwork for future experimental and clinical studies of the role of the PD-1/PD-L1 pathway in cardiovascular diseases. Further studies are required to find patients at potentially high risk of cardiovascular adverse events associated with immune checkpoint inhibitors therapy.

Keywords: ischemic heart disease, dilated cardiomyopathy, myocardial infarction, cardio-oncology, cardiotoxicity, checkpoint, PD-L1

INTRODUCTION

Programmed cell death receptor 1 (PD-1) and its ligand PD-L1 are involved in the regulation of T-cell activation, tolerance, and immune-mediated organ damage. Under physiological conditions, PD-1/PD-L1 signaling plays an important role in the prevention of autoimmune diseases. Apart from the expression on T- and B-cells, dendritic cells, and macrophages, PD-L1 could be expressed on non-hematopoietic cells, including cardiomyocytes and endothelial cells. Recently, it was found that a wide range of tumor cells express PD-L1 on their surface to prevent antitumor immune response (1). As a result, a new strategy for the treatment of advanced or metastatic cancer based on inhibition of PD-1 on the surface of T-cells or blocking PD-L1 on the tumor cells surface has appeared.

Therapy with immune checkpoint inhibitors (ICI) was associated with increased overall survival in patients with advanced cancer that previously had a poor prognosis. The results of KEYNOTE-042 trial comparing the effectiveness of ICI and standard chemotherapy in patients with advanced or metastatic non-small cell lung cancer (NSCLC) and PD-L1 tumor proliferation score (TPS) >50% showed that relapse-free survival was better in a group of ICI therapy—20 months vs. 12.2 months in the standard chemotherapy group (2). The KEYNOTE-522 trial confirmed the higher efficiency of combination therapy with pembrolizumab–chemotherapy against the placebo–chemotherapy group in patients with triple-negative breast cancer, as measured by relapse-free survival and a pathological complete response at the time of definitive surgery (3). The long-term outcomes of ICI therapy were measured for the CheckMate-017 and 057 trials and assessed 5-years overall survival and safety. Overall survival was longer in NSCLC patients receiving nivolumab than in NSCLC patients on chemotherapy (13.4% vs. 2.6%), and treatment-related adverse events were found in 25.8% of nivolumab-treated patients (4).

Cardiotoxic side-effects of ICI therapy have been reported since 2016. First publications described the development of fulminant myocarditis in patients receiving ICI (5–7). Moreover, there were cases of myopericarditis, takotsubo-like syndrome, and vasculitis with acute coronary syndrome symptoms (8–11). However, the true incidence of immune-related adverse events (irAEs) is still unknown and, according to some data, is in the range of 1 to 10.3% (12, 13). On the other hand, ICI-related myocarditis, one of the most common cardiac irAEs, has a relatively high mortality rate of 40–50% (14, 15).

Currently, there are no methods to identify patients at high risk for the development of ICI-associated cardiotoxicity. Moreover, the impact of pre-existing cardiovascular (CV) disease and traditional CV risk factors in cardiac irAEs occurrence is not yet fully understood. Histological and immunohistochemical analysis revealed high levels of membrane and cytoplasmic PD-L1 expression in samples from patients with ICI-associated myocarditis (5, 16). However, the role of PD-1/PD-L1 signaling in the development of non-cancer-related CV diseases is unclear. *In vivo* experiments performed by Grabie et al. discovered that IFN-γ-induced PD-L1 was mainly expressed on endothelium and its expression had an important cardioprotective effect against immune-related heart damage (17). Later, it was shown that PD-L1–/– knockout mice had a higher risk for the development of autoimmune myocarditis and pneumonitis with a more severe course of the disease and worse prognosis compared to PD-L1+/– and PD-L1+/+ animals (18). Baban et al. showed that in the model of ischemia-reperfusion injury and cryoinjured hearts, PD-L1 expression was markedly higher than in intact cells (19).

Up-regulation of PD-L1 may probably attenuate T-cell response against damaged cardiomyocytes, for example, in the course of ischemic heart disease (IHD), thus reducing the local inflammation in the myocardium. On the other hand, high PD-L1 myocardial expression in CV diseases might be associated with an increased risk of developing irAEs, since PD-L1 is a direct target for anti-PD-1 and anti-PD-L1 ICI. However, there is still no research demonstrating increased myocardial expression of PD-L1 in damaged human hearts due to different types of CV diseases.

To characterize the PD-L1 expression pattern in patients with CV diseases of different etiology, we analyzed PD-L1 myocardial expression in patients with documented IHD and dilated cardiomyopathy (DCM).

MATERIALS AND METHODS

We examined 12 autopsy samples of left ventricular (LV) myocardium obtained from patients with a history of myocardial infarction (MI). Nine patients died in an acute period of MI. Cardiac pathology specimens from an infarct-related artery were used for further immunohistochemical evaluation. The comparison group included seven samples of LV from patients with DCM who underwent orthotopic heart transplantation. In this group, IHD was excluded according to coronary angiography results. Echocardiography was carried out for all patients at one clinic. The control group included 10 LV samples collected from cancer patients without known CV pathology who died in the early postoperative period and had not received neoadjuvant

TABLE 1 | Clinical, echocardiographic, and immunohistochemical characteristics of patients.

	IHD ($n = 12$)	DCM ($n = 7$)	p
Age, years	66.1 ± 7.0	52.1 ± 9.8	**0.008**
Male sex, n (%)	12 (100)	4 (57.1)	0.361
LVEF, %	34.9 ± 7.2	20.3 ± 7.1	**0.005**
LVEDV, ml	194.3 ± 64.2	275.0 ± 82.2	0.071
Membrane PD-L1, n (%)	5 (41.7)	0 (0)	0.068
Cytoplasmic PD-L1, n (%)	10 (83.3)	7 (100)	0.386
Endothelial PD-L1, n (%)	4 (33.3)	0 (0)	0.127
ICD PD-L1, n (%)	7 (58.3)	7 (100)	0.068
PD-L1 ICDPS, %	1 [0;32.5]	90 [85;100]	**0.003**
PD-L1 CMPS, %	10 [5;17.5]	90 [30;100]	**0.001**

IHD, ischemic heart disease; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end diastolic volume; ICD, intercalated discs; CMPS, cardiomyocyte positive score. Bold values are $p < 0.05$.

chemotherapy and/or immunotherapy and 4 LV samples from patients received ICI and died without intravital data for CV irAEs (two without CV diseases and two with known IHD). The tissue was fixed in 10% neutral buffered formaldehyde and then embedded in paraffin. We used hematoxylin and eosin (HE) staining to visualize the myocardial structure and immunohistochemistry to investigate the expression of PD-L1 and distribution of $CD3^+$ T-cells and CD68+ macrophages. Additionally, the correlation analysis between echocardiographic parameters, complete blood count and histological results has been conducted (**Figures 1A,D,G**).

Ethics Approval

The study was approved by the local ethics committee (Protocol Number: 12032020 of March 16, 2020).

PD-L1 Expression and T-Cells Immune Infiltration Assessment

Immunohistochemistry was performed on the automated immunostaining platform Autostainer Link 48 (Dako, USA) for PD-L1 and Ventana Benchmark Ultra (Roche, Switzerland) for CD3. Tissue sections were immunostained with mouse monoclonal anti-PD-L1 antibody (clone 22C3, 1:50, Dako), rabbit monoclonal anti-CD3 antibody (clone 2GV6, Ventana) and mouse monoclonal anti-CD68 antibody (KP1, Abcam). All slides were scanned using a Pannoramic 1000 scanning microscope (3D Histech) with a x60 objective lens. Assessment of PD-L1 was performed by an experienced board pathologist. CD3 and CD68 expression was quantify with digital image analysis of scanned by QuPath software. We assessed membrane, cytoplasmic, and endothelial PD-L1 expression in all groups.

To characterize the expression level of PD-L1 in the myocardium, we developed a combined cardiomyocyte positive score (CMPS). CMPS was calculated as a percentage of PD-L1 positively stained cardiomyocytes with membrane and/or cytoplasmic expression of any intensity. To additionally evaluate the PD-L1 expression in intercalated discs (ICD), we determined the PD-L1 ICD positive score (ICDPS), which was defined as a percentage of positively stained ICD from all cardiomyocytes cut in a longitudinal section.

Statistical Analysis

Data were expressed as mean and standard deviation (Mean ± SD) or median with 25th and 75th percentiles (Median [25;75]). Clinical and expression data were analyzed using the Mann-Whitney U test for continuous variables and Fisher Exact test for dichotomous variables. Correlations were calculated with Spearman's rank correlation coefficient for non-parametric samples. p-values <0.05 were considered significant. All statistical analyses were performed using R Statistical Software—R studio version 1.3.1093.

RESULTS

The mean age in the MI group at the time of death was 66.1 ± 7.0 years. For patients who died from acute MI ($n = 9$), the mean time interval between symptom onset and death was 7.9 ± 4.3 days. During hospitalization, 10 of 12 patients underwent percutaneous transluminal coronary angioplasty, and one patient underwent coronary artery bypass graft surgery. The mean age in the DCM group at the time of heart transplantation was 52.1 ± 9.8 years, which was significantly lower than in the MI group ($p = 0.008$). There were no age differences between the experimental and control groups. Control group age was 59.5 ± 12.4 ($p = 0.197$ for MI and $p = 0.186$ for DCM). Clinical, echocardiography, and immunohistochemistry data of studied groups (MI and DCM) are summarized in **Table 1**.

In all patients from the DCM group, according to histopathological evaluation, <7 $CD3^+$ T-cells per mm^2 were detected [4 (3; 5) cells per mm^2], so inflammation cardiomyopathy was excluded (20). The median number of $CD3^+$ T-cells in MI group was 15.3 [8; 19] cells per mm^2 and 62 [50,5;93] cells per mm^2 for CD68.

In both studied groups, according to immunohistochemical evaluation, cytoplasmic and ICD PD-L1 expression was found. Membrane and endothelial PD-L1 expression was identified only in patients with ischemic myocardial damage. Furthermore, in this group, cytoplasmic and membrane PD-L1 expression in perivascular zones was more pronounced (**Figures 1B,C,E,F**). In samples from the control group without ICI treatment, there was a lack of membrane, endothelial, and ICD expression, accompanied by infrequent cytoplasmic PD-L1 expression, which appears to be a non-specific finding (**Figures 1H,I**). In the control group receiving ICI pronounced PD-L1 expression was found only in samples with pre-existing CVDs ($n = 2$; CMPS = 50 and 70%; ICDPS = 40 and 20%). In contrast, PD-L1 expression was not detected in patients without CVDs in ICI therapy group (**Figure 2**). The median number of CD3 and CD68 cells in control ICI group was 17 [11;102] and 149 [129;180] cells per mm^2 respectively.

There were no statistically significant differences between experimental groups in the presence or absence of different expression patterns. However, DCM group had significantly

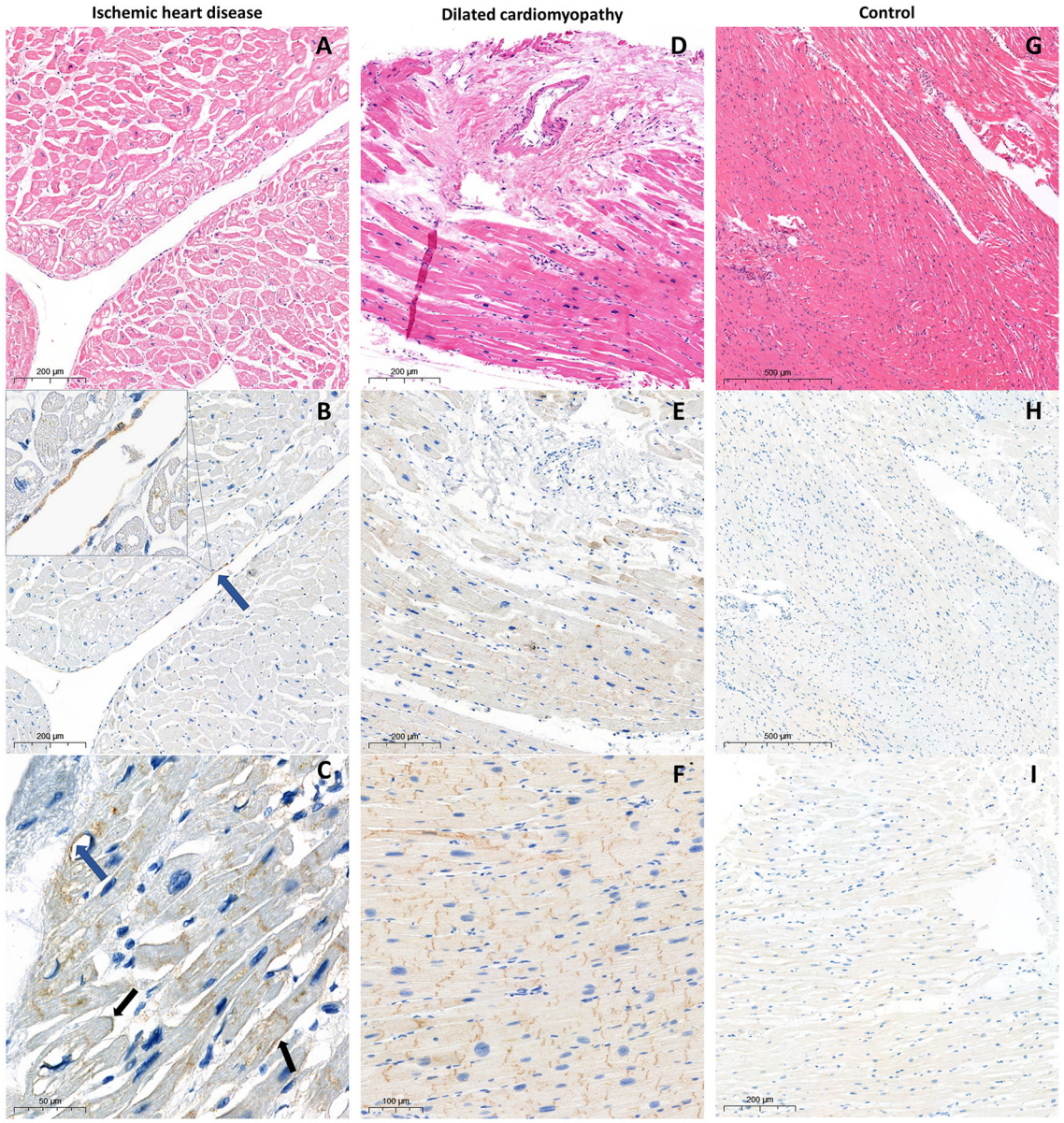

FIGURE 1 | Histological and immunohistological examination of the myocardium samples from the patient with ischemic heart disease **(A)** hematoxylin and eosin staining; **(B,C)** PD-L1 staining with CMPS 5% and 20% respectively. Blue arrows indicate positive endothelial PD-L1 expression, black arrows indicate positive membrane PD-L1 expression; from the patient with dilated cardiomyopathy **(D)** hematoxylin and eosin staining; **(E,F)** PD-L1 expression in cytoplasm and intercalated discs, without endothelial, perivascular, and membrane patterns with ICDPS 70% and 100% respectively; from the control without ICI treatment **(G)** hematoxylin and eosin staining; **(H)** absence of PD-L1 expression; **(I)** extremely poor cytoplasmic PD-L1 expression.

higher CMPS (90 [30;100] vs. 10 [5;17.5], $p = 0.001$) and ICDPS (90 [85;100] vs. 1 [0;32.5], $p = 0.003$) compared to the MI group.

According to correlation analysis between immunohistochemistry and echocardiography data, we got the following results (**Figure 3**). In all patients with CV diseases (IHD + DCM), there were significant negative correlations between CMPS and LVEF ($R = -0.628$, $p = 0.005$) and between ICDPS and LVEF ($R = -0.680$, $p = 0.002$), and significant positive correlations between CMPS and LVEDV ($R = 0.670$, $p = 0.003$) and between ICDPS and LVEDV ($R = 0.539$, $p = 0.026$). After dividing patients into subgroups, a significant negative correlation between ICDPS and LVEF ($R = -0.861$, $p = 0.013$) remained only in the DCM group. In the group of MI, only the tendency to a positive correlation between ICDPS and LVEDV was found ($R = 0.605$, $p = 0.064$). The lack of other significant correlations between studied parameters in subgroups may be attributed to a low number of analyzed samples.

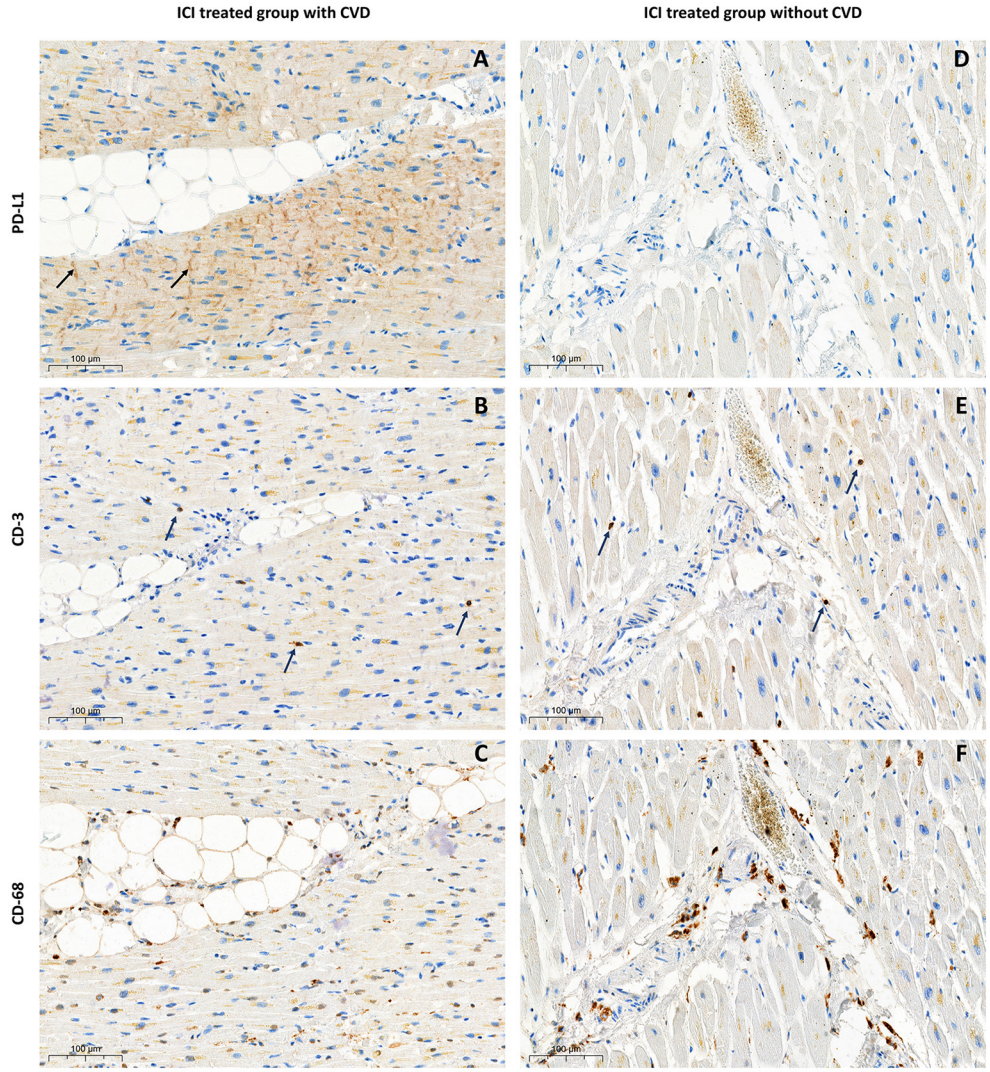

FIGURE 2 | Immunohistological examination of the myocardium samples from patients treated with ICI. With pre-existing CVD—left column **(A)** PD-L1 expression in ICD with CMPS 50%, ICDPS 40%; **(B)** CD-3 infiltration 7 cells per mm^2; **(C)** CD-68 infiltration 142 cells per mm^2; Without pre-existing CVD—right column **(D)** absence of PD-L1 expression; **(E)** CD-3 infiltration 17 cells per mm^2; **(F)** CD-68 infiltration 92 cells per mm^2.

Also, we have indicated strong positive correlations between myocardial PD-L1 expression (CMPS, ICDPS) and complete blood count (WBC, neutrophils) for patients with IHD (**Supplementary Figure 1**). No correlation between PD-L1 expression and monocytes or lymphocytes count was found. In DCM group PD-L1 expression didn't correlate with complete blood count data.

DISCUSSION

The present study aimed to examine the expression profiles of PD-L1 in the myocardium of cardiac patients without a history of cancer. In damaged myocardium from patients with IHD and DCM, we found several patterns of PD-L1 expression compared to the myocardium of patients without CV diseases. The presence of membrane and endothelial expression was more specific for patients with MI history than those without ischemic damage. The reason for this result is not yet fully understood. Previously, PD-L1 endothelial expression was described in the mouse model of CD8 T-cell myocarditis (17). Therefore, endothelial expression we found in ischemic injured myocardium could be caused by chronic inflammation, which is evidenced by an increase in the number of CD3+ cells.

Further analysis revealed PD-L1 expression in intercalated discs in all groups of cardiac patients, but predominantly PD-L1 was observed in the DCM group. This matches well with the recently described strong but diffuse staining of PD-L1 in ICD of cardiac allograft vasculopathy hearts. However, the

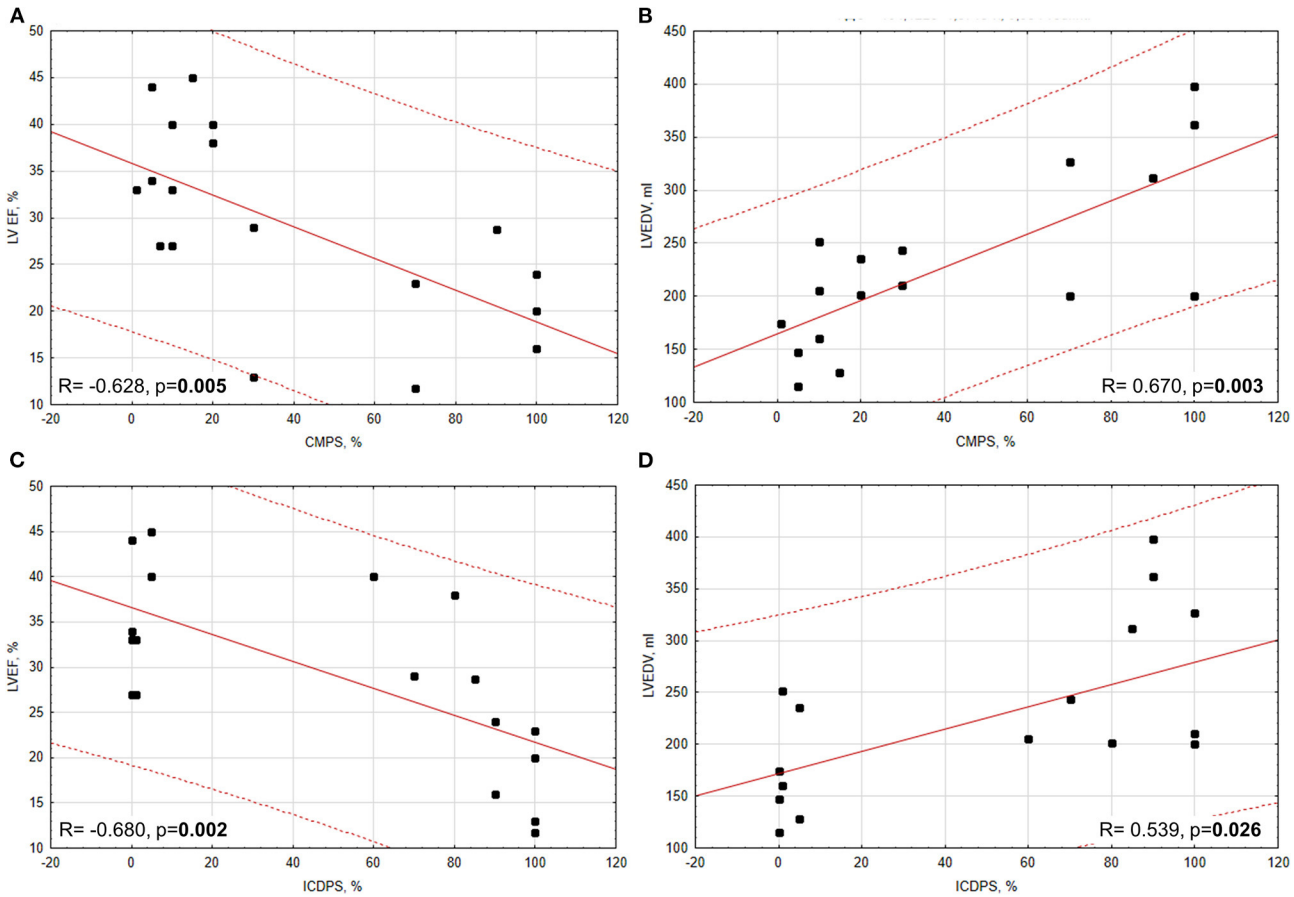

FIGURE 3 | Correlations between PD-L1 expression (CMPS, ICDPS) and echocardiographic parameters (LVEF and LVEDV). **(A)** Significant moderate negative correlation between CMPS and LVEF; **(B)** significant moderate positive correlation between CMPS and LVEDV; **(C)** significant moderate negative correlation between ICDPS and LVEF **(D)** significant moderate positive correlation between ICDPS and LVEDV.

staining was considered by the authors as non-specific and insignificant (21).

The crucial role in ICI toxicity development plays activation of inflammatory response which damage tissues and organs. Cytokines that are secreted by immune cells such as macrophages, activated T-cells, B-cells and NK cells take a lead in irAEs pathophysiology. Increased levels of IFN-γ and IFN-γ pathway genes are positive biomarkers of tumor response on ICI treatment and irAEs and IL-8, IL-6, and TGF-β are negative biomarkers (22). Experimental study evaluated cardiomyocyte cell line showed an increase of IL-1β, IL-8, IL-6, and TGF-β after Nivolumab and Ipilimumab affection (23). There are no experimental studies described features of cytokines levels and PD-L1 expression in human hearts after ICI administration. We showed PD-L1 expression in ICD in patients with history of CVD treated by ICI with significantly more pronounced CD-68 infiltration compared with those who had CVD but didn't receive ICI ($p = 0.01$). The limitation of our study is that we used archived material presented by paraffin blocks which makes it impossible to conduct flow cytometry or ELISA assay to describe T-cell immunophenotyping and cytokines levels.

In large retrospective study Oren et al. showed the increasing of ICI-related myocarditis risk from 0.13% to 4.5% in patients with history of MI, HF and age >80 years (24). But the mechanism of such risk increasing is unknown. In our studied groups, we found a negative correlation of PD-L1 expression prevalence in ICD, calculated as ICDPS, and LVEF and a positive correlation between ICDPS and LVEDV. These results may partially explain previously published clinical data. LV dilation occurred due to ischemia or cardiomyopathy likely resulted in disruption of intercellular contacts. Thus, PD-L1 expression can be considered as one of the possible cardioprotective mechanisms against myocardial injury. Also, there is experimental study shown that hyperglycemia increased cardiomyocyte damage during anti-CTLA4 ICI (Ipilimumab) administration (25). Another clinical study showed that diabetes is associated with an increase in PD-L1 positivity and recurrence in NSCLC (26). But there is no experimental data about direct anti-PD-L1 treatment influence on cardiomyocyte damage in condition of hyperglycemia. Nevertheless, based on the known data we may hypothesize that known CV comorbidity with diabetes may be a combined risk factor in

patient treated with anti-CTLA4+anti-PD-1 immunotherapy. But to prove it another experimental and clinical investigations are needed.

To sum up, our work described an increase of PD-L1 expression in the myocardium of cardiac patients and revealed a correlation between PD-L1 expression (CMPS, ICDPS) and echocardiographic parameters of left ventricular size and function (LVEDV and LVEF). The findings of this study lay the groundwork for further investigations aimed to identify the high risk patients for CV irAEs and give us a reason to pay more attention to patients with LV dysfunction and heart chambers enlargement.

We are aware that our research may have the limitation of a small sample size that did not allow us to investigate additional correlations in distinct subgroups of cardiac patients.

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 study was approved by the local ethics committee (Protocol Number: 12032020 of March 16, 2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EK performed data collection, statistical analyses, and wrote manuscript. VK performed immunohistochemical analysis. AA, LM, and OM provided the critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.759972/full#supplementary-material

Supplementary Figure 1 | Correlations between PD-L1 expression (CMPS, ICDPS) and white blood cells (WBC) count in peripheral blood. **(A)** Significant strong positive correlation between CMPS and WBC; **(B)** significant strong positive correlation between CMPS and neutrophils; **(C)** significant strong positive correlation between ICDPS and WBC; **(D)** significant strong positive correlation between ICDPS and neutrophils.

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Cardio-Oncology in the COVID Era (Co & Co): The Never Ending Story

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The pathophysiology of some non-communicable diseases (NCDs) such as hypertension, cardiovascular disease (CVD), diabetes, and cancer includes an alteration of the endothelial function. COVID-19 is a pulmonary and vascular disease with a negative impact on patients whose damaged endothelium is particularly vulnerable. The peculiar SARS-CoV-2-induced "endothelitis" triggers an intriguing immune-thrombosis that affects both the venous and arterial vascular beds. An increased liability for infection and an increased likelihood of a worse outcome have been observed during the pandemic in patients with active cancer and in cancer survivors. "Overlapping commonalities" between COVID-19 and Cardio-Oncology have been described that include shared phenotypes of cardiovascular toxicities such as left ventricular dysfunction, ischemic syndromes, conduction disturbances, myocarditis, pericarditis and right ventricular failure; shared pathophysiologic mechanisms such as inflammation, release of cytokines, the renin-angiotensin-aldosterone-pathway, coagulation abnormalities, microthrombosis and endothelial dysfunction. For these features and for the catalyst role of NCDs (mainly CVD and cancer), we should refer to COVID-19 as a "syndemic." Another challenging issue is the persistence of the symptoms, the so-called "long COVID" whose pathogenesis is still uncertain: it may be due to persistent multi-organ viral attacks or to an abnormal immune response. An intensive vaccination campaign is the most successful pharmacological weapon against SARS-CoV-2, but the increasing number of variants has reduced the efficacy of the vaccines in controlling SARS-CoV-2 infections. After a year of vaccinations we have

also learned more about efficacy and side-effects of COVID-19 vaccines. An important byproduct of the COVID-19 pandemic has been the rapid expansion of telemedicine platforms across different care settings; this new modality of monitoring cancer patients may be useful even in a post pandemic era. In this paper we analyze the problems that the cardio-oncologists are facing in a pandemic scenario modified by the extensive vaccination campaign and add actionable recommendations derived from the ongoing studies and from the syndemic nature of the infection.

Keywords: SARS-CoV-2, COVID-19, cancer, cardiovascular disease, cardiotoxicity, syndemic, telehealth

"*COVID-19 is not a pandemic. It is a syndemic. The syndemic nature of the threat we face means that a more nuanced approach is needed if we are to protect the health of our communities" (1).*

INTRODUCTION

SARS-CoV-2 causes primarily pulmonary disease due to a high expression of ACE2, the entry receptor of the virus, in many epithelial cell types of the respiratory tract such as alveolar epithelial type II cells in the lungs (2, 3). ACE 2 is also expressed in extrapulmonary tissues such as nasal goblet secretory cells, cholangiocytes, colonocytes, esophageal keratinocytes, gastrointestinal epithelial cells, pancreatic β-cells, renal proximal tubule and podocytes, as documented by many studies (4–6). This widespread expression of ACE2 leads to the numerous extrapulmonary manifestations of SARS-CoV-2 infection outlined in a recent paper as thrombotic complications, myocardial dysfunction and arrhythmias, acute coronary syndromes, acute kidney injury, gastrointestinal symptoms, hepatocellular injury, hyperglycemia and ketosis, neurologic illnesses, ocular symptoms and dermatologic complications, thus making COVID-19 a truly systemic disease (7). As far as cardiovascular system is concerned, SARS-CoV-2 targets endothelial cells that abundantly express ACE2 and dysregulate the endothelium balance affecting immune competence, inflammatory balance, tight junctional barriers, hemodynamic stability and the thrombosis/fibrinolysis equilibrium (8, 9).

The COVID-19 pandemic has affected the healthcare systems throughout the world, directly by the virus-related morbidity and mortality, and by the rapid shift of resources to the infective emergency, limiting the healthcare offer for unrelated pathologies (including cardiovascular diseases and cancer). As of December 17th, 2021, patients infected by SARS-CoV-2 are over 270 millions and deaths from COVID-19 over 5 millions (10).

The first pandemic wave in the first months of year 2020 was followed by a second wave after about 6 months and, in early 2021, by a third one whose peak has been overcome in several countries thanks to the massive vaccination campaign. However, the vaccination coverage is still <50% worldwide with countries such as Russia, Venezuela and some states in the USA where 60% of the population is unvaccinated and others such as the UK and Germany with <70% of people fully vaccinated and/or not applying strict social rules such as wearing masks or limiting accesses to public events, still facing the emergency of an increasing rate of cases (11). The low vaccination coverage, the high contagiousness of new variants and the decreased efficacy of vaccines over time have contributed to the advent of the fourth wave that is now spreading all over the world at an unprecedented speed. In addition we have to struggle with new problems, such as the post-COVID syndrome (12).

The ANMCO (National Association of Italian Cardiologists) published some months ago a Position Paper (13, 14). analyzing the peculiar problems of Cardio-oncology in the COVID-19 pandemic era. In this paper we will update the previous Position Paper and recommendations according to the new scientific achievements in the field, and to the new scenario after the start of vaccination campaign.

COVID-19, CANCER AND CARDIOVASCULAR SYSTEM: WHAT WE LEARNED IN 2021

Cancer and COVID-19

During COVID-19 pandemic, cancer patients showed a higher risk of serious events compared to non-cancer patients, including a more frequent need of invasive ventilation while admitted in the intensive care unit and higher mortality; patients treated with chemotherapy in the previous 2 weeks required more frequent admissions to the intensive care unit (15). In a recent study including more than 20,000 cancer patients a significantly increased risk of COVID-19 infection was observed among cancer patients, especially among older individuals and males; treatment with chemotherapy or immunotherapy was associated with a 2.2-fold increased risk of infection (16). Not only patients with active cancer but also cancer survivors have been shown to be more susceptible to COVID-19, in this population it has been shown that advanced age is the only risk factor for serious events (17).

In the era of immune check-point inhibitor (ICI) treatment, the question has been raised whether ICI treatment could affect protection from the virus and on the possible toxicity associated with COVID-19 vaccination. Indeed, the vaccine could "overload" the immune system and trigger a "cytokine storm," leading to severe toxicity or even fatal events. However, in the real world the results have been controversial. A recent study of 134 cancer patients who received ICI treatment and two doses of a COVID-19 vaccine reported a similar side effect

TABLE 1 | COVID-19, cancer and cardiovascular system.

COVID-19 and Cancer

- Potential susceptibility of the cancer population to COVID-19 and higher risk of serious events (15, 16).
- Not only patients with active cancer but also cancer survivors have been shown to be more susceptible to COVID-19 (17).
- Treatment with ICI is acceptable in COVID-19-infected cancer patients, except in those with severe disease (18).
- Patients receiving ICI treatment might benefit from COVID-19 vaccination and they might also benefit from increased efficacy (18).
- Rituximab-induced immunosuppression can lead to persistent SARS-CoV-2 viraemia and pneumonia, but a large meta-analysis did not show a worse outcome (19–22). In a more recent retrospective cohort study an increased risk of mechanical ventilation or in-hospital death was observed in patients treated with rituximab, especially female patients with cancer (23).

COVID-19 and cardiovascular system

- Hypertension is associated with a higher risk of severity and mortality of COVID-19 (24).
- Diabetes correlates with an increased susceptibility to infection and an increased propensity for disease progression (25).
- Obesity implies greater susceptibility to the virus, greater severity of disease, higher incidences of hospitalization, intensive care unit admission, and death (25).
- Incidence of acute cardiac injury in COVID-19 cases is 20–40% and mortality rate is up to 10-fold higher in patients with myocardial injury at presentation (26–28).
- Myocarditis is rare (<2%) (29).
- Myocarditis and pericarditis after vaccination are rare events and the balance of risk and benefit is decidedly in favor of vaccination (30, 31).

COVID-19 and Cardio-oncology

- Overlap phenomena exist between COVID-19, tumor complications and cardiovascular effects of cancer treatments (32).
- COVID-19- and anticancer drug-induced myocardial damage might have an additional effect leading to a rise in cardiovascular adverse outcomes through a "two-hit" model (33).

Long COVID-19

- It could be the effect of a direct result of persistent multi-organ viral attack or a chronic low grade inflammation brought about the immunomodulatory effects of the virus in the long term (34).
- A persistent endotheliopathy seems to occur independently of the response to the acute phase and is accompanied by increased thrombin production (35).
- It has recently been proposed that long COVID-19 may predispose to the development of cancer and accelerate its progression (36).

profile between cancer patients and healthy controls (18). It has been therefore hypothesized that patients receiving ICI treatment might benefit from COVID-19 vaccination and that they might also benefit from increased efficacy.

Another question has been raised regarding rituximab, an anti CD20 antibody that represents an effective treatment in many B-cell lymphomas. In patients treated with rituximab a persistent SARS-CoV-2 viraemia, an atypical COVID-19 dynamic and a persistent COVID-19 pneumonia with failure to develop anti SARS-CoV-2 antibodies have been reported, but a large meta-analysis of over 3,000 patients with hematological neoplasms did not show a correlation between concurrent treatment and worse outcome (19–22). The immunosuppressive treatment could indeed blunt the hyperinflammation and reduce the incidence of severe pneumonitis. In a more recent retrospective cohort study 12,841 immunosuppressed patients were compared to 29,386 non-immunosuppressed patients. No increased risk of mechanical ventilation or in-hospital death from the rheumatological, antineoplastic or antimetabolite therapies was observed, with the exception of patients treated with rituximab, especially female patients with cancer (23). Since rituximab-induced chronic hypogammaglobulinemia could also blunt the immune response to SARS-CoV-2 vaccination, a tailored vaccination is suggested in patients treated with rituximab (37, 38). A recent study assessed m-RNA-based COVID-19 vaccine effectiveness in patients treated with rituximab for rheumatic diseases and found that anti-CD20 treatment weakens humoral responses but does not impair T-cell responses to the vaccine (39) (**Table 1**).

COVID-19 and Cardiovascular System

Since the early studies published in China, patients hospitalized for COVID showed a high prevalence of CVD risk factors and CVD and this accounted for a more severe course of the disease and higher case fatality rates (24). The pandemic has highlighted a higher risk of severity and mortality of COVID-19 in hypertensive patients and a peculiar infectious risk in diabetic and obese patients (25). Individuals with diabetes generally suffer from chronic low-grade inflammation, which may facilitate cytokine storms, contributing to the inauspicious prognosis of COVID-19. Recently, a meta-analysis demonstrated in diabetic patients not only an increased susceptibility to the infection but also an increased disease progression of COVID-19 (40).

We are constantly learning more and more on the impact of COVID-19 on the cardiovascular system. COVID-19 has been placed in the context of the broader critical care landscape. SARS-CoV-2 infection causes myocardial injury that has a relevant role in the occurrence of severe clinical phenotypes or adverse events in affected patients. Elevated cardiac troponin is the hallmark of cardiac injury and the biomarker gives a prevalence of 20–40% of cardiac damage; myocardial injury at presentation accounts for a 10-fold increase of mortality rate (26–28).

There are many mechanisms potentially involved in the elevation of troponin in COVID-19, including thrombotic and plaque rupture events, supply-demand mismatch, direct cardiac viral toxicity, hypoxia, hypoperfusion, and tachycardia. In addition to acute myocardial infarction, troponin elevation may occur in other kinds of COVID-19 cardiovascular involvement such as viral myocarditis, cardiac damage secondary to cytokine storm, stress cardiomyopathy, heart failure (HF), pulmonary embolism, and arrhythmias (41). Myocarditis is an uncommon cause of cardiac injury, clinical and imaging markers are often suggestive of myocarditis, but the definite diagnosis requires an endomyocardial biopsy (EMB) that is rarely performed. A true autopsy- or EMB-proven diagnosis occurs in 4.5% of cases, but if we take into account some bias of autopsy studies, the percentage is even lower (42). A recent review of 22 publications with a total of 277 autopsied hearts found myocarditis in 7.2% of hearts, but a closer examination of the cases revealed that most cases were not functionally significant and the authors conclude that the true prevalence is <2% (29). Evidence of a myocarditis directly

caused by the SARS-CoV-2 is scarce. Virus particles found in cardiac macrophages have been considered the result of a viremic phase or the migration of infected alveolar macrophages outside the pulmonary tissues (43–46). The risk of mortality and adverse events follows a continuous linear trend with the degree of troponin increase; therefore, troponin measurement has been incorporated into routine clinical practice in hospitalized COVID- 19 patients. A recent study has challenged previously acquired certainties, myocardial damage in severe COVID-19 has been shown to be driven by underlying comorbidities, advanced age, and multisystem organ dysfunction. These findings raise a new question: does myocardial damage evidenced by troponin represent a mediator or a marker of adverse outcome? (47).

Furthermore, in an international, retrospective multicenter study of echocardiographic findings in more than 300 patients admitted with COVID-19, a significantly higher risk of in-hospital mortality was observed only in patients with troponin elevation and echocardiographic abnormalities, not just elevated troponin (48).

During the early phase of the pandemic, there was initially theoretical uncertainty about the safety of using angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin II receptor blockers (ARBs) in patients with COVID-19. ACE2 is a receptor for SARS-CoV-2, therefore concern was initially raised in the medical and scientific community that the use of ACEIs and ARBs could result in increased mortality and severity of COVID-19. Since 12-day administration of losartan or both losartan and lisinopril induced an increase in cardiac Angiotensin Converting Enzyme 2 (ACE2) mRNA and in cardiac membrane ACE2 activity in rats (49), it was hypothesized that ACEIs and ARBs could increase the entrance receptors for SARS-CoV-2 infection leading to a more severe infection and higher mortality. Subsequent studies have allayed initial fears, demonstrating not only the potential benefit of ACEI/ARB treatment in hospitalized patients with hypertension and COVID-19, but also a reduction in COVID-19 all-cause mortality in treated vs. untreated patients (50). A special report described the uncertain effect of renin-angiotensin-aldosterone system (RAAS) inhibition in humans due to the paucity of studies regarding the effect of RAAS inhibition on ACE2 expression confirming that RAAS inhibitors should be continued in hypertensive patients at risk for or with COVID-19 (51). A recent meta-analysis of 26 studies confirmed that treatment with ACEIs and ARBs compared with other antihypertensive drugs or no treatment was associated with reduced mortality as well as a lower risk of ventilatory support among COVID-19-infected hypertensive patients (52).

Major scientific Societies have provided recommendations in favor of continued treatment with ACEIs and ARBs in patients with hypertension, HF, and ischemic heart disease (53–55) (**Table 1**).

Myocarditis and Pericarditis After Vaccination for COVID-19

Although the physiopathology of myocarditis is still unclear, it has been hypothesized that vaccine mRNA can be identified as an antigen by the immune system that activates pro-inflammatory cascades and immunological pathways that may have a relevant role in the development of a systemic reaction of which myocarditis is an important component. Another mechanism could be related to molecular mimicry between the coronavirus spike protein and self-antigens whereby a cross-reaction may occur between antibodies against SARS-CoV-2 spike glycoproteins and structurally similar peptide protein sequences, such as α-myosin (56). A possible association between COVID-19 mRNA vaccines and myocarditis, mainly in younger male individuals within a few days after the second vaccination, has been recently reported by the Centers for Disease Control and Prevention, with an incidence of ∼4.8 cases per 1 million (30). According to a recent report on 2,000,287 vaccinated subjects, myocarditis developed in 20 young patients, a median of 3.5 days after vaccination, especially after the second dose of vaccine. Pericarditis affected 37 patients with a median onset of 20 days after the most recent vaccination (31). Despite these rare events, the balance of risk and benefit is decidedly in favor of vaccination against COVID-19 (**Table 1**).

Cardio-Oncology and COVID-19

In the cardio-oncology population, additional diagnostic complexity has been observed due to "overlap" phenomena between COVID-19, tumor complications, and cardiovascular side effects of cancer treatments. Cardiovascular toxicities shared by COVID-19 and cardio-oncology include myocardial injury, cardiomyopathy, myocarditis, pericarditis, ischaemia, conduction disturbances involving immune system activation, cytokine release syndrome, arterial and venous coagulopathy (32). It should be emphasized that in this population, the increased troponin assumes an even more intriguing significance since it may be also indicative of subclinical cardiotoxicity induced by treatments with anthracyclines and/or anti-HER2 agents, and it can be observed in patients receiving tyrosine kinase inhibitors at high prothrombotic risk or fluoropyrimidines. Studies are needed to define whether cardiac injury deriving from SARS-CoV-2 infection and from anticancer drugs might have an additional effect leading to a rise in cardiovascular adverse outcome through a "two-hit" model, both in cancer patients and survivors (33). A recent analysis of an AHA COVID-19-based CVD registry did not show a significant difference of in-hospital mortality among cancer patients with or without preexisting CVD, on the other hand (and in contrast to previous studies), a strong independent association of oncologic treatment with in-hospital morbidity was observed. The combination of these data provides the cue for a delicate reflection that should involve both oncologists and cardiologists inviting them to share with their patients the definition of the optimal timing of anti-cancer therapies according to the necessity to cope with limited health resources and an infection breakdown, obviously balancing the possible need for urgent therapy according to cancer type and cancer status (57) (**Table 1**).

Post-acute COVID-19 Syndrome "(Long COVID)"

Several outpatients' clinics are flooded by patients affected by long-lasting symptoms: the so-called "long COVID" syndrome. This syndrome is better defined as "post-acute COVID-19 syndrome (PACS)" if the symptoms last more than 3 weeks and

"chronic COVID-19" if they last more than 12 weeks (58, 59). The National Institutes of Health has defined "long COVID" as post-acute sequelae of SARS-CoV-2 infection (PASC) (60). Initial reports, currently confirmed, have highlighted the following residual effects of SARS-CoV-2 infection: fatigue, dyspnea, chest pain, cognitive impairment, arthralgia, and decline in quality of life (61). Symptomatic tachycardia, either presenting as postural orthostatic tachycardia syndrome or inappropriate sinus tachycardia, is also frequently reported in post-acute COVID-19 syndrome (62). All these symptoms may pose problems of differential diagnosis with symptoms originating from a primary cardiovascular problem. The overdrive of host immunity in response to the virus may contribute to severe disease. Long COVID-19 could be a chronic low-grade inflammation brought about by the immunomodulatory effects of the virus in the long-term (34). It has recently been proposed that long COVID-19 may predispose to the development of cancer and accelerate its progression. The hypothesis comes from an increased evidence of a relevant role of SARS-CoV-2 in modulating oncogenic pathways, promoting chronic low-grade inflammation and causing tissue damage. Responses in COVID-19 patients are governed by proinflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α), which are also drivers of oncogenesis.

Hypoxia due to inflammation can induce oxidative stress that synergistically with chronic inflammation can lead to DNA damage and subsequent tumorigenesis (36). A recent study has shown a frequent prolonged activation of endothelial cells (up to 10 weeks after acute SARS-CoV-2 infection) and this sustained endotheliopathy seems to be independent from the response to the acute phase and is accompanied by increased thrombin production (35). These data open a new scenario that raises a question about the stratification of thrombotic risk after the resolution of the acute infection and the possible need for prolonged thromboprophylaxis. Multidisciplinary collaboration is essential to provide appropriate outpatient care for COVID-19 survivors (**Table 1**).

Cardio-Oncological Counseling in COVID-19 Pandemic

The Very Early Phase

Shortly after the pandemic spread we learned that patients with cardiovascular disease and cancer were at a higher risk of acquiring the infection and of experiencing poorer outcomes (63). Cardio-oncology focuses on the intersection of two pathologies that both affect, by definition, "fragile" patients. For these reasons Cardio Oncology Services have faced a series of issues, which have influenced both the clinical and organizational areas:

- The subgroups most at risk seem to be patients on active therapy, in particular those with signs/symptoms attributable to cardiotoxicity; patients being treated with immunosuppressive drugs and patients who have undergone autologous or allogeneic haematopoietic stem-cell transplantation (64–66). For this reason, the absolute need to protect these subgroups of patients from the possibility of contracting COVID-19 has emerged since the very beginning.
- Cancer patients with or without pre-existing cardiovascular disease were in any case indirectly involved in the profound reorganization of both territorial and hospital health services that the pandemic urged to make, as well as by the reallocation of human and structural resources to the management of COVID-19 patients. This has led to the postponement and reprogramming of diagnostic tests and treatments with a clear impact on cancer outcome (67, 68).

What Have We Learned so Far?

The COVID-19 pandemic has represented and still represents a unique opportunity for a reasoned review on the appropriateness of our clinical cardio-oncology practice which still lacks shared guidelines and is frequently anchored to local habits (69). During pandemic our watchwords have become appropriateness and optimization of therapeutic and follow-up paths. We therefore learned that risk stratification of our cardio-oncology patients played a key role. Identifying truly low-risk patients makes it possible to concentrate the limited resources available on patients at higher cardiological risk, for whom the deferral of clinical and instrumental controls could actually have negative consequences.

Recommendations for a modified screening and monitoring schedule to detect cardiac dysfunction, and judicious use of multimodality imaging and biomarkers to identify heart involvement during pandemic are actually available from three international groups (70–72) and have been variously applied in order to minimize the outpatient accesses to hospital. The central issue is to obtain baseline LVEF assessment and to keep standard monitoring by means of trans-thoracic echocardiography only in those patients considered to be at high risk for cardiotoxicity and to reserve additional imaging to selected cases.

The COVID-19 pandemic has propelled the use of telemedicine because it can be accessed by people directly from home and may reduce the probability of viral transmission by limiting hospital accesses and interpersonal contacts. Over the course of <1 year, many centers have shifted the majority of follow-up cancer care to virtual modality, a dramatic transformation in the way our patients' care is delivered. The video-visit volume at the University of California, San Francisco Comprehensive Cancer Center expanded from <20 to 72% in a brief time at the beginning of the pandemic (73). In the first months of the pandemic a national survey evaluated the impact of COVID-19 on Canadian medical oncologists, 82% of medical oncologists reported the implementation of telemedicine for many cancer patients: telephone call was utilized in 100% of cases, videoconferencing was used in 42% and e-mail in 12% of cases (74). An early implementation of Virtual Care was reported as feasible in a high volume cancer center in Ontario Canada from March to May 2020 with a preserved quality of care (75). Even though multiple barriers, including cost-effectiveness, security of communication links for personal data (including health), limitations/unreliability of internet connections, concerns regarding the impact of telemedicine on doctor-patient relationship, liability and legal issues, time constraints, and financial (e.g., billing) obstacles have slowed progress of telemedicine, the data collected in this period make

TABLE 2 | The four pillars of counseling.

- Limitation of hospital accesses
- Spread of telemedicine
- Restriction of imaging sessions
- More extensive and reasoned use of biomarkers

telemedicine a valuable component of our clinical practice that will last beyond the pandemic (76, 77) (**Table 2**).

Cardio-Oncological Consulting in Outpatients

For cardio-oncological patients, a first distinction must be made between the outpatient and the hospital level, with a further differentiation, between COVID-free "Cancer Centers" and general hospital. At first, the only effective strategy to contain the spread of the disease appeared to be social distancing (78) and, for cancer patients, this translates into the need to limit hospital access to selected cases.

In cancer patients with no previous CVD, an accurate risk stratification could be based on the anamnestic criteria only, by a shared cardiological and oncological evaluation. The cardiologist's task is to provide the oncologist with simple flowcharts to identify low-risk patients, for whom cardiological consultation in presence is not necessary, once a baseline electrocardiogram and a pretreatment echocardiogram (if needed) have been acquired. For patients with known CVD it is not always possible to safely defer or to skip cardiological checks.

In order to restrict accesses to hospital to high-risk patients only, an appropriate triage for patients with new cancer diagnosis and cancer survivors is mandatory and telemedicine can fulfill this purpose. A first approach can include a cardiologist's telephone contact aimed at ascertaining the clinical stability of the patient. This evaluation can possibly be integrated by telemedicine tools, as the transmission of the instrumental tests held by the patient. This preliminary "virtual visit" assesses cardiac risk; if the risk is high an "in person" cardio-oncology visit is suggested, if the cardiac risk is low a "virtual" cardio-oncology visit is planned (72). Telemedicine is indeed in the spotlight, especially in the USA, where in 2020 Congress approved a regulation (79) which allows certain providers to charge Medicare for some services provided through telemedicine. In spring 2020, there was an increasing use of online platforms, as a tool to keep patients out of the hospital (80, 81). However, in many countries the regulatory framework and the possibility of reimbursement for telemedicine activities are still very poor. Furthermore, the unavailability of technology and the lack of digital literacy could accentuate the inequalities in access to specialized medical care. And this is an issue that affects mainly the most disadvantaged population groups, such as patients of low socioeconomic status, the elderly and immigrants (82). Actually *"equitable"* care is one of the 6 quality dimensions of telehealth interventions provided by the Institute of Medicine's report: "*care that is safe, effective, patient-centered, timely, efficient and equitable*" (83).

As far as telemedicine in the cardio-oncology field is specifically concerned, an international survey conducted between March and April 2020, which involved over 1,400 cardiologists and oncologists from 43 countries, showed a rapid growth in telemedicine already in the first months of the pandemic. Of note, cardiologists more often than oncologists reported the need to cancel or postpone elective visits or treatments, and that can partly be explained by the fact that cardiologists were more often directly involved in the care of COVID-19 patients (84) (**Table 2**).

Cardio-Oncological Counseling in Hospitalized Patients

In this context too, the primary need is to protect "fragile" patients, minimizing the chances of contagion. Within non-COVID-free general hospitals, it is necessary to provide and organize protected pathways for cancer patients. More extensive use of biomarkers to reduce imaging sessions and the use of portable hardware (POCUS, point-of care ultrasound) could find application in hospitalized patients even more than in outpatients. In hospitalized patients, a problem that could arise from a wider use of biomarkers is represented by the differential diagnosis between manifestations of cardiotoxicity and a possible SARS-CoV-2-related cardiac involvement in the course of infection, considering, however, that the former is much more frequent than the latter. Finally, the clinical and instrumental pre-surgery operative cardiological evaluation of patients to be sent to oncological surgery which, especially in Cancer Centers, is widely used, should even more be limited to cases in which the results of the consultation is able to modify the surgical choices and/or treatment (85) (**Table 2**).

ADAPTED CARDIAC MONITORING IN THE VACCINATION ERA

Basal cardiovascular screening and on-treatment monitoring in cancer patients receiving potentially cardiotoxic therapies are of fundamental importance to reduce cardiac toxicity and improve outcome (87). The costs of pandemic both in terms of the direct impact on healthcare system and by the huge amount of cumulated backlogs in elective diagnostic procedures impose a deep reflection about how to improve both sustainability and equity in healthcare (88). The need to recover unperformed cardiac evaluations/tests together with an increasing number of tests required by new diagnoses suggests a common strategy to harmonize cardiac surveillance protocols avoiding unnecessary tests and reducing the frequency of examinations under certain circumstances. The modifications applied to cardiac monitoring protocols during the first wave of pandemic could offer some solutions to be implemented even in the vaccination era. The central idea of a careful stratification of the risk of cardiac toxicity should get more and

TABLE 3 | Proposal for a risk-based approach to planned cardiac monitoring during anthracycline and trastuzumab treatment in the vaccination era.

Treatment	Recommendations before pandemic	Recommendation during pandemic	Recommendation during vaccination
Anthracyclines: basal evaluation	• Cardiological visit only in intermediate and high-risk patients* • Echocardiography to all patients	• Cardiological visit only in high-risk patients* • Echocardiography only in high-risk patients*	• Cardiological visit only in high-risk patients* • Echocardiography only in high-risk patients*
Anthracyclines: on treatment	• Echocardiography at mid-cycle if high CV risk • Echocardiography at the end of treatment to all patients	• No screening in asymptomatic patients • Echocardiography if high-dose RT, high cumulative anthracycline dose (>400 mg/m^2) or with doses of 250 mg/m^2 in presence of CV risk factors or cardiopathy	• Echocardiography at the end of treatment to all patients (OOH) • Early assessment if high-dose RT, high cumulative anthracycline dose (>400 mg/m^2) or with doses of 250 mg/m^2 in presence of CV risk factors or cardiopathy
Anthracyclines: follow-up	• If no cardiotoxicity echocardiography at 6– 12 months and after 2–3–5 years • If cardiotoxicity echocardiography at 3–6–12 months and each year until 5 years	• In asymptomatic patients defer the echo-imaging	• If no cardiotoxicity echocardiography at 12 months and after 2–5 years in intermediate and high-risk patients* • If no cardiotoxicity echocardiography at 12 months in low-risk patients** (OOH) • If cardiotoxicity echocardiography at 3–6–12 months and each year until 5 years
Trastuzumab: basal evaluation	• Echocardiography to all patients	• Echocardiography only in high-risk patients	• Echocardiography only in intermediate and high-risk patients
Trastuzumab: during treatment	• If LVEF is normal, echocardiography every 3 months. • If LVEF 40–49%, optimize HF therapy. Continue treatment if LVEF stable after 4 weeks and repeat echocardiography after 4 weeks. • If LVEF <40% stop treatment, optimize HF therapy and evaluate after 4 weeks	• In low-risk** patients with no previous anthracyclines, echocardiography at 6–12 months; if metastatic disease echocardiography every 6 months • In high-risk patients* echocardiography every 3 months • If LV dysfunction or signs and symptoms of HF follow pre-pandemic recommendations	• In low-risk** patients with no previous anthracyclines, echocardiography every 6 months (OOH) • In high-risk patients* echocardiography every 3 months • If LV dysfunction during treatment or signs and symptoms of HF follow pre-pandemic recommendations
Trastuzumab: follow-up	• The same as anthracyclines	• If asymptomatic defer the echo imaging	• If no cardiotoxicity echocardiography at 12 months and after 2 years in intermediate and high-risk patients* • If no cardiotoxicity echocardiography at 12 months in low-risk patients** (OOH) • If cardiotoxicity echocardiography at 3–6–12 months and each year until 5 years

*Adapted from Calvillo-Arguelle et al. (86) and Bisceglia et al. (13, 14) for before pandemic and during pandemic sections. CV, cardiovascular; RT, radiotherapy; OOH, out-of-hospital; LVEF, left ventricular ejection fraction. *Two or more of the following risk factors: age ≥60 years, cardiopathy, high-dose radiotherapy, ≥2 cardiovascular risk factors, high-dose anthracyclines. **No risk factors.*

more relevance. Limited healthcare resources should be focused on people with a higher baseline risk of toxicity and in this setting the frequency of cardiac consultations should be kept unchanged. On the other hand we could safely increase the time period between visits in very-low and low risk population. An additional solution could be the relocation of some routine activity in low-risk patients in out-of-hospital (OOH) facilities in close collaboration with general practitioners. Baseline and on-treatment cardiac monitoring are ideal candidates to test this new risk-based model.

General Considerations

The proposed post-COVID recommendations on cardiac monitoring are focused on the general surveillance schedule for patients receiving anthracyclines and anti-HER2 agents. Cardiac surveillance in those cancer patients with a higher probability to develop cardiotoxicity and/or when an appropriate early cardiological treatment is advisable to avoid delays or interruptions of anticancer treatment program must continue unchanged. Cardiological visits should coincide with cancer therapy administration to reduce the need of hospital accesses. Cardiac imaging monitoring should be focused on the predicted toxicity. Alternative imaging techniques [as computed tomography (CT) scan, cardiac magnetic resonance, and nuclear medicine techniques] (89, 90), should be reserved to selected cases based on cardio-oncologist consultation only.

In subsequent visits in asymptomatic low-risk patients, it could be reasonable to reduce the general duration of echo examination. In centers with specific expertise

in monitoring cardiac toxicity by means of serial troponin and/or brain natriuretic peptide, the frequency of imaging could be reduced in asymptomatic patients with persistent normal values (<99th percentile) of biomarkers given their high negative predictive value (91). In those centers where biomarkers are routinely tested, we suggest to use routine cancer treatment-related blood draws to minimize exposures. **Table 3** summarizes recommendations for an adapted risk-based imaging and clinical assessment schedule.

Baseline Evaluation of Cancer Patient

Anthracyclines

Baseline cardiac imaging should be offered to patients with a history of significant CVD, with signs or symptoms of cardiac dysfunction, with two or more cardiovascular (CV) risk factors for cardiotoxicity (age ≥60 years, hypertension, diabetes mellitus, dyslipidaemia, smoking, or obesity). If the execution of baseline evaluation is not feasible before treatment, it may be reasonable to postpone it during treatment in asymptomatic and low-risk patients. For adult patients whose only risk factor is a planned high cumulative doxorubicin dose (≥250 mg/m^2), it may be reasonable to delay imaging until this threshold dose is reached or at the end of treatment (86).

Trastuzumab

Basal screening should be reserved to patients with a known CVD, with signs or symptoms of cardiac dysfunction, with 2 or more CV risk factors for cardiotoxicity (age ≥60 years, hypertension, diabetes mellitus, dyslipidaemia, smoking, or obesity), prior exposure to anthracyclines. In patients without valvular disease and a normal ventricular function (LVEF ≥55%) assessed in the previous 6 months, it is reasonable to avoid basal evaluation (86).

Surveillance During Treatment

Anthracyclines

The majority of cardiac dysfunction observed during anthracyclines therapy are mild and moderate with a very low mortality rate. Therefore, in the general population it could be reasonable to delay routine imaging during anthracycline therapy and perform a single final evaluation except for the following cases: signs and symptoms of HF or anthracycline dosages >400 mg/m^2 or cardiac risk factors and need for anthracycline therapy >250 mg/m^2, especially when there is a potential clinical impact of cardio-protective strategies. In those centers that routinely use biomarkers, cardiological evaluation should be performed in case of significant rise of biomarkers (86).

Trastuzumab

In the adjuvant setting, asymptomatic women without CV risk factors and not previously treated with anthracycline may undergo echocardiography at a reduced schedule of evaluation at 6 and 12 months only. In the metastatic setting, an echocardiogram could be performed every 6 months in the first year; beyond first year cardiac imaging may be deferred in asymptomatic patients. In patients with risk factors for cardiotoxicity (prior anthracycline exposure, CV risk factors) it is necessary to keep cardiac surveillance every 3 months. Patients with borderline ejection fraction (EF) 50–55% or reduced LVEF or with signs or symptoms of HF must continue to have a closer imaging schedule. In those centers that routinely use biomarkers, cardiological evaluation should be performed in case of significant rise of biomarkers (86).

Follow-Up

Routine cardiac follow-up in asymptomatic survivors of pediatric, adolescent, and young adult cancers could be moved to OOH facilities. Immediate cardio-oncological consultation will be provided in case of symptoms or signs of toxicity.

Perspectives

COVID-19 pandemic has forced the cardio-oncology community to make a re-evaluation on how to deliver the best clinical care. In addition to the aforementioned leading role of the appropriateness issue, one of the most important byproducts of COVID-19 pandemic has been the growth of telemedicine platforms across different care settings. In an era of digital technologies in many aspects of our life, COVID-19 has accelerated digital transformation, this impressive transition has been called "techcelleration" (92). For clinicians this paradigm shift from an interactive empathic "face to face" visit to a mere decoding of data from a smart screen has been challenging, some of them accept these changes, but others are troubled by this profound transformation.

Moreover, multi-organ point-of-care ultrasound (PoCUS), including lung ultrasound (LUS) and focused cardiac ultrasound (FoCUS), has impacted greatly on the management of COVID-19 patients both at triage and at subsequent clinical management. An expert panel has developed a consensus document on the use of PoCUS in COVID-19 patients. PoCUS was useful in nine clinical domains (diagnosis of SARS-CoV-2 infection, initial triage and risk stratification, diagnosis of Covid-19 pneumonia, diagnosis of cardiovascular disease, screening for venous thromboembolic disease, respiratory support strategies, management of fluid therapy, clinical monitoring of patients with COVID-19, and infection control to reduce the environmental spread of infection and risk of infection for health care providers) (93).

In the future we will also have to be able to minimize the disparities in accesses to care that the pandemic has highlighted. This will enable us to better face future pandemics and limit their spread using models that have proven effective against COVID-19, without losing contact with our patients and compromising the effectiveness of cancer and cardiological treatments. The rapidly accumulating data and patients' follow-up we are accompanying through the storm of the COVID-19 pandemic will allow us to refine our approach to what increasingly resembles "precision cardio-oncology." The "digital future is now" is the warning of the editors of JACC Heart Failure (92), therefore we must be ready to support the valuable components of this transition and their "*potential for a better tomorrow*" (92).

Finally, the tremendous impact of the virus on CVD and cancer patients should fuel a vigorous campaign to implement healthy lifestyles that will reduce the burden of CVD and cancer, improve the health of our planet and eventually stop the syndemic.

AUTHOR CONTRIBUTIONS

IB, MC, GG, FT, and CL wrote sections of the manuscript. All authors contributed to the conception of the work, manuscript revision, read, and approved the submitted version.

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Corrigendum: Cardio-Oncology in the COVID Era (Co & Co): The Never Ending Story

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A Corrigendum on

Cardio-Oncology in the COVID Era (Co & Co): The Never Ending Story

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In the published article, there was an error in affiliation 13. Instead of "Cardiology Department, Santa Maria Nuova Hospital, Reggio Emilia, Italy," it should be "Divisione di Cardiologia, Arcispedale S. Maria Nuova, Azienda Unità Sanitaria Locale-IRCCS di Reggio-Emilia, Reggio Emilia, Italy."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Percutaneous Coronary Intervention in Patients With Gynecological Cancer: Machine Learning-Augmented Propensity Score Mortality and Cost Analysis for 383,760 Patients

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Background: Despite the growing number of patients with both coronary artery disease and gynecological cancer, there are no nationally representative studies of mortality and cost effectiveness for percutaneous coronary interventions (PCI) and this cancer type.

Methods: Backward propagation neural network machine learning supported and propensity score adjusted multivariable regression was conducted for the above outcomes in this case-control study of the 2016 National Inpatient Sample (NIS), the United States' largest all-payer hospitalized dataset. Regression models were fully adjusted for age, race, income, geographic region, cancer metastases, mortality risk, and the likelihood of undergoing PCI (and also with length of stay [LOS] for cost). Analyses were also adjusted for the complex survey design to produce nationally representative estimates. Centers for Disease Control and Prevention (CDC)-based cost effectiveness ratio (CER) analysis was performed.

Results: Of the 30,195,722 hospitalized patients meeting criteria, 1.27% had gynecological cancer of whom 0.02% underwent PCI including 0.04% with metastases. In propensity score adjusted regression among all patients, the interaction of PCI and gynecological cancer (vs. not having PCI) significantly reduced mortality (OR 0.53, 95%CI 0.36–0.77; $p = 0.001$) while increasing LOS (Beta 1.16 days, 95%CI 0.57–1.75; $p < 0.001$) and total cost (Beta $31,035.46, 95%CI 26758.86–35312.06; $p < 0.001$). Among gynecological cancer patients, mortality was significantly reduced by PCI (OR 0.58, 95%CI 0.39–0.85; $p = 0.006$) and being in East North Central, West North Central, South Atlantic, and Mountain regions (all $p < 0.03$) compared to New England. PCI reduced

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mortality but not significantly for metastatic patients (OR 0.74, 95%CI 0.32–1.71; $p = 0.481$). Eighteen extra gynecological cancer patients' lives were saved with PCI for a net national cost of $3.18 billion and a CER of $176.50 million per averted death.

Conclusion: This large propensity score analysis suggests that PCI may cost inefficiently reduce mortality for gynecological cancer patients, amid income and geographic disparities in outcomes.

Keywords: gynecologic malignancies, gynecological tumors, PCI, percutaneous coronary intervention, cardio oncology

INTRODUCTION

Cardiovascular disease (CVD) and cancer remain the two most common causes of mortality among non-communicable diseases in Western countries (1). The bidirectional relationship between the two, with cancer patients or survivors having a significant burden of CVD and patients with CVD posing an increase in cancer incidence, has become more evident over the last decade and is reflected by the heightened interest in the discipline of cardio-oncology (2, 3). Common risk factors such as tobacco use, poor diet, and chronic inflammatory state are implicated in both disease states (4). Cancer commonly induces a pro-thrombotic state, which can be compounded by side effects of surgical interventions, chemotherapy, radiotherapy, and immunotherapy (3, 5) and trigger cardiovascular events. The recent improvement in overall long-term survival of cancer patients (6), likely related to the progress in cancer therapies, has been paralleled by an increase in the number of percutaneous coronary interventions (PCI) performed in cancer patients (7). Knowing the prevalence of acute coronary syndrome (ACS) in the general population requiring PCI, CVD burden in these cancer patients appears to be vastly underestimated.

Treatment of ACS in cancer patients is challenging, as each type of cancer has a unique clinical presentation and underlying physiology that calls for personalized care. The primary organ site, stage, and presence of metastases are all modifying factors that can influence post-PCI outcomes. Historically this understanding has not been reflected in clinical practice, partly due to the exclusion of patients with cancer from cardiovascular clinical trials and vice versa (8–10). While there is now limited data exploring the overall prognostic impact of cancer on PCI outcomes (11–14), there is no data regarding PCI outcomes in gynecological cancer patients. Reported incidence of gynecologic malignancies in the U.S. is approximately 94,000 cases per year (15), with the most common malignancy being uterine cancer (26.82 cases per 100,000) and the least common vaginal cancer (0.66 per 100,000).

Gynecological cancer patients have special considerations when determining risk for ACS and potential intervention with PCI. Women with endometrial cancer, a population particularly characterized by significant rates of obesity and diabetes mellitus, have been found to have a 1.5-fold increased 10-year risk of CVD when compared to the general population (16). As many as 22% of endometrial cancer patients present at diagnosis with three or more risk factors of coronary artery disease (CAD) (16). Furthermore, death from CVD has been found to be more prevalent in patients with endometrial cancer (17). In women who have undergone debulking procedures for epithelial ovarian carcinoma, the highest risk for hospital readmission perioperatively is a cardiopulmonary event (18). Platinum-based chemotherapeutic agents are frequently utilized for the treatment of ovarian and cervical cancer and are associated with multiple cardiotoxic side effects, with such cardiotoxic drugs as anthracyclines (including doxorubicin and cyclophosphamide) being frequently used for recurrent ovarian cancer (19). While the safety of common cardiovascular interventions such as percutaneous coronary intervention (PCI) in gynecologic cancer patients is not well-described, coronary artery bypass grafting (CABG) is considered a relative contraindication in patients with cancer due to an increased risk of metastatic dissemination during extracorporeal circulation (20). To bridge this knowledge gap, we used a large contemporary national database and examined the outcomes and economics of revascularization procedures in patients with gynecologic malignancies, stratified by specific type of cancer and stage.

METHODS

We defined gynecologic cancer in this analysis as any cancer involving the female reproductive system and further classified it based on specific anatomic location, including cancers of the ovaries, cervix, uterus, vagina, and vulva.

Data Source

The data source for this study was the 2016 United States (U.S.) National Inpatient Sample (NIS) for hospital discharges, the largest all-payer inpatient dataset in the nation, sponsored by the U.S. Department of Health and Human Services' Agency for Healthcare Research and Quality and maintained within the Healthcare Cost and Utilization Project (HCUP). The NIS currently accounts for approximately 1 in 5 discharges from all community hospitals in the U.S. To reduce sampling bias, the sampling strategy has been modified in the most recent data to produce results more generalizable to all inpatient discharges in the country. In 2016, the NIS data coding adopted the International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM). Diagnoses of cancer and CVD were maded up to and including the index hospitalization period per patient based on the reported ICD-10. Cardiotoxic oncological treatment both prior and active were not reported in the dataset.

Study Design

This is the first nationally representative multicenter analysis of inpatient mortality and total cost among all eligible hospitalized adults with CAD by PCI (yes/no) and PCI and cancer (yes/no), including overall and by primary organ site. The 2016 NIS dataset was selected for this study as it is the among latest available datasets and the first to use ICD-10 coding and thus betterreflects current clinical trends in PCI use compared to prior available datasets. Study inclusion criteria was all NIS hospitalizations for adults age 18 years or older during 2016. This study used de-identified data and was conducted according to the ethical principles in the Declaration of Helsinki.

Subjects undergoing PCI were identified by the ICD-10 procedure codes of 00.66 (percutaneous transluminal coronary angioplasty), 36.06 [insertion of non-drug-eluting coronary artery stent(s)], or 36.07 [insertion of drug-eluting coronary artery stent(s)]. ICD-10 diagnosis were used to identify gynecological cancers: C540, C541, C542, C543, C548, C549, C55, D070, Z8542, C530, C531, C538, C539, D060, D061, D067, D069, R87610, R87611, R87612, R87613, R87614, Z8541, Z86001, C561, C562, C569, Z8543, C510, C511, C512, C518, C519, C52, C5700, C5701, C5702, C5710, C5711, C5712, C5720, C5721, C5722, C573, C574, C577, C578, C579, C58, D071, D072, D0730,D0739, R87620, R87621, R87622, R87623, R87624, Z8540, Z8544. ICD-10 codes were used to identify demographics, comorbidities, and outcomes. HCUP tools such as the Clinical Classification Software, which had been used prior to the NIS 2016 dataset for such purposes as classifying cancer (e.g., by primary type and current vs. historical), were not used in this study because they were found by HCUP as a beta version to be unreliable when applied to the 2016 dataset's ICD-10 data.

Bivariable Statistical Analysis

Descriptive statistics for demographics and comorbidities were performed for the full sample. Comorbidities were selected for analysis (and identified in the dataset by their ICD-10 scores) based on their clinical and/or statistical significance for similar studies in the existing literature. The comorbidities included in this study were diabetes, hypertension, peripheral vascular disease, hyperlipidemia, smoking, obesity, poor diet, stroke, congestive heart failure, cardiac arrest, myocardial infarction, cardiogenic shock, valvular disease, HIV, alcohol abuse, opioid abuse, anemia, chronic obstructive pulmonary disease, coagulopathy, depression, cirrhosis, chronic kidney disease, and malignancy (overall and by primary malignancy type).

Bivariable sub-group analysis was then conducted among gynecological malignancy patients according to the following: (a) inpatient all-cause mortality (yes/no); (b) PCI (yes/no) among the overall sample, stratified by metastases (yes/no) and in subgroup analyses among patients with malignancy; (c) PCI vessel number (multi- vs. single-vessel); (d) malignancy (yes/no) in subgroup analyses among patients who died with non-ST segment elevation myocardial infarction (NSTEMI) and separately among those with ST segment elevation myocardial infarction (STEMI); (e) length of stay by gynecological malignancy type; (f) total cost by gynecological malignancy type. For continuous variables, independent sample *t*-tests were performed to compare means and Wilcoxon rank sum tests were performed for medians. For categorical variables, Pearson chi square tests or Fisher exact tests were performed to compare proportions.

Regression Statistical Analysis

To optimize the likelihood of validated and replicable results, the performance of the final multivariable regression models in sub-group analysis among gynecological malignancy patients was first assessed by backward propagation neural network machine learning by accuracy and root mean squared error (RMSE) to ensure they were comparable based on an integrated hybrid methodology of traditional statistics reinforced by machine learning (21, 22). Variables found to be statistically significant in the bivariable analysis were included in forward and backward stepwise regression to augment decision-making on which variables should be included in the final multivariable regression models. This regression analysis adjusted for the PCI propensity score was conducted to assess the following outcomes: (a) inpatient all-cause mortality (by logistic) and (b) total hospital costs (by linear, adjusting with the additional variable of total all-cause length of stay) using the predictor of the interaction term between PCI and malignancy (to provide separate estimates of the associations of mortality and PCI, mortality and malignancy, and mortality with PCI and malignancy). The regression models separately assessed these outcomes according to the following major predictors: (a) historical or active malignancy (yes/no), and gynecological malignancy type (uterus, cervical, ovarian, other). Sub-group analysis without propensity score adjustment was conducted separately according to history of CAD (additionally with stratified analysis by ACS and active or prior malignancy), active malignancy, prior malignancy, presenting diagnosis of ACS, NSTEMI, unstable angina, UA), and STEMI. All models adjusted for age, race, income, geographic region, metastases, and mortality risk by diagnosis-related group (DRG). Other variables were excluded based upon the machine learning analysis and diagnostic testing to produce the most clinically and statistically justifiable models.

Next, machine learning-backed propensity score–adjusted multivariable regression was conducted for mortality and controlled for age, race, income, presence of metastases, and mortality risk by diagnosis-related group in addition to the likelihood of undergoing PCI and the NIS weights accounting for the cluster sample data structure. The propensity score was then created for the likelihood of undergoing PCI [the treatment, utilizing the same above variables used in the final regression model to given the double propensity score adjustment method (23–25)], balance was confirmed among blocks, and then the propensity score was included in the final regression models as an adjusted variable. This causal inference approach (propensity score adjustment) was selected because it is a widely accepted methodology to reduce but not eliminate selection bias and the effect of confounding variables. Such competing causal inference approaches as fixed, random, and mixed effects were not appropriate, though these have the added advantage of reducing unobserved variable bias,

because the dataset lacked adequate repeated hospitalizations from the same subjects. Propensity score adjustment was used rather than covariate adjustment without the propensity score to enable a more complicated propensity score model (i.e., able to test interactions and higher order terms to produce the best estimated probability of treatment assignment) without risking over-parameterizing while still permitting diagnostic analysis of the final models to be done to confirm superior performance to simple covariate adjustment without the propensity score. Finally, propensity score adjustment rather than competing propensity score techniques was used because of its superior performance in the appropriate context (confirmed by current statistical theory and adequate diagnostic quantitative testing of the final models in cardiovascular studies) (23, 24), and because its inclusion in the final regression models had sufficient performance confirmation the below diagnostic tests.

To modify the final models until optimal performance was achieved, performance was first assessed relative to results from backward propagation neural network machine learning to ensure comparability by root mean squared error and accuracy. Regression model performance was additionally assessed with correlation matrix, area under the curve, Hosmer-Lemeshow goodness-of-fit test, Akaike and Schwarz Bayesian information criterion, variance inflation factor, and tolerance, multicollinearity, and specification error.

The utility of this above hybrid analytic approach, which integrates the traditional statistical method of frequentist-based multivariable regression (supported by propensity score-based causal inference analysis) and supervised learning-based machine learning has been previously demonstrated, as causal inference results which are more familiar to medical science audiences can be confirmed and replicated automatically through machine learning (and thus may accelerate real-time findings on larger high-dimensional datasets as they already increasingly do for other economic sectors outside of medicine), while producing more rapid and accurate results compared to traditional statistics (25–30). An academic physician-data scientist and biostatistician (DJM) confirmed that the final regression models were sufficiently supported by the existing literature and clinical and statistical theory. Fully adjusted regression results were reported with 95% confidence intervals (CIs) with statistical significance set at a 2-tailed p-value of < 0.05.

Cost Effectiveness Analysis

Cost-effectiveness analysis was conducted according to the methodology detailed by the Centers for Disease Control and Prevention (cdc.gov/policy/polaris) and applied to PCI (intervention) vs. medical management alone (comparator): the net cost was calculated as the cost of implementation minuts the averted cost which then produced the ratio of net costs over change in health outcome or the cost-effectiveness ratio (CER), with a negative value in the ratio representing cost savings and a positive value indicating increased cost. The implementation cost was determined by the higher end of the cost of inpatient PCI taken from the National Cardiovascular Registry CathPCI Registry (31) and then multiplied by the number of procedures in the specified sub-group of cardio-oncology patients below in this study's principle dataset (NIS). The averted cost was determined by the 2016 World Bank average life expectancy (worldbank.org/world-development-indicators) minutes the average 2016 NIS age in this study multiplied by the 2016 Quality Adjusted Life Year ($50,000/year/patient) and the cases of mortality averted with the treatment vs. the comparator. The net national cost was calculated as the above implementation cost minus the averted cost. The CER was the above net national cost divided by the number of averted costs by the treatment vs. the comparator.

Software

Statistical analysis was performed with STATA 14.2 (STATACorp, College Station, Texas, USA), and machine learning analysis was performed with Java 9 (Oracle, Redwood Chores, California, USA).

RESULTS

Descriptive Statistics and Bivariable Analysis

Of the 30,195,722 hospitalized patients meeting criteria, 383,760 (1.3%) had gynecological cancer. Among those, mean age was 63.3 years (standard deviation [SD] 15.7), 73.53% were Caucasian, 38.07% had uterine cancer, 29.95% had cervical cancer, 29.51% had ovarian cancer, and 2.47% had other gynecological malignancy (**Table 1**). Out of the 383,760 patients with gynecological cancer, 7,215 (1.9%) underwent PCI; of those who underwent PCI, 2,875 (39.8%) had active malignancy and 460 (6.4%) had metastases. Significantly patients with gynecological cancer vs. those without it underwent PCI (1.88 vs. 4.04%, $p < 0.001$) even when matched by age and mortality risk as calculated by the NIS according to DRGs (2.35 vs. 5.52%, $p < 0.001$). Among patients receiving PCI, patients with vs. without gynecological were significantly less likely to have CAD (71.56 vs. 78.22%, $p < 0.001$) and presenting STEMI (10.24 vs. 15.09%, $p < 0.001$), but had comparable likelihood of diabetes, hypertension, and presenting NSTEMI.

A total of 794,147 (2.6%) deaths were recorded, out of which 20,807 (2.6%) were from gynecological malignancy (**Table 1**). Patients with gynecological cancer had significantly lower mortality when compared to non-gynecological cancer patients (2.30 vs. 4.54%, $p = 0.004$). Furthermore, in patients with gynecological malignancy, mortality (yes/no) was significantly lower for Caucasian (69.09 vs. 73.65%) but higher for African American patients (16.1 vs. 11.8%) (both $p < 0.001$) and those with metastases (54.5 vs. 22.6%, $p < 0.001$).

Among patients with gynecological malignancy, the median all-cause length of stay (LOS) was 3 days (range 2–6, $p < 0.001$) and median cost of hospitalization in U.S. dollars was 34,657 (18,894–62,952; $p < 0.001$). The highest mortality (yes/no) percentage was ovarian vs. non-ovarian gynecoloical malignancy (0.60 vs. 0.37%) followed by uterine vs. non-uterine (0.59 vs. 0.48%) (**Table 2**). The longest mean LOS was ovarian cancer (5.39 days [SD 5.57]), followed by other gynecological malignancy (5.25 days [SD 7.87]), and the most expensive total

TABLE 1 | Descriptive statistics and bivariable analysis by inpatient mortality (N = 383,760 admissions).

Variables	Sample	Inpatient mortality		*P*-value
		No (373,1695; 97.38%)	Yes (10,065; 2.62%)	
Demographics, No. (%)				
Age, years, mean (SD)	63.31 (15.68)	63.19 (15.71)	67.82 (13.82)	<0.001
Race				
All groups				<0.001
White	73.53	73.65	69.09	
Black	11.89	11.78	16.05	
Hispanic	8.99	9.02	7.89	
Asian	2.60	2.57	3.72	
Native American	0.46	0.46	0.52	
Other	2.54	2.53	2.73	
Non-white	26.47	26.35	30.91	<0.001
Income quartile				0.461
First	28.78	28.74	30.11	
Second	25.63	25.65	24.61	
Third	24.58	24.60	23.90	
Fourth	21.02	21.01	21.38	
Insurance				
Type				<0.001
Commercial	25.24	25.30	23.12	
Medicare	55.41	55.31	58.96	
Medicaid	15.01	15.11	11.38	
VA	1.95	1.89	4.49	
None	2.39	2.39	2.05	
Non-commercial	74.76	74.70	76.88	0.026
Admission, No. (%)				
Non-elective	73.00	72.55	89.44	<0.001
Weekend	18.42	18.26	24.64	<0.001
Medical history				
Diabetes	19.55	19.54	19.72	0.843
Hypertension	59.09	59.10	58.97	0.907
PVD	3.26	3.26	3.43	0.669
HLD	32.25	32.34	29.11	0.002
Obesity	18.70	18.86	12.67	<0.001
Smoking	1.40	1.42	0.60	0.002
Poor diet	0.13	0.13	0.05	0.320
CVA/TIA	3.05	2.95	6.66	<0.001
CHF	4.49	4.45	5.66	0.010
HFrEF	1.53	1.51	2.14	0.025
Exacerbation	4.15	4.08	6.51	<0.001
Cardiac Arrest	0.51	0.15	13.91	<0.001
Myocardial Infarction	1.92	1.82	5.86	<0.001
STEMI	0.31	0.26	2.19	<0.001
NSTEMI/UA	1.62	1.56	3.78	<0.001
Cardiogenic shock	0.17	0.11	2.48	<0.001
Valvular disease	4.83	4.82	5.02	0.686
HIV	0.32	0.32	0.20	0.335
Alcohol abuse	2.01	2.02	1.69	0.303
Opioid abuse	1.52	1.55	0.60	0.001
Anemia	29.90	29.61	40.64	<0.001
COPD	15.27	15.26	15.45	0.819
Coagulation disorder	6.93	6.62	18.48	<0.001
Depression	15.87	16.01	10.73	<0.001
Cirrhosis	1.71	1.68	2.98	<0.001

SD, standard deviation; VA, Veteran Affairs; PVD, peripheral vascular disease; HLD, hyperlipidemia; CVA, cerebrovascular disease; TIA, transient ischemia attack; CHF, congestive heart failure; HFrEF, heart failure with reduced ejection fraction; STEMI, ST segment elevation myocardial infarction, NSTEMI, non-ST segment elevation myocardial infarction; UA, unstable angina; HIV, human immunodeficiency virus; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft.

TABLE 2 | Summary bivariable outcome results by malignancy (N = 383,760 admissions).

Malignancy	Outcomes			
	Mortality, No. (%)*		LOS, days, mean (SD)**	Cost, USD, mean (SD)**
	No	Yes		
Gynecological	1.27	1.52	5.03 (5.71)	52925.20 (69153.44)
Uterus	0.48	0.59	4.99 (5.57)	53907.51 (69559.61)
Cervix	0.38	0.30	4.74 (5.51)	48644.10 (64795.88)
Ovarian	0.37	0.60	5.39 (5.57)	56708.13 (72440.59)
Other	0.09	0.07	5.25 (7.87)	52326.71 (67357.82)

*LOS, length of stay; SD, standard deviation; USD, US dollars; *$p < 0.05$ for mortality (yes vs. no); **$p < 0.05$ for malignancy (yes/no).*

hospitalization cost was ovarian (USD 56,708 [SD 72440.59]) followed by uterine (53907.51 [SD 69559.61]).

Multivariable Regression

In propensity score-adjusted regression among all patients, the interaction of PCI and gynecological cancer (vs. not having PCI) was associated with significantly reduced mortality (OR 0.53, 95%CI 0.36–0.77; $p = 0.001$; marginal effects likelihood: −0.87%). Among gynecological cancer patients, mortality was similarly significantly reduced by PCI (OR 0.58, 95%CI 0.39–0.86; $p = 0.007$) as well as hospitalization in East North Central, West North Central, South Atlantic, and Mountain regions (all $p < 0.05$) compared to New England. PCI reduced mortality but not significantly for patients with metastatic cancer (OR 0.74, 95%CI 0.31–1.75; $p = 0.493$) (**Table 3**). There were no significant racial or income disparities.

TABLE 3 | Machine learning–augmented propensity score adjusted multivariable regression of inpatient mortality among gynecological malignancy patients (N = 383,760 admissions).

Variable	OR (95% CI; *P*-value)
Age by 10 years	1.00 (0.99–1.00; $p = 0.136$)
Non-white race	**1.22 (1.08–1.36; $p = 0.001$)**
Region	
Mid-Atlantic	0.95 (0.73–1.24; $p = 0.715$)
East North Central	**0.73 (0.56–0.95; $p = 0.021$)**
West North Central	**0.71 (0.50–0.99; $p = 0.044$)**
South Atlantic	**0.75 (0.57–0.98; $p = 0.038$)**
East South Central	1.04 (0.73–1.48; $p = 0.830$)
West South Central	1.03 (0.76–1.36; $p = 0.851$)
Mountain	**0.61 (0.42–0.88; $p = 0.008$)**
Pacific	0.99 (0.76–1.29; $p = 0.935$)
Zip code income	
1st quartile	Reference
2nd quartile	0.94 (0.82–1.08; $p = 0.416$)
3rd quartile	0.89 (0.78–1.03; $p = 0.124$)
4th quartile	0.87 (0.75–1.02; $p = 0.086$)
PCI	**0.58 (0.39–0.86; $p = 0.007$)**
Malignancy	
Metastases	**2.03 (1.84–2.24; $p < 0.001$)**
Mortality risk by DRG	**7.12 (6.54–7.75; $p < 0.001$)**

OR, odds ratio; CI, confidence interval; PCI, percutaneous coronary intervention; DRG, diagnosis-related group. The bold values are statistically significant.

In sub-group analysis by individual gynecological malignancy type, PCI significantly decreased all-cause mortality for uterine cancer (OR 0.49, 95% CI 0.25–0.96; $p = 0.038$) but not ovarian, cervix, or other. In sub-group analysis by ACS (including separately NSTEMI/UA vs. STEMI) and active malignancy (yes/no) among gynecological malignancy patients, PCI reduced mortality for all sub-groups but only significantly for patients with non-ACS active malignancy patients (OR 0.37, 95% CI 0.15–0.89; $p = 0.027$) and NSTEMI/UA prior malignancy patients (OR 0.19, 95%CI 0.05–0.72; $p = 0.014$) (**Figure 1**). In sub-group analysis by gynecology cancer by primary organ site and cancer status (without metastasis, with metastasis, and historical diagnosis all vs. no cancer), the highest mortality reductions with PCI were for patients with ovarian metastasis (**Figure 2**).

Cost Effectiveness

In propensity score adjusted regression among all patients, the interaction of PCI and gynecological cancer (vs. not having PCI) significantly increased LOS (Beta 1.16 days, 95% CI 0.57–1.75; $p < 0.001$) and total cost of stay (Beta $31035.46, 95% CI 26758.86–35312.06; $p < 0.001$). Of the 7,215 gynecological cancer patients who underwent inpatient PCI, 0.25% or 18 extra gynecological cancer patients' lives were saved with PCI for a net national cost of $3.18 billion and a cost effectiveness ratio (CER) of $176.50 million per averted death.

DISCUSSION

Our study demonstrated that inpatient PCI can be safely performed in patients with gynecological cancer, including those with metastatic disease, albeit with increased cost and length of stay amid significant geographic disparities in mortality. This is the first known nationally representative, comprehensive machine learning-augmented, propensity score analysis of mortality and cost for patients with gynecological cancer vs. non-gynecological cancer patients in terms of PCI vs. medical management (including overall and by ACS).

Our analysis reveals that PCI does not increase mortality in patients with gynecologic cancer, regardless of the unique risks in this population. When analyzed by specific type of malignancy, PCI significantly reduced mortality for uterine cancer, while ovarian, cervical, and other gynecologic cancers had a non-statistically significant reduction in mortality. This may at least be in part because patients with uterine cancer in contrast to the other gynecological cancers in this dataset had greater CVD risk factors (i.e. older with higher prevalence of hypertension and diabetes) and thus may be positioned to best benefit from PCI. The lack of increased mortality rate across all cancer types is likely not just statistical in nature and could suggest that routine/standard of care PCI if applied to this patient population would not translate in a significant increase in mortality.

Furthermore, this analysis shows that even when patients with gynecological vs. non-gynecological cancer have comparable age and mortality risk, they undergo PCI significantly less than patients without this cancer type, suggesting that inpatient PCI may be withheld from these patients (further research is required to clarify the reasons why which likely are multifactorial and can include lower clinical suspicion or more non-specific symptoms for CVD given typically younger age and less CVD risk factors). This finding was consistent across a wide range of age and mortality risk groups. While PCI may be offered less to cancer patients due to concerns of safety and efficacy, previous literature indicates that PCI is safe and beneficial in such population (11–14), and our real-world analysis shows PCI is safe to perform in gynecological cancer patients as well. The results presented here should promote the inclusion of patients with gynecological cancer undergoing cancer treatment and with acceptable medium- and long-term survival (least 6 months and preferably 1 year expected >50% survival) in future cardiovascular trials and encourage physicians to more frequently utilize PCI in this patient population.

Other factors worth considering in future analyses are the type of stent used and medication used in gynecologic cancer patients. Standard balloon angioplasty or percutaneous old balloon angioplasty (POBA) has been shown to have overall worse outcomes compared to drug-eluting stents in the general population, and was considered a possible option in gynecologic cancer patients as the reduced duration of aspirin and Plavix or dual antiplatelet therapy (DAPT) with POBA may be beneficial to patients with an increased bleeding risk (32). Evolution of stent platforms, polymers and eluting medications over the last decade has translated in an abbreviated DAPT course, for certain indications (stable angina, abnormal stress

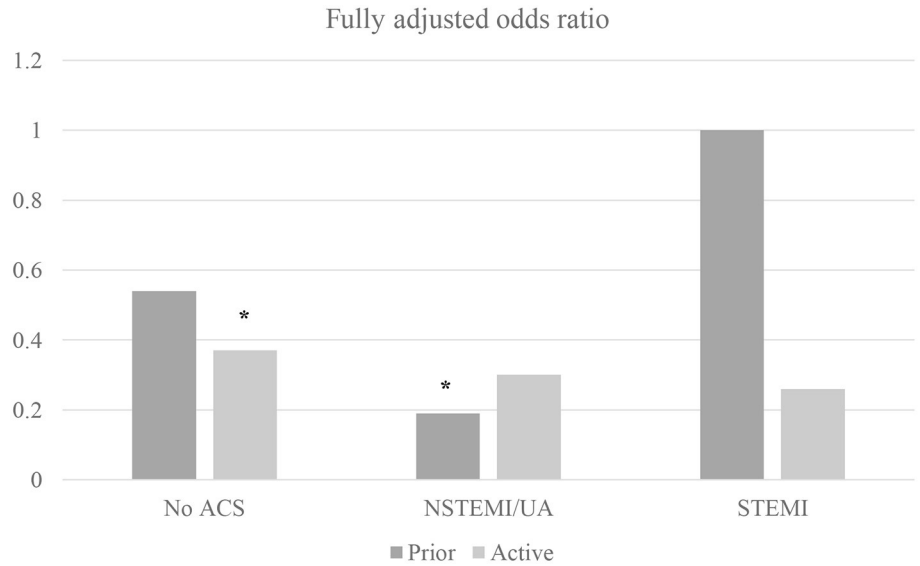

FIGURE 1 | Machine learning–augmented propensity score adjusted multivariable regression of inpatient mortality among gynecological malignancy patients (N = 383,760 admissions). Multivariable regression fully adjusted for age, race, income, metastases, and mortality risk by Diagnosis Related Group; NSTEMI/UA, non-ST elevation myocardial infarction/unstable angina; STEMI, ST-elevation myocardial infarction; *$p < 0.05$.

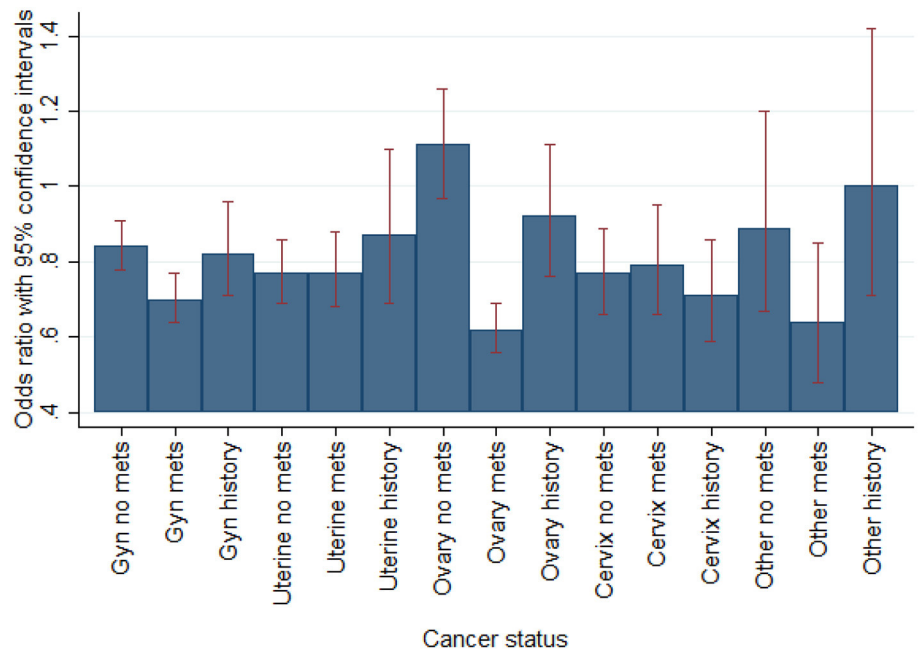

FIGURE 2 | Multivariable regression of mortality by gynecological oncology status vs. no cancer (N = 383,760 admissions). Fully adjusted for age, race, income, region, PCI, PCI likelihood, and NIS-calculated mortality risk by DRG.

test) several stent have been approved for 1–3 months of DAPT. Patients with metastatic disease would require additional stratification that impacts decision making in these complex clinical challenges.

Our results should be interpreted with caution in the context their limitations, which include a non-randomized design with administrative data limited to inpatient variables without longitudinal individual follow-up data, particularly 3-month and 12-month mortality which can affect cost-effectiveness analysis. This study sought to overcome such limitations on its external and internal validity by utilizing multicenter nationally representative data with robust causal inference analysis to allow for the most reliable and reproducible results possible for this nuanced clinical topic.

CONCLUSION

This study provides evidence that the clinical benefit of PCI may be safely extended to gynecological cancer patients, albeit with an increase in cost. There is also evidence of mortality disparity by geography and PCI underutilization in gynecological cancer patients despite clinical indication. This first known granular sub-group analysis by malignancy type, and active vs. prior cancer status suggests PCI significantly decreases mortality by type of gynecological cancer.

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

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

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BRIEF RESEARCH REPORT
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BRCA1/2 Mutations and Cardiovascular Function in Breast Cancer Survivors

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Objective: Animal models suggest that *BRCA1/2* mutations increase doxorubicin-induced cardiotoxicity risk but data in humans are limited. We aimed to determine whether germline *BRCA1/2* mutations are associated with cardiac dysfunction in breast cancer survivors.

Methods: In a single-center cross-sectional study, stage I-III breast cancer survivors were enrolled according to three groups: (1) *BRCA1/2* mutation carriers treated with doxorubicin; (2) *BRCA1/2* mutation non-carriers treated with doxorubicin; and (3) *BRCA1/2* mutation carriers treated with non-doxorubicin cancer therapy. In age-adjusted analysis, core-lab quantitated measures of echocardiography-derived cardiac function and cardiopulmonary exercise testing (CPET) were compared across the groups. A complementary *in vitro* study was performed to assess the impact of *BRCA1* loss of function on human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) survival following doxorubicin exposure.

Results: Sixty-seven women with mean (standard deviation) age of 50 (11) years were included. Age-adjusted left ventricular ejection fraction (LVEF) was lower in participants receiving doxorubicin regardless of *BRCA1/2* mutation status ($p = 0.03$). In doxorubicin-treated *BRCA1/2* mutation carriers and non-carriers, LVEF was lower by 5.4% (95% CI; −9.3, −1.5) and 4.8% (95% CI; −9.1, −0.5), respectively compared to carriers without doxorubicin exposure. No significant differences in VO_{2max} were observed across the three groups ($p_{overall} = 0.07$). Doxorubicin caused a dose-dependent reduction in viability of iPSC-CMs *in vitro* without differences between *BRCA1* mutant and wild type controls ($p > 0.05$).

Conclusions: *BRCA1/2* mutation status was not associated with differences in measures of cardiovascular function or fitness. Our findings do not support a role for increased cardiotoxicity risk with *BRCA1/2* mutations in women with breast cancer.

Keywords: anthracycline, *BRCA1/2*, breast cancer, cardiomyocyte, heart failure, HER2 therapy

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INTRODUCTION

BRCA1/2 genes play a critical role in multiple cellular processes governing genome stability including DNA repair. In addition to suppressing tumor growth, *BRCA1/2* genes may play a role in the maintenance of cardiomyocyte survival and function (1). In animal models, loss of cardiomyocyte-specific *BRCA1/2* is associated with DNA damage, apoptosis, cardiac dysfunction, and cardiac mortality following doxorubicin exposure (1, 2). *BRCA1/2* genes may potentially mitigate against anthracycline-induced genotoxic stress and cardiomyocyte apoptosis and thus serve a cardioprotective role. However, whether these preclinical findings translate to humans is unclear (3–5).

In a single-center, cross-sectional study, we investigated differences in cardiac function and cardiopulmonary fitness through comprehensive phenotyping of breast cancer survivors with and without *BRCA1/2* mutations. Furthermore, we performed an *in vitro* study using human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to assess the impact of *BRCA1* loss on cardiomyocyte survival following doxorubicin exposure.

METHODS

Study Population

The Genetics and Heart Health After Cancer Therapy (Gene-HEART) study (NCT03510689) evaluated stage I-III breast cancer survivors older than 18 years old treated at the University of Pennsylvania Abramson Cancer Center (Philadelphia, Pennsylvania). Three groups of breast cancer survivors were enrolled at least ∼12 months after initiation of chemotherapy. These included: (1) *BRCA1/2* mutation carriers treated with 240 mg/m^2 of doxorubicin; (2) *BRCA1/2* mutation non-carriers treated with 240 mg/m^2 of doxorubicin; and (3) *BRCA1/2* mutation carriers treated with non-doxorubicin cancer therapy. Exclusion criteria included stage IV disease, genetic testing confirming a variant of unknown significance or benign polymorphism in *BRCA1/2* genes, contraindications to VO_2 testing, or pregnancy. The study was approved by the University of Pennsylvania Institutional Review Board, and all participants provided written informed consent.

Echocardiography Quantitation

Participants underwent comprehensive phenotyping with echocardiography-derived measures of systolic and diastolic cardiac function (TomTec Imaging Systems platform, Unterschleissheim, Germany). Quantitative echocardiography was performed by a single blinded observer at the University of Pennsylvania Center for Quantitative Echocardiography (Philadelphia, PA). Intra-observer coefficients of variation were 4.5, 9.0, and 9.7% for LVEF, longitudinal strain, and circumferential strain, respectively, and 4–5% for mitral inflow and tissue Doppler velocities. The absolute values of longitudinal and circumferential strain are presented, whereby a greater absolute value represents improved function.

TABLE 1 | Baseline characteristics according to exposure group.

Baseline characteristics	*BRCA1/2* Carriers, doxorubicin (*n* = 39)	*BRCA1/2* Carriers, no doxorubicin (*n* = 14)	*BRCA1/2* Non-carriers, doxorubicin (*n* = 14)
Age at study enrollment (years)	46.0 (10.1)	57.0 (10.1)	54.6 (7.9)
Race			
White	30 (76.9)	14 (100)	14 (100)
Black	4 (10.3)	0 (0)	0 (0)
Asian	2 (5.1)	0 (0)	0 (0)
Unknown	3 (7.7)	0 (0)	0 (0)
Years from breast cancer diagnosis	5 (3, 8)	7 (6, 7)	4 (3, 6)
Breast cancer stage			
I	11 (28.9)	10 (71.4)	3 (21.4)
II/III	27 (71.1)	4 (28.6)	11 (78.5)
Disease site			
Left	18 (46.1)	6 (42.9)	8 (61.5)
Right	20 (51.3)	8 (57.1)	5 (38.5)
Lymph nodes only	1 (2.6)	0 (0)	0 (0)
HER2 status			
Positive	2 (5.1)	1 (7.7)	3 (21.4)
ER status			
Positive	19 (48.7)	11 (84.6)	9 (64.3)
PR status			
Positive	20 (51.3)	11 (84.6)	8 (57.1)
Triple negative breast cancer	18 (46.2)	1 (7.7)	4 (28.6)
Trastuzumab with or without pertuzumab	3 (7.7)	0 (0)	3 (21.4)
Tamoxifen	11 (29.3)	7 (53.8)	3 (21.4)
Aromatase inhibitors	18 (47.4)	9 (69.2)	6 (42.9)
Radiation therapy	18 (51.4)	5 (35.7)	9 (69.2)
Mastectomy	29 (78.4)	11 (84.6)	7 (50.0)
Bilateral salpingo-oophorectomy	28 (75.5)	12 (85.7)	2 (15.4)
Body mass index (Kg/m^2)	27.4 (5.7)	26.4 (5.1)	24.4 (2.8)
Systolic blood pressure (mmHg)	118.6 (3.6)	117.7 (17.8)	116.5 (11.1)
Current or past smoking	12 (30.8)	3 (23.1)	6 (42.8)
Diabetes mellitus	1 (2.6)	0 (0)	1 (7.1)
Hypertension	8 (20.5)	2 (14.3)	2 (14.3)
Hyperlipidemia	10 (25.6)	4 (28.6)	3 (21.4)
ACEI/ARBs or Beta-blockers	4 (10.3)	2 (16.7)	2 (14.3)
Statins	6 (15.4)	2 (16.7)	1 (7.1)

ACE, Angiotensin converting enzyme; ARB, Angiotensin receptor blocker.
For baseline characteristics, categorical variables are summarized using count (proportion); age, body mass index and systolic blood pressure are summarized using mean (standard deviation); Years from diagnosis is summarized using median (Q1, Q3).

Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing (CPET) was performed based on the modified Bruce protocol with continuous measurement of breath-by-breath gas sampling oxygen consumption (VO_2) using a calibrated metabolic cart (ParvoMedics TrueOne® 2400, Sandy, UT).

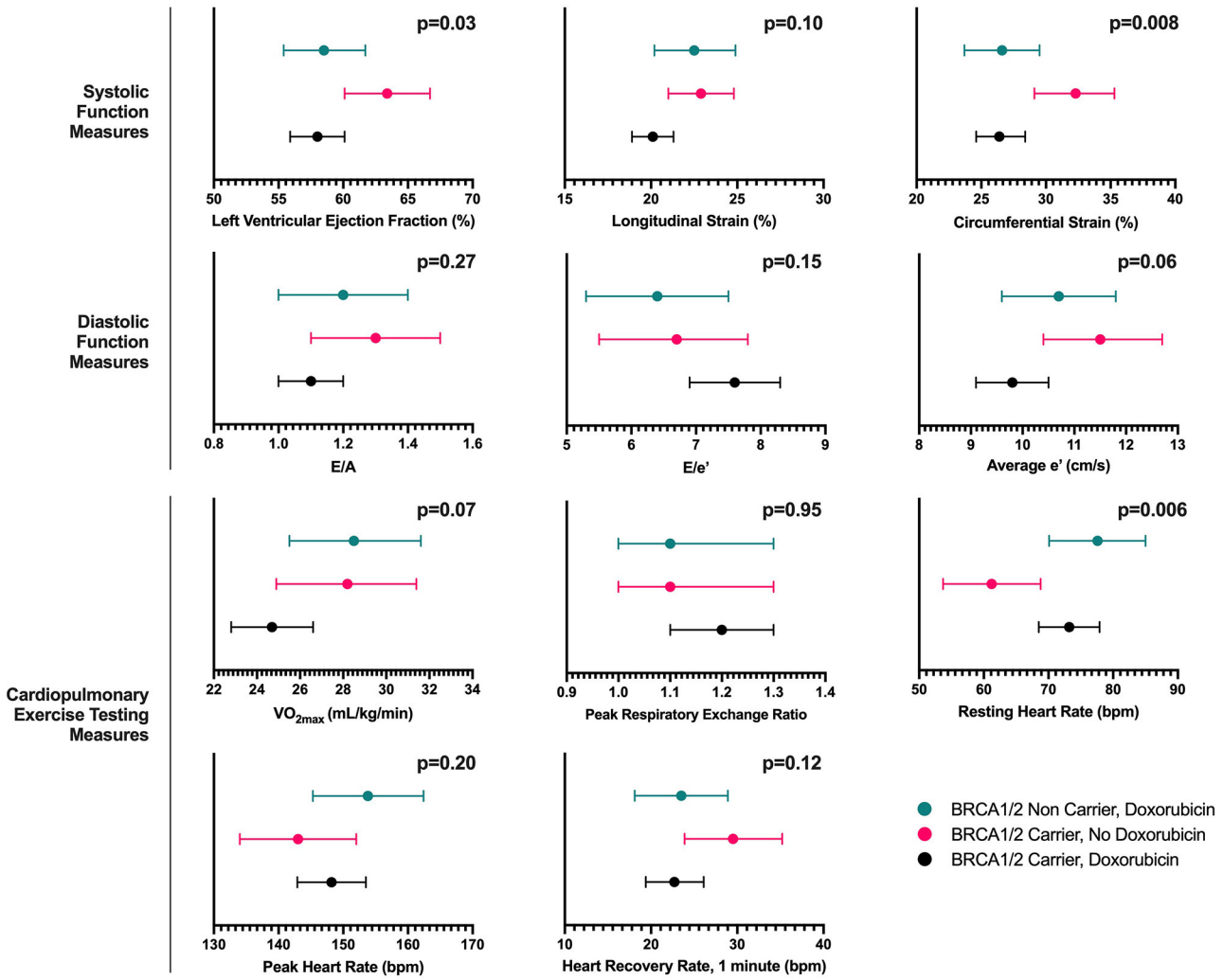

FIGURE 1 | Age-adjusted marginal mean (95% Confidence Interval) estimates of echocardiography and cardiopulmonary exercise testing measures according to exposure group. The figure presents the age-adjusted marginal mean (95% confidence interval) estimates based on analysis of covariance for measures of systolic function, diastolic function and cardiopulmonary exercise testing according to exposure group including (a) *BRCA1/2* mutation carriers exposed to doxorubicin, (b) *BRCA1/2* mutation carriers not exposed to doxorubicin, and (c) *BRCA1/2* mutation non-carriers exposed to doxorubicin. For longitudinal and circumferential strain, absolute values are presented, where by a higher value represents greater function.

Statistical Analysis

Baseline characteristics were summarized according to exposure group using proportions for categorical variables while mean (standard deviation [SD]) and median (quartile 1 [Q1], quartile 3 [Q3]) were utilized for normally and non-normally distributed continuous variables, respectively. In cross-sectional analysis, measures of cardiac function and cardiopulmonary fitness were compared across the three groups. Age-adjusted marginal means and their respective 95% confidence intervals (CI) were estimated for each parameter, and group differences were tested using analysis of covariance. We performed sensitivity analysis by excluding HER2-positive breast cancer participants who received trastuzumab to determine the potential effect of targeted cardiotoxic cancer therapy. Statistical significance was evaluated at a two-sided alpha level of 5%. Analyses were performed using R 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

Experimental Design

For the *in vitro* study, a premature stop codon was introduced via CRISPR-Cas9 into one *BRCA1* allele in a healthy donor-derived iPSC line (**Supplementary Figure 1**). Cells which were transfected but not mutated were retained as wild type controls. *BRCA1* mutant and wild type iPSCs were differentiated into cardiomyocytes (iPSC-CMs) using an established protocol (6). At 25 days post-differentiation, cardiomyocytes received varying concentrations of doxorubicin (1–500 nM). Cell viability was assessed using alamarBlue Cell Viability Reagent.

RESULTS

The mean (SD) age of the 67 breast cancer survivors in the study cohort was 50 (11) years, 87% were White and 64% had stage II/III disease. The median (Q1, Q3) time

from diagnosis at enrollment was 6 (3, 7) years. **Table 1** summarizes baseline characteristics according to *BRCA1/2* status and doxorubicin exposure.

Participants were assessed at a median (Q1, Q3) of 4 (2, 6) years after completion of chemotherapy. The age-adjusted left ventricular ejection fraction (LVEF) was significantly lower in participants treated with doxorubicin, regardless of *BRCA1/2* mutation status ($p = 0.03$). In doxorubicin-treated *BRCA1/2* mutation carriers and non-carriers, estimated differences were lower by 5.4% (95% CI; −9.3, −1.5) and 4.8% (95% CI; −9.1, −0.5), respectively, compared to carriers without doxorubicin exposure. These findings were consistent across additional cardiac function measures including circumferential and longitudinal strain, although less pronounced for the latter. There were no differences in diastolic function measures E/A, e', and E/e' (**Figure 1**, **Supplementary Table 1**). These findings remained consistent in a sensitivity analysis excluding 6 participants who had received HER2-targeted therapy (**Supplementary Table 2**).

Among CPET measures, the age-adjusted resting heart rate was significantly higher in the doxorubicin-treated groups regardless of *BRCA1/2* status. However, we did not find significant differences across the three groups in VO_{2max}, peak heart rate or peak respiratory exchange ratio (**Figure 1**, **Supplementary Table 1**). Similar findings were observed in a sensitivity analysis excluding participants who received HER2-targeted therapy (**Supplementary Table 2**). We also performed additional sensitivity analysis comparing echocardiography and CPET measures across the groups using a non-parametric test (i.e., Kruskal-Wallis test) and the findings were largely similar.

In vitro, doxorubicin caused a dose-dependent reduction in cell viability with no differences between *BRCA1* mutant and wild type iPSC-CMs ($p>0.05$). Estimates of cell viability (doxorubicin concentration) in *BRCA1* mutant compared with wild type iPSC-CMs were 97.3 vs. 92.4% (1 nM), 91.9 vs. 96.7% (10 nM), 36.0 vs. 34.0% (50 nM), 4.4 vs. 4.1% (100 nM), and 4.1 vs. 4.1% (500 nM) (**Figure 2**).

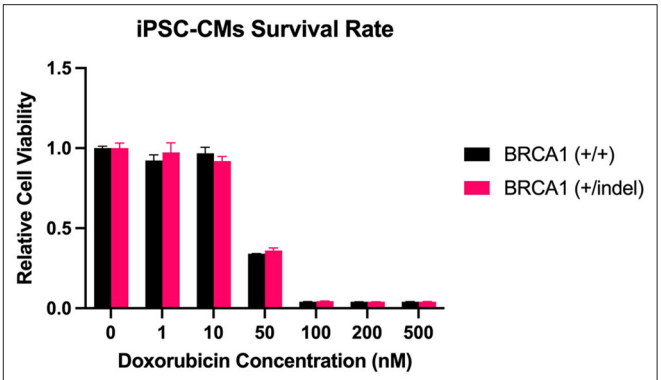

FIGURE 2 | *BRCA1* mutation and cardiomyocyte cell viability following doxorubicin. The figure presents comparisons of cell viability between *BRCA1* mutant [*BRCA1* (+/indel)] and wild type [*BRCA1* (+/+)] human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) following exposure to 1–500 nM doxorubicin concentration.

DISCUSSION

Overall, our results suggest that women with breast cancer who have *BRCA1/2* mutations are not at increased risk of anthracycline-induced cardiotoxicity relative to those with sporadic breast cancer. This is based on several lines of evidence. First, although we observed significantly lower left ventricular systolic function in breast cancer survivors treated with doxorubicin compared to those without doxorubicin exposure, we did not find differences in age-adjusted estimates of echocardiography-derived measures of systolic or diastolic dysfunction according to germline *BRCA1/2* mutation status. Second, there were no significant differences in cardiopulmonary fitness measures as determined by CPET based on *BRCA1/2* status. Third, complementary *in vitro* experiments showed a comparable dose-dependent reduction in cell viability in both loss of function *BRCA1* mutant and wild type iPSC-CMs receiving doxorubicin.

BRCA1/2 mutations may be associated with increased risk of doxorubicin cardiotoxicity, but human data are limited. One prior exploratory study of 401 patients, including *232 BRCA1* and 159 *BRCA2* mutation carriers, showed an increased risk of heart failure based on self-reported symptoms elicited on an anonymous survey, relative to historical controls drawn from the general population (3). In this study, however, the authors were unable to verify reported symptoms using objective confirmatory data such as echocardiogram reports in most participants, and there was no direct comparator control group. In contrast, two other studies found no significant differences between rates of cardiomyopathy in *BRCA1/2* mutation carriers vs. wild type controls receiving anthracyclines, though each had limitations (4, 5). One prospective study was underpowered to assess for differences in cardiac dysfunction between groups, excluded participants with hypertension or those who received trastuzumab, and did not demonstrate expected LVEF declines among *BRCA1/2* mutation carriers receiving anthracyclines (4). A second retrospective study only evaluated the incidence of either asymptomatic decline in LVEF to <50% or heart failure and lacked detailed assessment of subclinical measures of cardiovascular function (5). Only a minority of participants included in the study underwent follow-up LVEF assessment after completion of anthracycline therapy limiting the ability to detect asymptomatic declines in cardiac function. Our study fills an important evidence gap by comprehensively characterizing cardiac function using both quantitative echocardiography and CPET and performing complementary *in vitro* experiments using iPSC-CMs.

Our human data contrast with the results of murine studies, where loss of *BRCA1/2* in cardiomyocytes was associated with worse cardiac function and increased mortality following doxorubicin exposure (1, 2). There are several possible explanations for this. First, significant differences exist in the physiology of human and murine cardiomyocytes including calcium cycling, expression of ion channels, energetics, and myofilament composition (7). Second, cardiomyocyte specific

BRCA1/2 knockouts in mice are biologically distinct from inherited germline *BRCA1/2* mutations in humans. Third, mice used in preclinical studies were either exclusively male or the sex was not disclosed and administered relatively higher anthracycline doses compared to standard chemotherapy dosing regimens, potentially contributing to discrepancies in results (1, 2).

Our study has limitations. Though the study is one of the few studies to date to assess the impact of *BRCA1/2* mutations on detailed measures of cardiac function in breast cancer patients receiving anthracyclines, statistical power was limited due to sample size. Our analyses were adjusted for age alone given the relatively small sample size, and confounding remains possible. Furthermore, limitations related to unequal group sizes should be considered. Our *in vitro* experiments do not incorporate hemodynamic or neurohormonal stressors inherent to *in vivo* studies, which may diminish observed differences, particularly with respect to *BRCA1* status (8). In addition, we focused on cell viability in the *in vitro* study, and other measures related to iPSC-CM structure and function were not evaluated.

In conclusion, we present both detailed phenotypic characterization of cardiac function, including echocardiography and CPET, in breast cancer survivors with and without *BRCA1/2* mutations treated with anthracyclines, and *in vitro* characterization using anthracycline-treated, wild type vs. gene-modified human iPSC-CMs with a loss of function mutation in *BRCA1*. Overall, we found no strong evidence to support associations between *BRCA1/2* mutations and anthracycline-induced cardiac dysfunction based on echocardiography, CPET or *in vitro* data. Our study fills an important evidence gap and adds support to the lack of increased cardiotoxicity risk in breast cancer patients with *BRCA1/2* mutations.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Pennsylvania Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BK, PS, and AS contributed to conception and design of the study. BK, AS, KS, and KMS contributed to clinical data collection. WL, CM-R, BK, and KM contributed to the design and execution of the *in vitro* study. BD and BK performed statistical analysis. BD, NW, and BK wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REVIEW
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Etiology and Management of Dyslipidemia in Patients With Cancer

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Patients with cancer are now living longer than ever before due to the growth and expansion of highly effective antineoplastic therapies. Many of these patients face additional health challenges, of which cardiovascular disease (CVD) is the leading contributor to morbidity and mortality. CVD and cancer share common biological mechanisms and risk factors, including lipid abnormalities. A better understanding of the relationship between lipid metabolism and cancer can reveal strategies for cancer prevention and CVD risk reduction. Several anticancer treatments adversely affect lipid levels, increasing triglycerides and/or LDL-cholesterol. The traditional CVD risk assessment tools do not include cancer-specific parameters and may underestimate the true long-term CVD risk in this patient population. Statins are the mainstay of therapy in both primary and secondary CVD prevention. The role of non-statin therapies, including ezetimibe, PCSK9 inhibitors, bempedoic acid and icosapent ethyl in the management of lipid disorders in patients with cancer remains largely unknown. A contemporary cancer patient needs a personalized comprehensive cardiovascular assessment, management of lipid abnormalities, and prevention of late CVD to achieve optimal overall outcomes.

Keywords: dyslipidemia, cholesterol, cancer, cardiovascular risk reduction, cancer survivor

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INTRODUCTION

The development of highly effective anticancer therapies over the past few decades has favorably changed the landscape of patients with cancer, who can now achieve high cure rates in early stages of disease and long-term remission in others (1). This oncologic progress, however, has generated a unique patient population who are at a high risk of experiencing a myriad of chronic comorbidities, among which CVD is one of the most important (2). Cancer and CVD share several common risk factors, including advanced age, chronic inflammation, obesity, hyperlipidemia, poor diet, smoking history, and physical inactivity (3, 4). A multi-disciplinary team comprising of primary care, oncology, pharmacy, and cardio-oncology is best poised to serve this special cohort of patients who often pose challenging diagnostic and management dilemmas (5).

Dyslipidemia has been associated with poor outcomes in patients with cancer by promoting tumor invasion and metastasis (6), producing resistance to cancer drugs (7), and enhancing the cardiac and vascular toxicity of anticancer therapies (8). In this review, we discuss the emerging literature on the relationship between lipid abnormalities and carcinogenesis, review anticancer treatment-associated hyperlipidemia, discuss CVD risk assessment and risk reduction in patients with cancer, and highlight the current evidence to support the use of antilipidemic agents in this special patient population.

HYPERLIPIDEMIA, METABOLIC SYNDROME, AND CANCER

It is well known that dyslipidemia is a strong predictor of CVD (9, 10). Emerging data suggest that hyperlipidemia may also play a role in carcinogenesis (11). Tumor cells have been shown to require large amounts of sterol metabolites to sustain rapid growth and proliferation (12, 13). A key regulatory transcriptional factor in lipid synthesis and uptake, sterol regulatory element-binding protein (SREBP), has been identified to be dysregulated in various cancer types to accelerate endogenous cholesterol and fatty acid production (14, 15). Another mechanism reported in prostate cancer is reduced cholesterol efflux through ABCA1 (ATP-binding cassette class A) transporters (16). Additional pathways connecting cholesterol and cancer are phosphatidylinositol 3-kinase (PI3-K)/Akt pathways that are part of hedgehog signaling, which when dysregulated can lead to abnormal cell proliferation and tumor growth (11).

Higher levels of saturated and monounsaturated phospholipids in cell membranes have been shown to protect cancer cells from oxidative damage (17). Lipids also serve an important role in cell signaling and migration, as well as post-translational modification of proteins (18, 19). Angiogenesis, a hallmark of cancer, occurs through the secretion of prostaglandin E2, a sterol compound in breast cancer cells (18, 20). All of these functions highlight the importance of lipids in oncogenesis and tumor spread.

Hyperlipidemia is a common comorbidity among cancer patients and survivors. Ray and Husain demonstrated that patients with breast cancer had significant elevations in plasma total cholesterol (TC), low density lipoprotein (LDL)-cholesterol, and triglyceride (TG) levels (21). Shah et al. reported similar findings in patients with breast cancer when compared to patients with benign breast disease (22). In a large cross-sectional study, there was a significant difference in the lipid profiles among different types of cancers (23). Patients with ovarian cancer were observed to have the highest serum TG levels, while those with colorectal cancer had the lowest TG (23). Breast cancer patients had the highest TC and LDL levels, while gastric cancer patients had the lowest values (23). Interestingly, serum LDL levels greater than 110 mg/dL correlated with lymphatic metastasis (23).

Not only hyperlipidemia but also metabolic syndrome (MetS) has been associated with the development of cancer (24). Within the United States, nearly 33% of all adults and about 50% of adults older than 60 have MetS (25). In a systematic review and meta-analysis of 43 studies including 38,940 cancer cases, metabolic syndrome was found to be associated with an increased risk of several cancers including colorectal, liver, pancreas, endometrial, and postmenopausal breast cancers (26). Survivors of childhood cancer (e.g., acute lymphoblastic leukemia) were observed to have roughly two-fold higher prevalence of MetS compared with general adult population (27). Obesity, a key component of MetS, has also been identified as a risk factor for developing cancer (28, 29).

A significant association between MetS and all-cause cancer mortality has been documented in a prospective study, where MetS was associated with a 56% greater age-adjusted risk for cancer mortality (30). Women with breast cancer and MetS had a higher incidence of partial response to therapy, and high blood sugar levels were predictive of a poor response to therapy (31). The American Society of Clinical Oncology has identified obesity as one of the most important determinants of cancer mortality (28, 29).

Statins may play a role in reducing the risk of cancer development and/or progression. Lochhead et al. described the benefits of statin therapy for colorectal cancer patients (32). Patients who used statins for more than 3 years prior to their colorectal cancer diagnosis had a lower tumor stage, lower prevalence of metastasis, and higher five-year cancer-specific survival compared with statin non-users (32). There is also preclinical evidence that statins may directly block the adhesion and migration processes of cancer cells, supporting the anti-carcinogenic potential of statins (33). Anti-angiogenic effect of statins has also been reported in patients with chronic liver disease (34). Statins have been shown to induce apoptosis of hepatoma cells, inhibit intrahepatic angiogenesis, and interfere with tumor cell adhesion in hepatocellular carcinoma (34). A meta-analysis of 26 studies found that long-term statin use may reduce the risk of pancreatic cancer incidence (35). Ahern et al. reviewed the basic science and epidemiologic evidence that statins, particularly simvastatin, may reduce the risk of breast cancer recurrence. They described the broad range of existing literature that supports the anticancer effects of statins and the protective effect of statins on breast cancer prognosis (36).

ANTICANCER THERAPIES THAT HAVE THE POTENTIAL TO CAUSE DYSLIPIDEMIA

Various drugs used in cancer therapy have been associated with lipid abnormalities, either due to chemotherapy-related gonadal failure or as a direct adverse effect of the medication (**Table 1**). The National Cancer Institute (NCI) classifies the severity of hypertriglyceridemia and hypercholesterolemia resulting from cancer drugs as categorized in **Table 2** (37).

DYSLIPIDEMIA FROM GONADAL FAILURE

Various combinations of anticancer agents can lead to gonadal failure. Tian et al. examined lipid levels of over 800 patients with early-stage breast cancer in a retrospective study, during and after neoadjuvant or adjuvant chemotherapy and compared them to those of patients who underwent surgery-only therapy without any chemotherapy (38). They found that in individuals receiving chemotherapy, the serum TC, LDL and TG levels increased significantly during chemotherapy treatment, but returned to pre-chemotherapy range about 6 months after completion of therapy (38). There were no differences between the groups receiving different combination of chemotherapy regimens. In a subgroup analysis, it was noted that younger premenopausal women were more prone to dyslipidemia while

TABLE 1 | List of anticancer therapies associated with dyslipidemia, their adverse effects on lipid profile, and the proposed mechanisms of dyslipidemia.

Anticancer therapy	Effects on lipid panel	Proposed mechanism of dyslipidemia
Androgen deprivation therapy	↑ TC, ↑ LDL	Gonadal failure (32, 33)
Antiestrogen therapy	↑ TC	Unknown
Anthracycline	↑ LDL, ↓ HDL	Downregulates PPAR gamma nuclear receptors and decreases apo A1 levels (38)
Tyrosine kinase inhibitors	↑ TG	Unknown
Lorlatinib (ALK TKI)	↑ TC, ↑ TG	Unknown
mTOR inhibitor	↑ TC, ↑ TG	Increases apo CIII, suppressing LPL activity and reduces clearance of VLDL (45)
VEGF Inhibitor	↑ TG	Unknown
L- asparaginase	↑ TG	Increases apo CIII and decreases apo CII, inhibits activity of LPL (51)
JAK 1/2 inhibitor	↑ TC, ↑ LDL, ↑ TG	Unknown
Bexarotene	↑ TC, ↑ TG	Unknown
Capecitabine	↑ TG	Unknown

TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; apo CIII, apolipoprotein CIII; apo CII, apolipoprotein CII. ↑ indicates "increases the level"; ↓ indicates "decreases the level".

receiving chemotherapy (38). A similar study also demonstrated that premenopausal women had greater alterations in their lipid panel compared to post-menopausal women (39). This difference could be attributed to changes in lipid levels from a sudden drop in estrogen secondary to chemotherapy-induced ovarian failure (39).

Similarly, in a retrospective analysis, patients with metastatic testicular cancer receiving cisplatin-based chemotherapy were noted to have an increase in TC and LDL levels, along with increased subcutaneous fat deposition and insulin resistance (40). It was also noted that the serum estradiol level was increased in these patients which could contribute to partial hypogonadism, which in turn would affect fat and glucose metabolism (40).

ANDROGEN DEPRIVATION THERAPY (ADT)

ADT, including gonadotropin releasing hormones (GnRH) agonists and antagonists, is the mainstay of treatment for prostate cancer (41). They inhibit the production of endogenous testosterone, causing various metabolic effects (41).

GnRH agonists (leuprolide, gosarelin) stimulate the GnRH receptor continuously, resulting in downregulation of the receptor with reduction in luteinizing hormone (LH) and subsequently testosterone levels. In contrast, GnRH antagonists (degarelix) block the same receptors and reduce the release of LH, which in turn reduces the production of testosterone (42). Anti-androgen medications like bicalutaminde and flutamide block the androgen receptors and inhibit dihydrotestosterone (DHT) from binding to it. Abiraterone acetate is an oral agent that blocks testosterone production by inhibiting the cytochrome P enzyme, CYP17 (42). ADT can cause significant changes in lipid profiles.

In a prospective study by Torimoto et al., 39 patients with prostate cancer on ADT were followed for 12 months while on therapy, with serial monitoring of their body composition and lipid levels (43). There was consistent elevation of TC and LDL levels documented throughout the year on ADT (43). Similar findings were reported by Salvador and colleagues during a 6-month follow up in patients on ADT for prostate cancer (44). They also observed no difference in the lipid profile abnormality among patients receiving GnRH agonists or bicalutamide (44). Grossman and Zajac suggested that patients receiving ADT should have a fasting lipid profile checked prior to initiation of therapy and have serial monitoring of lipid levels every 6 months (45). The American Heart Association, American Cancer Society, and American Urological Association released a scientific advisory recommending that patients have interval follow-up within 3-6 months of ADT initiation to monitor blood pressure, lipid profile, and glucose levels (46).

TABLE 2 | National Cancer Institute (NCI) grading of hypertriglyceridemia and hypercholesterolemia secondary to anti-neoplastic agents.

Severity of adverse event	Hypertriglyceridemia	Hypercholesterolemia
Grade 1	150–300 mg/dL	>ULN–300 mg/dL
Grade 2	300–500 mg/dL	300–400 mg/dL
Grade 3	500–1000 mg/dL	400–500 mg/dL
Grade 4	>1000mg/dL	>500 mg/dL
Grade 5	Death	Death

ANTIESTROGEN THERAPY

Antiestrogen therapies are primarily used in patients with estrogen receptor positive breast cancer. Tamoxifen is a selective estrogen receptor modulator that binds to estrogen receptors on tumors and suppresses effects of estrogen in the tumor (47). Aromatase inhibitors (AIs), such as anastrazole, letrozole, and exemestane, are selective nonsteroidal aromatase inhibitor that prevent the conversion of androstenedione to estrone and testosterone to estradiol. They are used to treat postmenopausal women with hormone-receptor positive breast cancer. These medications can reduce the tumor mass and delay cancer progression (47).

AIs, but not tamoxifen, have been associated with an increased risk of lipid abnormalities and cardiovascular (CV)

events (48). In a meta-analysis by Amir et al., patients on AIs were found to have significantly higher odds of being diagnosed with hypercholesterolemia and CVD when compared with those on tamoxifen (48). Additionally, studies on mice have demonstrated that AIs can directly affect the endothelium and predispose to the development of atherosclerosis, findings which were also illustrated as attenuation of endothelial function in human studies (49). To date, there are no official recommendations for the management of hyperlipidemia secondary to antiestrogen therapies.

ANTHRACYCLINES

Doxorubicin has been associated with hyperlipidemia secondary to ovarian failure. However, anecdotal evidence suggests that anthracyclines could also directly affect lipid metabolism (50). Sharma et al. longitudinally followed patients with newly diagnosed breast cancer undergoing treatment with four cycles of doxorubicin and cyclophosphamide (+/- 5-fluorouracil), followed by treatments of paclitaxel and analyzed their serial serum lipid profiles. A continual increase in LDL and decrease in HDL were documented throughout the duration of therapy. *In vitro* analysis showed that doxorubicin downregulated PPAR gamma nuclear receptors and decreased apoA1 levels, which possibly reduced the production of HDL in the liver. Long-term follow up of cholesterol levels was not performed to assess for any permanent effects on lipid metabolism (50). There are no official recommendations for the management of dyslipidemia in patients receiving anthracycline treatment.

TYROSINE KINASE INHIBITORS (TKI)

Dyslipidemia has been mentioned as a possible side effect of TKI (51). Anlotinib is a TKI targeting vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet derived growth factor receptor (PDGFR), stem cell factor receptor (c-Kit), and Ret (52), and is used in the treatment of advanced non-small cell lung cancer (NSCLC) (53).

Early phase clinical trials showed a higher incidence of HTG (41 vs. 23.8%) and hypercholesterolemia (41.8 vs. 14%) in the anlotinib arm than the control arm (52, 53). The time of onset of HTG in the anlotinib group was around 20 days. Most patients were treated with fibrates to lower their triglycerides, and very few needed dose reductions of anlotinib. None required drug discontinuation because of HTG (52, 53). The mechanism for this dyslipidemia is not known.

LORLATINIB

This third-generation TKI targets anaplastic lymphoma kinase (ALK) gene with activity against NSCLC demonstrating resistant ALK mutations (54). In the phase two trial of lorlatinib in patients with NSCLC, the most common adverse effect was hypercholesterolemia (81%) and HTG (60%) with grades 3 and 4 severity of both observed in about 15% of patients (55). The median time to onset of hyperlipidemia from treatment initiation was 15 days (55). All of the 81% of patients were started on a lipid-lowering agent. In patients with grade four hypercholesterolemia, the dose of lorlatinib was held until the cholesterol level decreased to grade two severity, and then successfully reinitiated (55).

The Canadian Cardiovascular Society (CCS) recommends checking a lipid profile at baseline, 1, 2 and 3 months after starting lorlatinib and every 3 months thereafter. They also recommend starting lipid-lowering therapy when LDL is >3.5 mmol/L (∼135 mg/dL) with a goal to reduce LDL level by 50% or <2.0mmol/L (77 mg/dL). They recommend withholding lorlatinib if the total cholesterol level is above 12.92 mmol/L (∼500 mg/dL), until the levels decrease. The lipid-lowering therapies recommended were pravastatin or rosuvastatin as first-line therapy and ezetimibe for second-line therapy (54). Similar first-line therapy was recommended for isolated HTG (∼500 mg/dL). They also recommend holding the medication if the TG level exceeds 11.4 mmol/L (∼1,000 mg/dL). Fenofibrate or omega-3 fatty acids could be utilized as second-line therapy (54).

MECHANISTIC TARGET OF RAPAMYCIN (MTOR) INHIBITORS

mTOR inhibitors (e.g., sirolimus) inhibit signaling in the phosphoinositide 3 kinase (PI3K) – Akt-mTOR pathway, which plays a key role in tumor growth and lipid metabolism. While it is a useful anti-cancer therapy and anti-rejection treatment for transplant recipients, inhibition of this pathway leads to reduced clearance of LDL in the blood causing hyperlipidemia (56).

Dyslipidemia with sirolimus use usually begins 2–4 weeks after starting therapy (57, 58). In a retrospective study of renal transplant patients on immunosuppressive regimen including sirolimus, a significant increase in TG levels and a moderate increase in the total cholesterol levels was documented (57). Morrisett et al. demonstrated return of cholesterol levels to normal within 8 weeks after discontinuation of sirolimus (58). It is hypothesized that sirolimus inhibits heparin-induced lipoprotein lipase (LPL) activity resulting in increase of apo-CIII levels, which suppresses LPL activity, hence reducing the clearance of VLDL particles (57).

Given the high incidence of this adverse effect, it is recommended to check lipid panels at baseline and then serially at every cycle for patients on mTOR inhibitors. Some experts recommend checking a fasting lipid panel weekly in early phase trials (59). It is also recommended to start statins in the first month of therapy if the patient has elevated total cholesterol or triglyceride levels (57). Lipid-lowering therapy is typically started with a goal to keep fasting TG <300 mg/dL and LDL <190 mg/dL. For patients started on lipid-lowering medication, a lipid panel should be rechecked with each cycle of therapy (59).

VEGF/VEGFR INHIBITOR

VEGF/VEGFR inhibitors lead to dyslipidemia by interfering with the mTOR pathway (60). A meta-analysis revealed that

patients on VEGF/VEGFR inhibitors had a higher incidence of hyperlipidemia (41%) compared to placebo (60).

Tivozanib is a VEGFR inhibitor used in patients with renal cell carcinoma (61). In the phase Ib trial of Tivozanib among patients with renal cell cancer, 30% of the recipients of Tivozanib had elevated TG levels with a grade 3/4 degree of HTG documented in up to 11% of the patients. These patients were on a relatively higher dose of tivozanib compared to other patients suggesting a possible dose-related association with HTG (61). The management strategy recommended for Tivozanib related hyperlipidemia is similar to that for mTOR inhibitors (61).

L-ASPARAGINASE

L-asparaginase is used in the treatment of acute lymphoblastic leukemia in children with a well-known adverse effect of lipid abnormalities (62). Parsons et al. serially examined fasting lipid and lipoprotein levels in 38 patients diagnosed with ALL before, during and after asparaginase therapy. Nineteen percent of (7/38) patients had an elevation of TG level to higher than 1000 mg/dL that reverted back to normal at the end of 2 years following therapy (62). Further lipoprotein subclass analysis revealed a significant increase in VLDL levels from 30.5 mg/dL to 396 mg/dL during asparaginase therapy (62). The proposed mechanism is inhibition of LPL, increase in apo-CIII and decrease in apo-CII levels which all lead to an increase in serum TG-rich lipoproteins in the plasma. The onset of HTG is usually 8–14 days after asparaginase therapy (63).

It is recommended that TG levels should be checked in all patients prior to asparaginase therapy. Initiation of early conservative treatment with fibrates can prevent further increase in TG levels and reduce the risk of future complications, such as pancreatitis and sagittal sinus thrombosis (64).

JAK1/2 INHIBITOR

Ruxolitinib is an oral JAK1 and JAK2 inhibitor approved for treatment of myelofibrosis (MF) and polycythemia (PV) (65). The COMFORT -I study demonstrating the efficacy of ruxolitinib in MF also showed an increase in TC and LDL levels (66). Anecdotal reports have also described HTG manifesting as steatohepatitis and pancreatitis in patients treated with ruxolitinib (65, 67). It is recommended to monitor lipid levels after starting ruxolitinib, particularly if given in combination with sirolimus for graft-vs. host disease (65).

BEXAROTENE

This retinoid compound is used in the treatment of patients with refractory cutaneous T-cell lymphoma (68). HTG within 2–4 weeks of starting therapy is a known adverse effect of bexarotene seen in up to 70% of patients secondary to a rise in the production of VLDL (69). The HTG and elevated TC levels are often reversible with discontinuation of therapy. Patients are recommended to have a baseline fasting lipid panel checked prior to starting bexarotene and thereafter be checked weekly for 2–4 weeks. If stable, it can then be checked every 8 weeks. The goal is to maintain fasting triglycerides around ∼400 mg/dL. If triglyceride levels rise above 400 mg/dL, it is recommended to consider starting lipid lowering therapy like statins, and if necessary, reduce the dose or interrupt bexarotene (68).

CAPECITABINE

Capecitabine is a prodrug of 5-fluorouracil (5-FU) commonly used in patients with breast and colon cancer. Multiple case reports of capecitabine-induced HTG exist in the literature (70). Dumana et al. described the case of a patient with breast cancer on capecitabine who developed HTG with levels > 9,000 mg/dL requiring lipid apheresis (71). Following discontinuation of capecitabine, the lipid levels normalized with eventual discontinuation of lipid lowering therapy (71). It has been hypothesized that this HTG may be more prominent in patients with hereditary LPL deficiency (70).

MANAGEMENT OF DYSLIPIDEMIA IN PATIENTS WITH CANCER

The initial steps for the treatment of dyslipidemia, metabolic syndrome and obesity which are highly prevalent in patients with cancer are the promotion of lifestyle changes, including modification of diet and addition of an exercise routine. A diet that emphasizes consumption of fruits, legumes, nuts, whole grains, and fish is recommended. A heart healthy diet should avoid saturated and trans fats, high sodium intake, processed meats, refined carbohydrates, and sweetened beverages (72). The ACC/AHA 2019 guidelines also recommend that adults exercise at least 150 min of moderate-intensity physical activity or 75 min of vigorous-intensity aerobic physical activity per week. All adults should decrease sedentary behavior to reduce ASCVD risk (72).

Current guidelines recommend the use of statin therapy for the primary prevention of CVD in patients with LDL>190 mg/dL, diabetes mellitus, or elevated 10-year atherosclerotic cardiovascular disease (ASCVD) risk score in patients without diabetes mellitus (73). In addition, statins are recommended for all patients with established ASCVD for secondary prevention. Patients with an intermediate (7.5% to <20%) and high (>20%) 10-year ASCVD risk scores should be considered for moderate- and high- intensity statin therapy, respectively, in addition to lifestyle changes (73). The Canadian Cardiovascular Society (CCS) updated their guidelines in 2021 to propose similar recommendations to the ACC/AHA with the key difference being that they recommend risk stratification using the Framingham Risk Score (74). The Childhood Cancer Survivor Study (CCSS) developed a risk assessment tool that predicts the risk of heart failure, ischemic heart disease, and stroke by age 50 among survivors of childhood cancer (75).

The traditional CVD risk assessment tools, such as the ACC/AHA Risk Estimator/ Pooled Cohort Equation or the Framingham Risk Score, do not include cancer-specific parameters or history of cancer treatment and thus may underestimate the true long-term CVD risk in cancer survivors. A population-based cohort study showed an increase in the medium and long-term risks of CV diseases (including heart

TABLE 3 | List of the risk stratification tools currently available to identify patients with cancer who are at increased risk of developing late atherosclerotic CVD.

Tools to predict atherosclerotic CVD risk in patients with cancer
American College of Cardiology/American Heart Association ASCVD Risk Estimator/ Pooled Cohort Equation
Framingham risk score
Childhood Cancer Survivor Study Cardiovascular Risk Calculator
Coronary artery calcium scoring
Lipoprotein(a), apolipoprotein B, high sensitivity C-reactive protein

failure, coronary artery disease, arrhythmia, stroke, and venous thromboembolism) in the survivors of various adult cancers compared with the general population (76). The increased risks were most pronounced in individuals who had exposure to chemotherapy. Multiple myeloma, lung cancer, non-Hodgkin lymphoma, and breast cancer were associated with significantly higher CVD risk compared with noncancer controls. The increased risk was most pronounced in cancer survivors with two or more CV risk factors (77).

Coronary artery calcium (CAC) scoring may provide additional risk stratification in patients with cancer (78). In a population-based cohort study that evaluated 484 patients undergoing low-dose CT for lung cancer screening, higher CAC scores were associated with an increased risk of CAD (78). The CAC Consortium developed an equation to calculate the risk of death from CVD vs. from cancer (79). They found that the mortality risk from CVD exceeded that from cancer at age 50 if the CAC score is >115 and at age 70 if the CAC sore is > 570 (79). These studies suggest the utility of CAC scoring in identifying individuals with cancer who can benefit from early preventative measures.

To further refine the prediction of CVD risk in the cancer patient, additional measures such as advanced lipid markers (lipoprotein(a), apolipoprotein B) and inflammatory markers (hs-CRP) may be of benefit. **Table 3** summarizes the current tools available for cardiovascular risk stratification in cancer patients. Further research is needed to elucidate which tools are the most predictive of CVD risk in this population.

STATINS

Recommendations guiding the management of hyperlipidemia in patients who are actively undergoing, or have recently completed, cancer treatment are largely lacking. The treatment of hyperlipidemia, and the primary and secondary prevention of CVD, in patients with cancer largely follow the framework proposed by the 2019 ACC/AHA guidelines (72). The management of hypercholesterolemia focuses on lifestyle modification initially, then on the addition of statin therapy, followed by consideration of other medications, such as ezetimibe and proprotein convertase subtilisin/kexin type 9 serine protease (PCSK9) inhibitors. Given the unique characteristics of patients with cancer, including their exposure to potentially cardiotoxic cancer treatment, future research is imperative to determine the ideal strategies to reduce their long-term CV risk.

Statins have been found to have pleiotropic effects, including antioxidant, anti-inflammatory, and immunomodulatory effects, as well as atherosclerotic plaque stabilization (80). They may also have anticancer effects, as discussed in the "Hyperlipidemia, metabolic Syndrome, and cancer" section above. These pleiotropic effects support the importance of statin therapy in this patient population, in which further studies investigating its potential benefits are warranted.

EZETIMIBE

Ezetimibe is the current second line therapy for hyperlipidemia. The Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study investigated the effects of combination ezetimibe/simvastatin compared with placebo on the effects of CV events (81). Initial analyses raised concerns about ezetimibe having potential carcinogenic properties. Further sub-analyses dispelled this hypothesis and found that ezetimibe does not significantly increase the risk of cancer or overall mortality (81). Meta-analyses have demonstrated that ezetimibe has beneficial effects on CVD endpoints, including myocardial infarction and stroke, without increasing all-cause or CV mortality, nor cancer development (82, 83). The addition of ezetimibe to statin therapy has been shown to cause a greater LDL reduction than doubling the statin dose (84). Given its overall benefits and safety, ezetimibe should be the ideal second antilipidemic agent of choice for individuals with cancer who have increased CV risk.

OTHER ANTILIPIDEMIC AGENTS

PCSK9 inhibitors are novel cholesterol-lowering agents that act by attaching to the LDL receptor, reducing its degradation and thus increasing LDL clearance (85). Although the data supporting the use of PCSK9 inhibitors primarily as antilipidemic agent in patients with cancer is limited, preliminary data suggests that it may potentially assist anti-cancer therapy by boosting the effect of immunotherapy by upregulating the MHC-I expression and promoting intratumoral T-cell infiltration making the tumor more responsive to immunotherapy (86). More studies are needed to analyze its lipid-lowering activity in this specific subset of patients.

There is scant data on the use of new lipid-lowering therapies like bempedoic acid in patients with cancer. It (8-hydroxy-2,2,14,14- tetramethylpentadecanedioic acid) is a small molecule that inhibits ATP citrate lyase, a crucial step in the synthesis of cytosolic acetyl – CoA, which is the building block in cholesterol biosynthesis (87). Currently, it serves as an alternative lipid-lowering treatment in patients intolerable of frontline agents (88).

Icosapent ethyl is another newer agent that acts by reducing hepatic production of TG. There is limited data on the use of this medication in patients with cancer. The REDUCE-IT trial, which demonstrated the CV benefits of icosapent ethyl in patients with elevated TG levels, excluded patients on tamoxifen, cyclophosphamide, and patients with life expectancy of <2

TABLE 4 | Special considerations for the use of lipid-lowering therapy in patients with cancer.

	Special considerations and recommendations for patients with cancer
Drug-drug interactions	• Nilotinib and ribociclib are considered moderate inhibitors of CYP3A4; lorlatinib and pexidartinib are moderate inducers of CYP3A4. • Avoid statins metabolized by CYP3A4 (simvastatin, lovastatin, and atorvastatin). • Consider replacing with safer alternatives (e.g., pravastatin or rosuvastatin). • Collaborate with Pharmacy and Hematology-Oncology for a multi-disciplinary approach. • Check pharmacy references or websites for drug-drug interactions prior to prescription.
Cancer patients with liver disease	• Pravastatin, rosuvastatin, or pitavastatin are not metabolized by the liver. • Studies have found lovastatin to be safe for patients with known liver disease.
Potential side effects of lipid-lowering therapy	• Statins: hepatotoxicity, rhabdomyolysis, immune-mediated necrotizing myopathy, myalgias • Ezetimibe: hepatocellular injury, rhabdomyolysis, myopathy, myalgias, erythema multiforme, anaphylaxis, angioedema • PCSK9 inhibitors: local site reactions • Bempedoic acid: dose-related hyperuricemia, rare tendon rupture • Icosapent ethyl: increased risk of bleeding, atrial fibrillation, and atrial flutter

TABLE 5 | Future areas of investigation for mitigating cardiovascular risk in patients with cancer.

Areas of future investigation in the management of hyperlipidemia and CV risk reduction in patients with cancer
1. What is the best CV risk assessment tool to identify those patients with cancer who are at an elevated risk for developing late CVD?
2. What is the role of coronary artery calcium scoring in the CV risk stratification of patients with cancer?
3. What is the utility of serum markers (lipoprotein(a), apolipoprotein B, high sensitivity CRP) in these patients?
4. What is the role of non-statin therapies, including ezetimibe, PCSK9 inhibitors, bempedoic acid and icosapent ethyl, in the management of dyslipidemia in cancer patients?

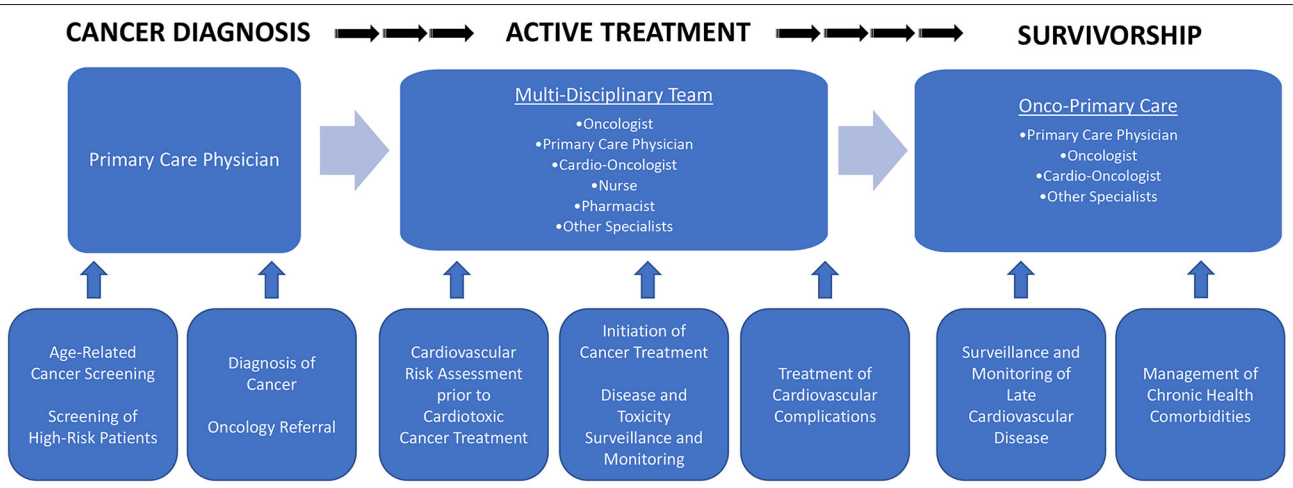

FIGURE 1 | The medical journey of cancer survivors.

years (89). Further research is needed to better define the role of non-statin therapies, including ezetimibe, PCSK9 inhibitors, bempedoic acid and icosapent ethyl, in the management of dyslipidemia in cancer patients.

SPECIAL CONSIDERATIONS FOR THE USE OF LIPID-LOWERING THERAPY IN PATIENTS WITH CANCER

There are special considerations to make when initiating patients on lipid-lowering therapy that are receiving active chemotherapy (**Table 4**). These include potential drug-drug interactions between dyslipidemia medications and chemotherapy, patients with liver disease, and patients that suffer adverse reactions from dyslipidemia medications.

DRUG-DRUG INTERACTIONS

Some statins are metabolized and cleared by the liver, predisposing potential drug-drug interactions. Simvastatin, lovastatin, and atorvastatin are metabolized by cytochrome p450 3A4 (CYP3A4). Thus, they can interact with other medications that are metabolized by CYP3A4, e.g., antibiotics, antivirals,

antiepileptics, calcium channel blockers, and antineoplastic agents (47). Most cases of drug interactions are reported with simvastatin likely arising from competitive effect of anti-cancer drugs on CYP3A4, resulting in hepatotoxity and rhabdomyolysis from augmentation of simvastatin through decreased clearance (90). Nilotinib and ribociclib are considered moderate inhibitors of CYP3A4; lorlatinib and pexidartinib are moderate inducers of CYP3A4 (47). In patients taking TKIs such as imatinib and dasatinib, or mitotane (used in adrenal carcinoma), hepatically metabolized statins should either be tapered to the safest tolerable dose or discontinued and replaced by safer alternatives (e.g., pravastatin or rosuvastatin) (91). Awareness of potential drug-drug interactions is critical in managing patients with cancer. Collaboration with a pharmacist and/or oncologist is important. Pharmacy references or websites that check for drug-drug interactions should be utilized prior to initiating antilipidemic medications in patients who are receiving anticancer drugs, particularly the novel targeted agents.

CANCER PATIENTS WITH LIVER DISEASE

As mentioned previously, statins are the cornerstone therapy for ASCVD risk reduction. However, myopathy and hepatotoxicity are its known adverse effects (92). This is especially concerning among patients with cancer and liver disease. Pravastatin, rosuvastatin, and pitavastatin are not metabolized by the liver and can be used for this special subset of patients (93). Statin-induced liver injury has primarily been observed with atorvastatin and simvastatin. Studies have found lovastatin to have no increased risk of hepatotoxicity in patients with known liver disease (93). Statins have pleiotropic effects, including potential inhibitory effect on the progression of liver fibrosis to cirrhosis and hepatocellular carcinoma (93). Thus, statins that are not metabolized by the liver can be safely used in patients with concomitant cancer and liver disease.

POTENTIAL ADVERSE REACTIONS OF LIPID-LOWERING THERAPY

Statins are generally well-tolerated medications. In addition to hepatotoxicity, rhabdomyoloysis, immune-mediated necrotizing myopathy, and myalgias are other known adverse reactions. It is important to monitor liver function tests and test for rhabdomyolysis if patients complain of myalgias. Less serious adverse reactions to statins include nasopharyngitis and diarrhea (47). Ezetimibe is also associated with hepatocellular injury, rhabdomyolysis, myopathy, and myalgias. Postmarketing studies have also found erythema multiforme, anaphylaxis, and angioedema associated with its use (47). PCSK9 inhibitors are well-tolerated with local injection site reactions, e.g., erythema, pain, or bruising, reported as the most common adverse reaction (47). Bempedoic acid is known to have a dose-related hyperuricemic effect and rarely associated with tendon rupture (47). Icosapent ethyl has been associated with an increased risk of bleeding and increased risk of developing atrial fibrillation and atrial flutter (47).

CONCLUSION

The burden of CVD is exceedingly high in patients with cancer because of a high prevalence of underlying risk factors, such as hyperlipidemia, hypertension, diabetes mellitus, and metabolic syndrome. In addition, anticancer therapies may exert cardio- and vasculo- toxic effects as well as adverse effects on lipid levels. It is essential for medical providers to be aware of these side effects and promptly institute appropriate therapy as well as other CV preventive strategies. Developing CV risk assessment tools that accurately identify cancer patients who are at an increased risk of CVD is needed. Coronary artery calcium scoring and serum markers can potentially aid with risk stratification and deserve further investigation to understand their utility in patients with cancer (**Table 5**). Statins are the mainstay of therapy in both primary and secondary CVD prevention as well as in the management of hyperlipidemia. The role for non-statin therapies for dyslipidemia management also need further investigation as they may contribute to overall CVD risk reduction (**Table 5**).

In summary, optimal cardiovascular care of the contemporary cancer patient requires a multidisciplinary approach to accurately define CVD risk, institute appropriate preventive measures, and address the potential adverse cardiometabolic effects of anticancer therapies. An integrative team comprised of primary care, oncology, cardio-oncology, nursing, and pharmacology devoted to the comprehensive and longitudinal care of patients from cancer diagnosis to treatment to survivorship is needed (**Figure 1**). This team of providers plays an integral role in cancer screening and diagnosis, monitoring for potential adverse events during cancer treatment, and management of chronic health comorbidities in survivorship (94). Understanding the myriad possible early and late side effects of cancer treatment is critical to improve overall morbidity and mortality of cancer survivors.

AUTHOR CONTRIBUTIONS

AK, MDJ, and TM made substantial contribution to the article design and conception of the work. MDJ, TM, MS, and JT contributed to the acquisition of data and drafting of the manuscript. AK and MDJ made major contributions to analysis, interpretation and editing of the manuscript, made critical revisions, and all authors read and approved the final manuscript.

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Intravenous Leiomyomatosis Complicated by Arteriovenous Fistula: Case Series and Literature Review

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Background: Uterine intravenous leiomyomatosis (IVL), a rare type of uterine leiomyoma, is defined by the intravascular proliferation of a histologically benign smooth muscle cell tumor. Pelvic arteriovenous fistula (AVF) is a rare vascular malformation that is most commonly congenital, post-traumatic, or iatrogenic. The link between leiomyomatosis and AVF has received little attention in the medical literature.

Results: We provide a case series of seven patients, four of whom were from our center, who had IVL complicated by a pelvic AVF. The symptoms of right heart failure were noted as swelling in the abdomen and two legs as well as a significant amount of ascites. Coil embolization of AVFs may be beneficial in minimizing bleeding during IVL surgery. A review of all accessible literature published on IVLs from 2000 to 2020 was conducted, and data were retrieved from 78 papers totaling 262 cases. Complications and recurrence were associated with pelvic mass excision and intravascular remnant tumor, respectively.

Conclusion: Intravenous leiomyomatosis combined with AVF aggravates congestion symptoms of surrounding organs. It is worth noting the uncommon combination of AVF and IVL, stressing the importance of a thorough assessment and surgical approach in IVL treatment.

Keywords: arteriovenous fistula (AVF), vascular surgery, coil embolization, pelvic mass, intravascular leiomyomatosis (IVL)

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INTRODUCTION

Uterine intravenous leiomyomatosis (IVL), a rare neoplasm defined by the intravascular proliferation of a histologically benign smooth muscle cell tumor, is an uncommon growth pattern of uterine leiomyoma (1). The clinical course varies depending on the severity of the condition. Vascular smooth muscle tumors can spread into the major veins and even the right atrium of the heart, obstructing blood flow and causing death (2–5).

Appropriate imaging examinations are required since IVLs could be misdiagnosed as intravascular thrombus, myxoma, or pancreatic tumors (6–8). Enhanced CT imaging can show the location, size, and full-scale extension pathway of IVL lesions, and it can be utilized in preoperative assessment (9). MRI could help with an accurate diagnosis, which is critical for deciding

on a surgical plan and achieving a positive outcome (6). Echocardiography can be used to assess extension into the right atrium (10).

Pelvic arteriovenous fistula (AVF) is a rare vascular malformation that is most commonly congenital, post-traumatic, or iatrogenic (11, 12). Massive AV shunting causes high output cardiac failure. This malformation is hard to eradicate completely because of a high recurrence rate. Because of the significant hemorrhage, surgical resection is often challenging. Although transcatheter embolization has recently become the treatment of choice for pelvic AVF, full embolization to stop the shunt flow is equally challenging (11–13). The link between leiomyomatosis and AVF has only been mentioned rarely in the medical literature. Three definite cases have been recorded since the first description in 1993 (14–16). We provide four further examples of fistula associated with leiomyomatosis, review the literature on the topic, and speculate on possible pathophysiological causes for the cooccurrence.

METHODS

For the literature review, PubMed, Embase, CNKI, and WanFang were utilized to conduct systematic searches of peer-reviewed literature published between 2000 and 2020. IBM SPSS was used to conduct the statistical analysis (IBM SPSS 26.0, SPSS Inc). For qualitative variables, Fisher's exact test and Pearson's chi-square test were utilized. All tests of statistical significance were two-sided, with $p < 0.05$.

RESULTS

Case Series

A total of seven patients, four of whom were admitted to Peking Union Medical College Hospital from 2018 to 2020, were discovered to have IVL combined with AVF. Cases 1, 4, 5, 6, and 7 were found to have developed IVL and AVF at the same time, while in cases 2 and 3, AVF was revealed a few years after IVL surgeries.

The first patient was a 58-year-old woman who was admitted with a 2-month history of right lower limb swelling. IVL was found incidentally 7 months before. Her past medical history included leiomyoma, which was treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH-BSO). Abdomen-pelvic enhanced CT showed a pelvic mass with abundant blood supply invading the right internal iliac vein, right common iliac vein, and soft tissue in the pelvis. Digital subtraction angiography (DSA) demonstrated multiple iliac AVF (**Figure 1**). After gynecological consultation, the patient underwent laparotomy and right common iliac venous tumor excision. A mass 2 cm in diameter and 10 cm in length was removed. However, the residual tumor within the right internal iliac vein was left untreated because of a tendency to bleed. On

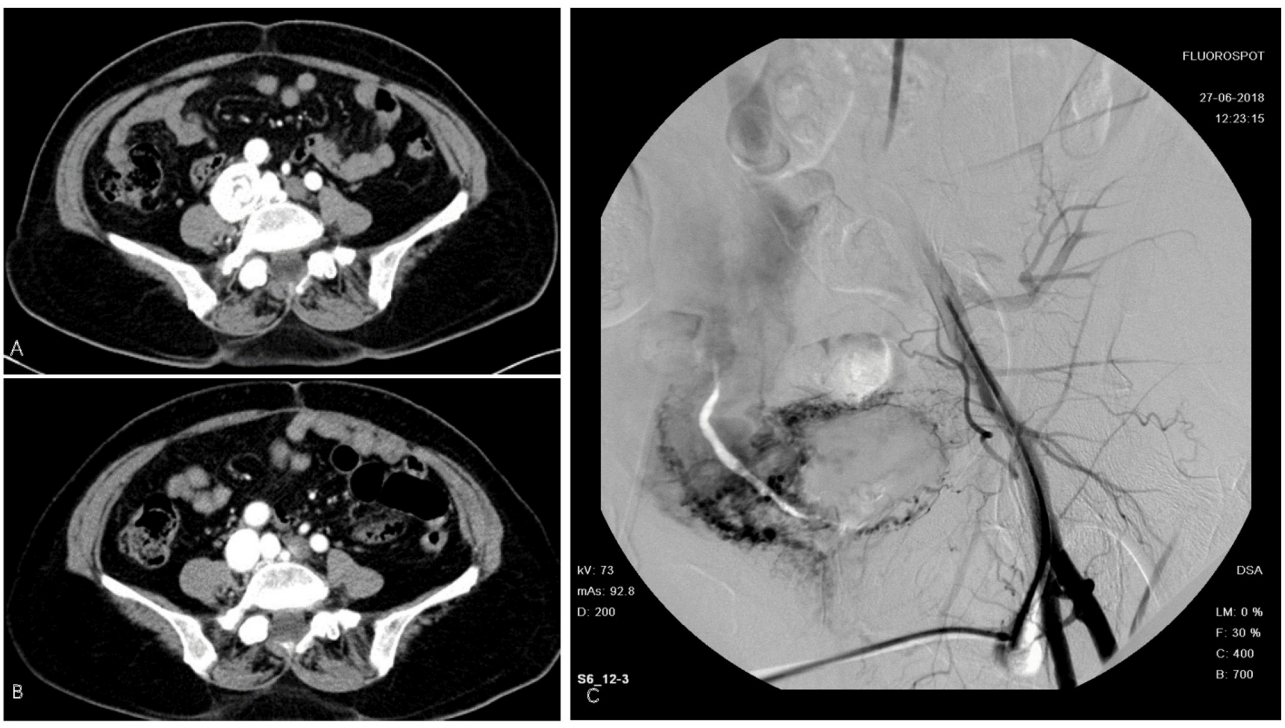

FIGURE 1 | Intravenous leiomyomatosis (IVL) in inferior vena cava (IVC). **(A)** Cord like tumor in IVC. **(B)** Tumor removed surgically. **(C)** Digital subtraction angiography (DSA) demonstrated a contrast agent entering the right iliac vein and IVC through vascular malformation.

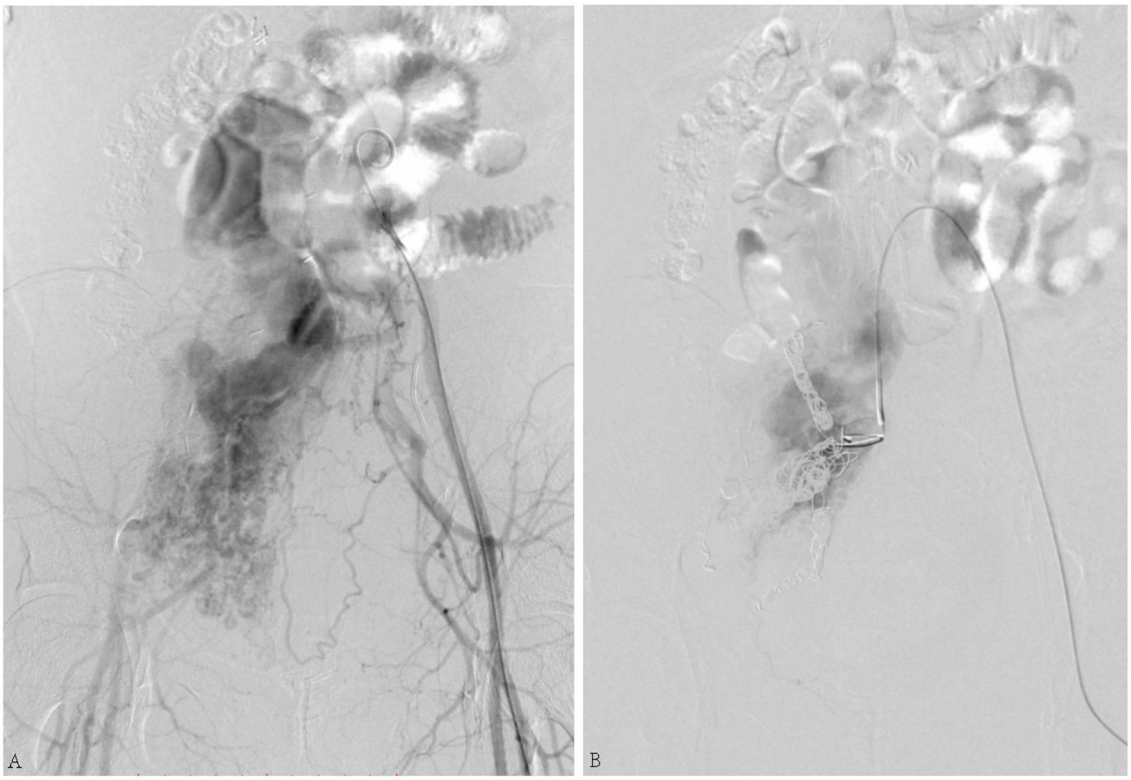

FIGURE 2 | (A) Arteriovenous fistula (AVF) connecting the right internal iliac artery and internal iliac vein. **(B)** Shunt flow disappeared after coil embolization.

outpatient follow-up in 6 months, IVL recurrence was revealed by ultrasound.

The second patient was a 49-year-old female who complained of swelling of the abdomen and two legs for 12 months. Iliac AVF was found 2 months before. Her past medical history included leiomyoma, which was treated by hysterectomy and left oophorectomy 17 years ago. She underwent vascular surgery for IVL 3 years before. Echocardiography (**Figure 2**) revealed enlargement of both atria and right ventricle. The arterial phase during angiography shows the enormous dilatation of both uterine and ovarian arteries to accommodate the high-volume shunting through the pelvic AVF. Coil embolization for pelvic AVF was performed successfully. On outpatient follow-up in 4 months, her condition was favorable and her heart function improved.

The third case was a 45-year-old female suffering from a large number of ascites. Her skin and mucosa were yellow. A vascular murmur could be heard in both femoral regions. The medical history was complicated by myomectomy and coil embolization for bilateral iliac aneurysms 8 years before. An ultrasound revealed enlarged liver. Pelvic AVF with high-volume shunting is obvious in angiography (**Figure 3**). She was treated with coil embolization. However, the iliac AVF still existed after 5 months.

The fourth case, 50 years old, had edema of both lower limbs, coughing, and blood-stained sputum for 3 months. She had a hysterectomy with right oophorectomy for over 7 years. Doppler ultrasound of iliac arteries and veins, echocardiography, PET-CT, and enhanced pelvic MRI were carried out (**Figure 4**), showing a giant tumor with enhancement in her inferior vena cava (IVC) and right atrium. The tumor was successfully extracted through IVC incision, yet the pelvic mass was not removed due to massive blood supply. Bilateral internal iliac veins were ligated to reduce the arteriovenous shunt flow. The patient recovered well.

After a thorough literature search, three case reports with a combination of AVF and IVL were found (**Table 1**). Lee et al. reported the first case in which high-output cardiac failure was caused by the development of arteriovenous shunting within the intravenous component of the tumor. Treatment by TAH-BSO and tumor mass excision was successful. This report pointed out that AVF could be formed within the tumor of leiomyomatosis. This was also shown in a few case reports, where arteries within the tumor of IVL revealed by computed tomography angiography (CTA) are parallel with the vena cava (5, 14).

Nishizawa et al. reported the second case. The patient had received a hysterectomy, whose AVF was extensive and was involved with the intravenous tumor. Therefore, the surgery was in danger of massive hemorrhage. In the end, the tumor was only partially resected, and AVF was left untreated (15).

Mizuno et al. reported the third case of lVL associated with a pelvic AVF. The patient had received a caesarian operation and a hysterectomy. However, the patient had a separate pelvic

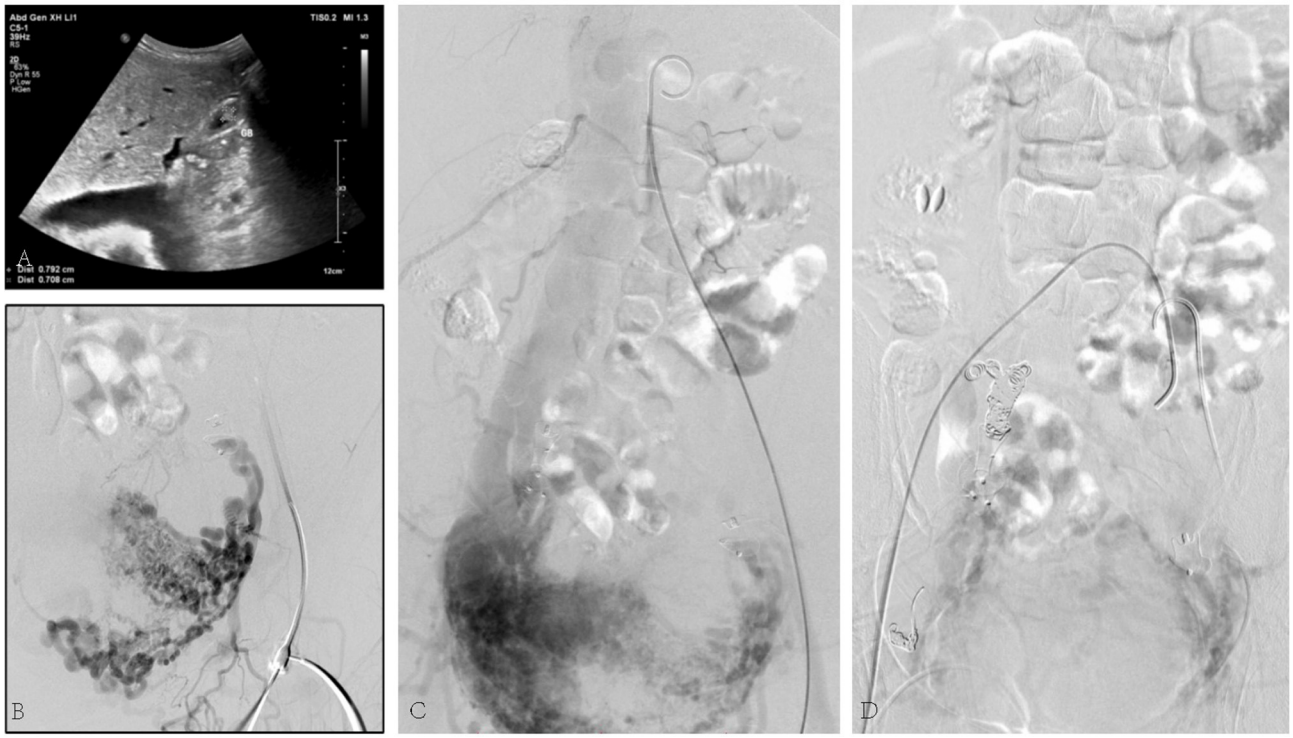

FIGURE 3 | (A) Ultrasound revealed congested liver. **(B)** Arteriovenous malformation. **(C)** Shunt flow entering the vein. **(D)** Abnormal flow stopped by the placement of embolization coils.

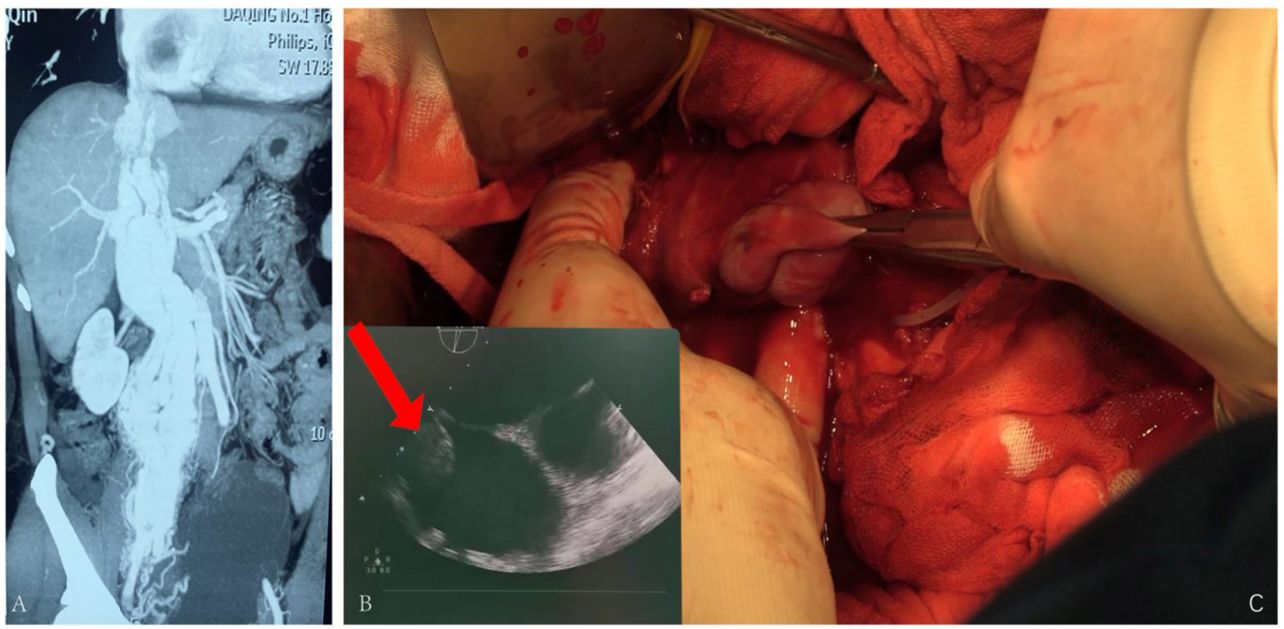

FIGURE 4 | (A) Tumor enhanced in the arterial phase. **(B)** Ultrasound revealing tumor being extracted during surgery. **(C)** Tumor being pulled out from the IVC.

AVF, which was not associated with IVL. Besides, the IVL was complicated by intracardiac extension. During the surgery, bleeding was hard to control due to the high venous return from the pelvic AVF at the time of removing the intravenous tumor (16). In these cases, AVF was found at the same time when IVL was diagnosed, yet few treatments were done for the AVF.

TABLE 1 | Review of intravenous leiomyomatosis (IVL) combined with arteriovenous fistula (AVF).

Publication	Past Medical History	IVL Location	AVF Location	Treatment		Outcome
Lee et al. (14)	/	Retroperitoneal and Ovarian veins	Retroperitoneal and Overian veins	IVL excision TAH-BSO	/	/
Nishizawa et al. (15)	Hysterectomy	Left Ovarian Vein	Bilateral RA Lumbar arteries Right IIA	IVL partial excision Hormonal therapy	AVF untreated	No recurrence
Mizuno et al. (16)	Caesarian operation Hysterectomy	Left Ovarian Vein	Right IIA	IVL excision TAH-LSO	AVF surgically removed	No recurrence

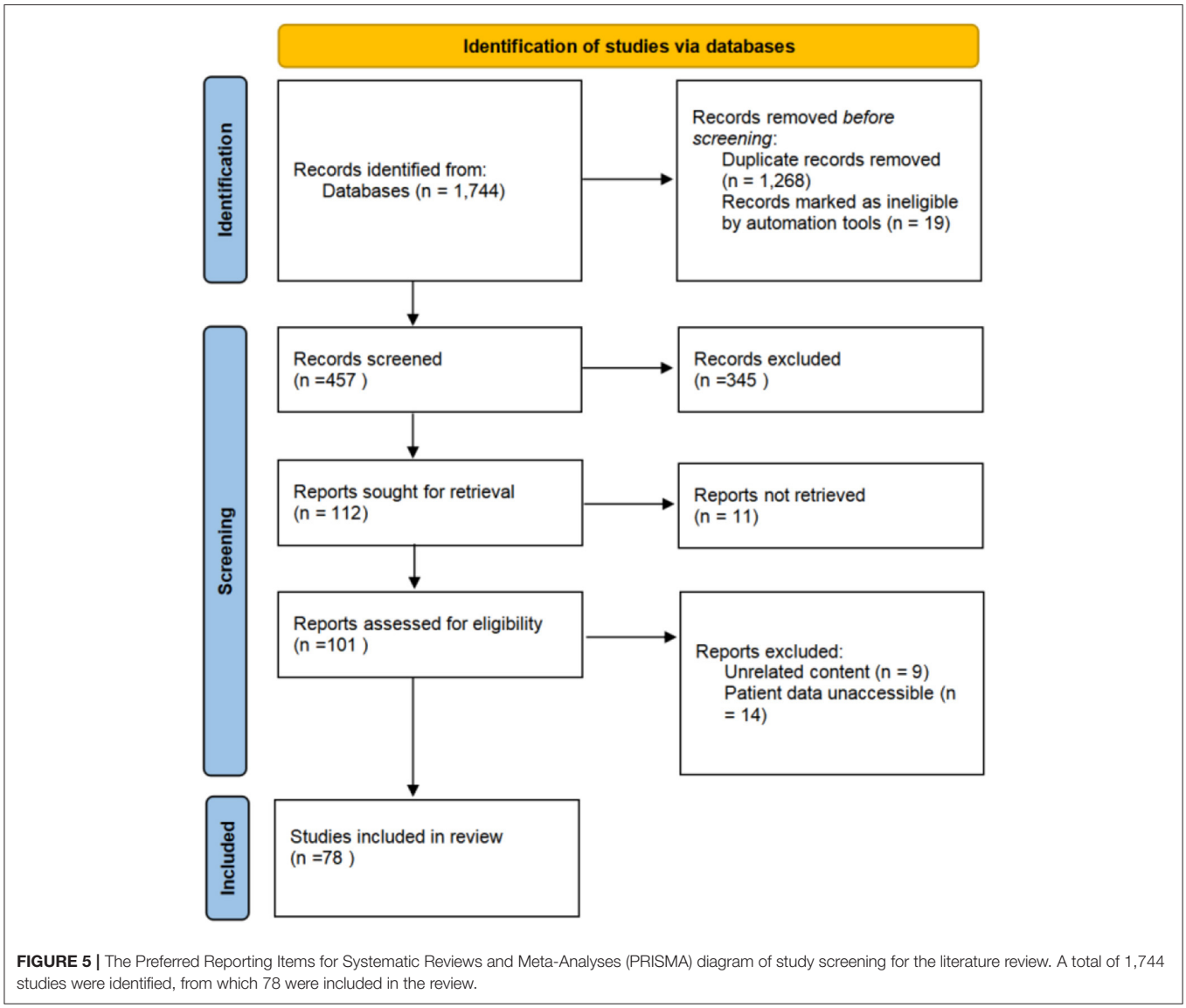

FIGURE 5 | The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram of study screening for the literature review. A total of 1,744 studies were identified, from which 78 were included in the review.

Literature Review

A total of 457 articles on IVLs were retrieved from the initial search from 2000 to 2020 (**Figure 5**). A total of 78 documents were chosen for complete analysis after abstract screening and full-text reviews, reporting a total of 262 cases.

Asian patients accounted for the majority of all reported IVL cases in literature. Cardiac involvement is one of the most common characteristics in patients with IVL, which could be found in 80.5% of all cases. Surgery is the primary strategy to remove IVL, both one-stage and staging surgeries could be

considered according to patient condition and tumor shape. It should be highlighted that the rate of misdiagnosis on IVLs was high, with 17 cases reported, accounting for 6.5% of total cases (**Table 2**).

Intravenous leiomyomatosis recurrences are prevalent. However, complete excision of intravascular mass is associated with a reduced recurrence rate ($p < 0.05$). Nevertheless, the incidence of complications was increased with complete excision, though not significantly compared with non-complete excision (**Table 3**). In contrast, intrapelvic mass resection might increase the incidence of complications ($p < 0.01$) but did not significantly reduce IVL recurrence. It is crucial to highlight that these comparisons have relatively little statistical efficacy, and do not reveal which types of patients would have severe complications and die.

DISCUSSION

The cause of AVFs formation in patients with IVL is unknown, and the association between AVF and IVL cannot be confirmed. Spontaneously developed pelvic vascular malformations are rare, and the most common causes include trauma, iatrogenic injury, aneurysm, and malignant tumor-related neovascularization (13, 17, 18). Vascular malformation induced by benign tumors, such as IVL, is seldom reported.

As demonstrated in cases 1 and 4, secondary vascular abnormalities developed around the tumor at the site of IVL invading the vein for unexplained reasons. The contrast agent in the vein appeared in advance under the angiography, indicating that the process of IVL invading the vein may promote local vascular malformation. Sometimes, this vascular malformation can even become the primary diagnosis in patients, masking IVL and leading to missed diagnosis and ignorant of the leiomyomatosis, resulting in tumor development (19).

There are several possible reasons why AVF would form in these patients with IVL. Six patients, including four cases from our center, who had leiomyomatosis complicated by pelvic AVF had received uterine surgery 7–15 years before. Thus, Iatrogenic AVF is to be suspected (15, 16). Furthermore, leiomyomatosis exhibits behaviors resembling malignant tumors; it invades blood vessels and induces angiogenesis. If the tumor invades and destroys both veins and arteries, fistulas could be formed during angiogenesis. AVF may originate from the nutritional artery of the tumor itself, and form AVFs as the tumor invades and grows into the vein. Such a process might be induced by iatrogenic injury during uterine surgery (20). The tumor inside the vein blocks the blood return, increasing local pressure, resulting in sphincter relaxation, and remodeling of the AVF. Some fistulas regressed after pressure relief (21, 22). The observation of pulmonary vascular malformation induced by benign metastasizing leiomyoma (BML) of the lungs increases the possibility of the hypotheses above.

Although pathological research provided evidence for AVF formation in the myometrium, such theories lack the support of long-term imaging surveillance (23, 24). Considering the risk of iatrogenic AVF, gynecologists are advised to avoid injury to

TABLE 2 | Summary of the clinical information of 262 patients with IVL.

Patients	262	**Involving vessel**	
Age	46.01 ± 7.13	Ovarian vein	69 (26.3%)
Race		Uterine vein	20 (7.6%)
Asian	189 (72.1%)	Internal iliac vein	80 (30.5%)
Caucasion	70 (26.7%)	Common iliac vein	13 (5.0%)
African	3 (1.1%)	Inferior vena cava	2 (0.8%)
Reproductive history		Nephrotic vein	5 (1.9%)
Yes	25 (9.5%)	NR	73 (27.9%)
None	9 (3.4%)	**Cardiac involvement**	
NR	228 (87.0%)	Yes	211 (80.5%)
History of fibroids		No	47 (17.8%)
Yes	161 (61.5%)	NR	4 (1.7%)
None	13 (5.0%)	**Tumor enhanced**	
NR	88 (33.6%)	Yes	8 (3.1%)
Myomectomy history		No	24 (9.2%)
Yes	113 (43.1%)	NR	230 (87.8%)
None	30 (11.5%)	**Surgery Staging**	
History of IVL surgery	15 (5.7%)	One-stage surgery	185 (70.6%)
NR	104 (39.7%)	Staging surgery	53 (20.2%)
Symptoms		Non-operative	3 (1.1%)
Dyspnea	87 (33.2%)	NR	21 (8.0%)
Palpitation	58 (22.1%)	**Cardiotomy**	
Chest pain	39 (14.9%)	Yes	118 (45.0%)
Syncope	40 (15.3%)	No	124 (47.3%)
Fatique	10 (3.8%)	NR	20 (7.6%)
Abdominal discomfort	41 (15.6%)	**Complete excision of intravascular mass**	
Lower limbs swelling	48 (18.3%)	Yes	138 (52.7%)
Pelvic mass	53 (20.2%)	No	30 (12.2%)
Menorrhagia	26 (9.9%)	NR	95 (35.1%)
None	34 (13.0%)	**Excision of pelvic mass**	
Imaging		Yes	191 (72.9%)
Enhanced CT	53 (20.2%)	No	11 (4.2%)
MRI	40 (15.3%)	NR	60 (22.9%)
CT	62 (23.7%)	**Complication**	
ECHO	67 (25.6%)	Yes	50 (19.1%)
Pelvic ultrasound	20 (7.6%)	No	54 (20.6%)
Laparoscopy	4 (1.5%)	NR	161 (66.8)
Others	10 (3.8%)	**Recurrence**	
NR	83 (31.7%)	Yes	18 (7.5)
		No	93 (38.6)
Misdiagnose	17 (6.5%)	NR	130 (53.9)

TABLE 3 | Factors associated with complications and recurrence of IVL.

		Complications	*p* value	Recurrence	*p* value
Reproductive history	Yes	7/11	1.000	4/21	1.000
	No	3/5		1/7	
History of fibroids	Yes	17/28	0.295	8/40	1.000
	No	1/4		0/4	
History of myomectomy or IVL	Yes	9/19	1.000	7/36	0.659
	No	4/10		1/11	
Complete excision of intravascular mass	Yes	18/28	0.236	7/53	0.024*
	No	3/8		6/10	
Excision of pelvic mass	Yes	30/42	0.007	11/49	0.673
	No	1/7		3/9	
Tumor enhanced	Yes	2/4	1.000	1/5	1.000
	No	6/9		1/9	
Cardiac involvement	Yes	11/28	0.062	9/49	0.500
	No	7/9		5/18	

*Fisher's exact test, *Calculated using Pearson χ2.*

TABLE 4 | Echocardiography of Case 2 and 3 before and after coil embolization.

Variable	Reference range	Case 2		Case 3	
		Before	After	Before	After
Right atrial vertical diameter	≤51 mm	83	80	87	79
Right atrial transveral diameter	≤41 mm	78	77	72	66
Right ventricle diameter	≤39 mm	63	53	NR	49
IVC diameter	≤21 mm	39	34	37	28
TAPSE	≥17 mm	11	18	21	16
Tricuspid regurgitation velocity		2.5	2.8	2.5	2.8

arteries and veins during pelvic surgery. Among the reported cases and those reported by us, there were three patients [Case 2∼3, and Mizuno et al. (16)] whose AVF and IVL occurred at different sites, which may have other reasons.

Magnetic resonance angiography (MRA) and DSA should be considered for the evaluation in IVL patients with heavy right cardiac load to exclude potential AVF. The advantage of MRA lies in its better ability to differentiate between soft tissue vascular malformations and soft tissue masses (25). Compared with CT, especially in patients with IVL, MRA can reduce the probability of misdiagnosing IVL as venous dissection or other hypervascular soft tissue tumors (19). The advantage of angiography is that it can clearly diagnose vascular malformations, which can be treated with coil embolization during the examination (11, 19). If a vascular malformation is found, it should be embolized from the arterial segment before the IVL surgery to reduce the blood flow in the vascular malformation and prevent bleeding during the IVL surgery, which contributes to a safe operation. If the vascular malformation is discovered during the operation, it is often difficult to remove due to tight adhesion and severe bleeding, as in Case 1. At this time, ligation of the artery supplying the AVF should be considered, as in Case 4.

Vascular malformation aggravates the symptoms of IVL and increases the risk of operation. High venous flow caused by vascular malformation increases the right ventricular pressure (Cases 2 and 3). Timely embolization may significantly improve the problem of right ventricular overload, reverse right heart failure, promote the recovery of right ventricular systolic pressure, and improve the blood supply of the lungs (**Table 4**). The rich blood supply of tumors caused by vascular malformations may promote IVL tumor growth in addition to increasing the risk of surgical bleeding. As in Case 4, the IVL significantly blocks IVC and reaches the heart.

According to statistics, the recurrence rate of intravascular tumor residue was much greater than that of patients with clean intravascular excision (**Table 3**). This discrepancy could be attributable to the bias introduced by the short sample size. Nevertheless, the ligation of the bilateral internal iliac vein and ovarian vein is helpful to prevent the recurrence or shedding of residual tumor embolus (26). Patients who have a pelvic tumor resection are more likely to experience complications than patients who do not have a pelvic tumor resection (**Table 3**), which could be owing to the abundance of the pelvic vascular bed, or possibly the creation of tumor-related arteriovenous malformations.

There is currently no consensus on the diagnosis and treatment of IVL, and only a few retrospective cohort studies in big centers with more cases were conducted (10, 27). Ma et al. presented an IVL staging system, categorizing IVLs into stages 1 through 4 based on the extent of intravenous tumor spread

(28). Liu et al. categorized IVLs that enter the heart chambers into types 1–5 and discussed surgical plans for each type of IVLs (29). Further classification methods and surgical techniques were discussed by Li et al., although the surgical categorization differs from that suggested by Liu et al. According to the relative diameter between tumor and IVC, Li et al. recommended four types of surgery. Such classification agrees with Ma et al.'s method on stage 3 IVLs, including IVLs reaching and not reaching the right atrium. However, effective imaging tools are still needed to determine whether the tumor in the heart can be retrieved from the IVC in advance according to their reports. Therefore, different institutes should share their surgical experiences and promote diagnostic guidelines to decrease misdiagnosis and missed diagnoses, improve complication management, and decrease significant complications and recurrence.

Limitations and Conclusions

To be clear, our study on IVL complicated by AVF, case series, and literature review has several limitations. First, the cases in the study were mainly from one medical center, some patients may have been overlooked in other places due to the rarity of this condition. The study was retrospective, providing less powerful evidence than prospective studies.

The symptoms of patients with IVL, caused by mass blocking venous reflux, mainly resemble that of right ventricular dysfunction and are easily misdiagnosed due to lack of experience or insufficient imaging examination. Incomplete intravascular IVL resection might contribute to recurrence; therefore, physicians should try to remove the tumor from the vessels completely. Some patients who had IVL complicated by pelvic AVF experienced a higher risk of bleeding in surgery, indicating the importance of dealing with the AVF in advance. Further studies on the above problems are necessary.

We point out that IVL combined with AVF aggravates congestion symptoms of peripheral organs, such as leg edema, ascites, hepatomegaly, jaundice, and intestinal bleeding, which can be seen in our cases and in the literature. Embolization of AVF in advance may reduce the risk of bleeding in IVL surgery. For patients with symptoms of right heart failure after pelvic surgery, imaging with a contrast agent (MRA and DSA) should be recommended to eliminate potential AVFs. In our cases, coil embolization was performed, the right ventricular function improved, and the short-term effect was satisfactory. Moreover, considering the possibility of iatrogenic AVF in patients with IVL, the tumor should be removed carefully, with attention to fine anatomical structure when ligating blood vessels, avoid ligation of arteries and veins together, and reduce secondary arteriovenous malformations.

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 study protocol was approved by the Research and Ethics Board of the Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study and for the publication of any potentially identifiable data included in this article.

AUTHOR CONTRIBUTIONS

HK and YCa analyzed the patient data and were major contributors in writing the manuscript. YZ and YCh made substantial contributions to the study design and gave final approval to the version to be published. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.878386/full#supplementary-material

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CASE REPORT
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Case Report: AL Amyloidosis Severe Restrictive Cardiomyopathy Associated With Multiple Myeloma—Diagnostic Difficulties

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Background: Cardiac AL amyloidosis as a complication of multiple myeloma (MM) is a formidable life-threatening condition. The first-line therapy for both MM and systemic AL amyloidosis is proteasome inhibitors (PIs). Unfortunately, the use of PIs may lead to cardiovascular toxicity development, which requires specific cardio-oncology supervision.

Case Report: A 57-year-old woman was admitted to a university hospital with clinical manifestation of progressive chronic heart failure. The patient had hypertension and no history of diabetes mellitus, myocardial infarction (MI), stroke, and arrhythmias. After a series of laboratory and instrumental examination methods, MM complicated by cardiac AL amyloidosis was proved. Upon specific cardio-oncology examination (NT-proBNP 4,274 pg/ml), ECHO showed systolic dysfunction, motion abnormalities in LV basal and middle segments, and a typical depositional myocardium pattern ("luminescence"); cardiac MRI revealed restrictive cardiomyopathy and specific hyperenhancement of the ventricles and atria; 24-h ECG showed QS-pattern in leads V1–V3 and unstable ventricular tachycardia (VT) paroxysms. Cardio-oncology consultation showed baseline cardiovascular risk was very high (≥20%), and cardioprotective therapy [iACE/ARBs, beta-blockers (BB), statins] was administered. The patient underwent VCD (bortezomib; cyclophosphamide; dexamethasone) chemotherapy (CMT) program. By the time of publication, the patient had received four CMT courses with a positive oncohematological and cardiovascular effect.

Conclusion: In this clinical case, we described a complication of MM, which was rare according to the severity and manifestation with restrictive cardiomyopathy due to secondary cardiac amyloidosis. The case's features were difficulties in verifying the underlying disease and its own complication, and the complexity of patient management according to modern principles of cardio-oncology.

Keywords: multiple myeloma, cardiotoxicity, vasculotoxicity, cardiac amyloidosis, restrictive cardiomyopathy (RCM), speckle tracking ECHO

INTRODUCTION

Multiple myeloma (MM) is a B-cell malignant tumor; morphological substrate—plasma cells producing monoclonal Ig (1). The annual worldwide incidence of MM is growing steadily to 1.5 cases per 100,000 people.

All organs and systems, including the cardiovascular system, are involved in the clinical picture of MM. In addition to the underlying disease, 10–15% of patients with MM may develop formidable complications such as focal or systemic AL amyloidosis (2).

Amyloidosis is a disease caused by extracellular deposition of a specific protein-polysaccharide complex (amyloid) in various organs and tissues, which leads to cell dysfunction, damage, or death (3, 4). According to the United States National Center for Health Statistics, the prevalence of AL amyloidosis is 4.5 cases per 100,000 populations (5). The main target organs in AL amyloidosis are the heart (70–80%), kidneys (74%), liver (27%), and peripheral and autonomic nervous systems (22 and 18%, respectively) (6, 7). Moreover, in only 5% of cases, a rare manifestation of the disease, such as isolated cardiac amyloidosis, is observed (8, 9). AL cardiac amyloidosis may clinically manifest through progressive chronic heart failure (HF): severe rest dyspnea (in 80% of patients), peripheral edema (70%), pleural effusion, or ascites in the later stages (10). Diagnosis of cardiac amyloidosis is complicated and requires a series of laboratory and instrumental examination methods, but the final diagnosis can only be verified morphologically (8, 9).

The primary approach in treating both MM and AL cardiac amyloidosis is inhibition of pathological precursor protein synthesis and plasmocyte proliferation. According to guidelines for the diagnosis and treatment of MM and systemic AL amyloidosis, the first-line therapies are a combination of proteasome inhibitors (PIs; bortezomib, carfilzomib, and ixazomib) with other chemotherapy drugs (cyclophosphamide, melphalan, and dexamethasone) (9, 11).

However, proteasome inhibition occurs not only in pathological plasma cells but also in normal cardiomyocytes and/or endothelial cells, which may result in development of cardiovascular toxicity. Clinical symptoms may manifest through various rhythm/conduction disturbances, ischemia progression including myocardial infarction (MI), and decrease in systolic function (12, 13). Alkylating drugs (cyclophosphamide and melphalan) and glucocorticoids have similar cardiotoxic effects (13). The incidence of HF during bortezomib therapy is relatively low, up to 4%; however, it can increase by up to 15% with simultaneous use of glucocorticoids (13, 14).

Abbreviations: AH, arterial hypertension; ARBs, angiotensin II receptor blockers; BB, beta-blockers; BMI, body mass index; BNP/NT-proBNP, brain natriuretic peptide/N-terminal-pro brain natriuretic peptide; BP, blood pressure; CMT, chemotherapy; CVD, cardiovascular disease; ECG, electrocardiogram; ECHO, transthoracic echocardiography; EF, ejection fraction; GLS, global longitudinal strain; HF, heart failure; HR, heart rate; hsTr, high sensitive troponin; iACE, angiotensin-converting-enzyme inhibitor; Ig, immunoglobulin; LA, left atrium; LV, left ventricle; LVH, left ventricle hypertrophy; MI, myocardial infarction; MM, multiple myeloma; MRI, magnetic resonance imaging; PIs, proteasome inhibitors; RV, right ventricle; RVH, right ventricle hypertrophy; SVE, supraventricular ectopic beat; VE, ventricular ectopic beat; VT, ventricular tachycardia.

The present clinical case describes a patient suffering from MM; diagnostic difficulties were due to manifestation of severe cardiac AL amyloidosis (restrictive cardiomyopathy, biventricular HF, and life-threatening arrhythmias), and the complexity of patient management according to modern principles of cardio-oncology.

CASE DESCRIPTION

A 57-year-old woman was admitted to the hematology department of Sechenov University in January 2021. The patient complained of chest discomfort, shortness of breath during minimal exertion (walking up to 100–200 m), exercise intolerance, rare episodes of heartbeat interruptions without a provoking factor, hypotension (up to 90/55 mm Hg), and weakness.

It is known that the patient suffered from second-grade arterial hypertension (AH) for many years, managed by low doses of iACE, but she had no history of acute MI, stroke, atrial fibrillation/flutter, pulmonary embolism, or HF.

Carpal tunnel syndrome was verified in 2019. In order to exclude hereditary amyloidosis, direct sequencing of the entire coding sequence and regions of exon-intropic junctions of the transthyretin (TTR) gene was performed; pathogenic and probably pathogenic variants of the TTR gene nucleotide sequence were not found. After a neurological consultation, symptomatic therapy with pregabalin was prescribed for several months with a moderate effect.

The patient developed the above complaints in September 2020.

We found the following upon outpatient examination:

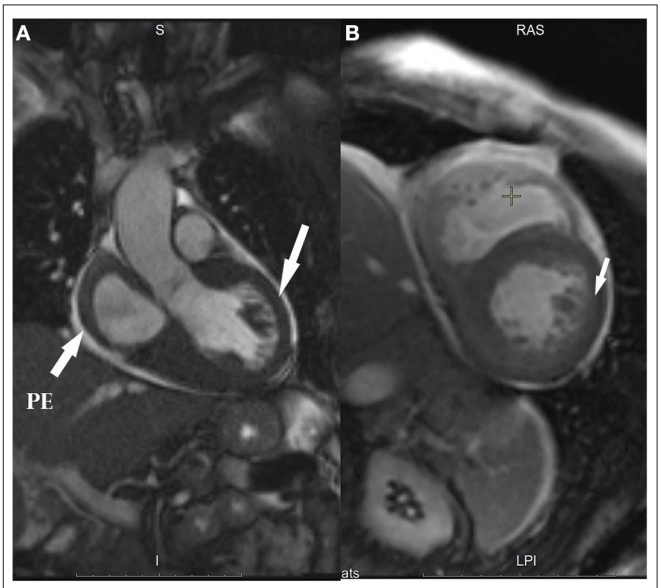

FIGURE 1 | Cardiac MRI images: **(A)** Left ventricle (LV) outflow tract (arrows show ventricular hypertrophy and pericardial effusion, PE); **(B)** Two-chamber views (arrow shows global subendocardial hyperenhancement).

- ECG and 24-h ECG showed sinus tachycardia, complete left bundle branch block, QS-pattern in leads V1–V3, frequent supraventricular extrasystoles (SVEs), ventricular extrasystoles (VEs), and unstable VT paroxysms without rhythm pauses or ischemia (at that time rhythm disorders were not interpreted, and anti-arrhythmic drugs were not prescribed);
- ECHO showed left atrium (LA) dilatation, concentric left ventricle (LV) and right ventricle (RV) hypertrophy (no zones of local contractility disorders), decreased global myocardial contractility, ejection fraction (EF) 48%, mitral regurgitation grade 2, tricuspid regurgitation grade 2, moderate pulmonary hypertension, physiological amount of fluid in the pericardial cavity, and increased echogenicity of the LV myocardium;
- We saw a significant increase in blood NT-proBNP level up to 4,274 pg/ml ($N = 0–125$);
- Cardiac MRI with late gadolinium enhancement showed restrictive cardiomyopathy, LV hypertrophy, moderate atria expansion, and specific contrasting of the ventricles and atria myocardium, which did not exclude cardiac amyloidosis (**Figure 1**).

Outpatient examination continued; serum and urine protein immunochemical study detected κ-type Bens-Jones protein [serum κ- free light chain (κ-FLC) 239 mg/L ($N = 3.3–19.4$), daily proteinuria 1.5 g], secondary hypogammaglobulinemia, increased serum β2-microglobulin levels, and dysproteinemia with α-1/α-2 fraction predominance.

The patient was consulted by a hematologist and in-charged with the above anamnesis. On admission (physical examination) the patient showed no obesity (BMI = 26.9 kg/m^2), no fever (T 36.7°C), no peripheral lymphadenopathy, no edema, normal lung breathing sounds, no wheezing, Sat O2 98% in room air, muffled heart sounds, arrhythmia due to single EXs, heart rate (HR) 86 bpm, blood pressure (BP) 100/60 mmHg, no hepatosplenomegaly, and no obvious disturbances of organs and systems.

Blood test abnormalities were as follows: AST 37 U/L ($N = 0–34$), γ-GT 123 U/L ($N = 0–73$), CPK 197 U/L ($N = 0–190$); potassium 5.5 mmol/L ($N = 3.4–5$), eGFR (CKD-EPI) 68 ml/min/1.73 m^2, LDH 590 U/L ($N = 240–480$), cholesterol 5.8 mmol/L ($N = 3.2–5.6$), triglycerides 2.44 mmol/L ($N = 0.4–1.7$), VLDL 95 mmol/L ($N = 0.19–0.77$), HDL.83 mmol/L ($N \geq 1.56$), troponin T (twice) negative, dysproteinemia with α-1 fraction predominance, secondary hypogammaglobulinemia, M–gradient negative, fibrinogen 5.12 g/L ($N = 1.8–4$), and daily proteinuria 1 g. All other parameters were in normal range.

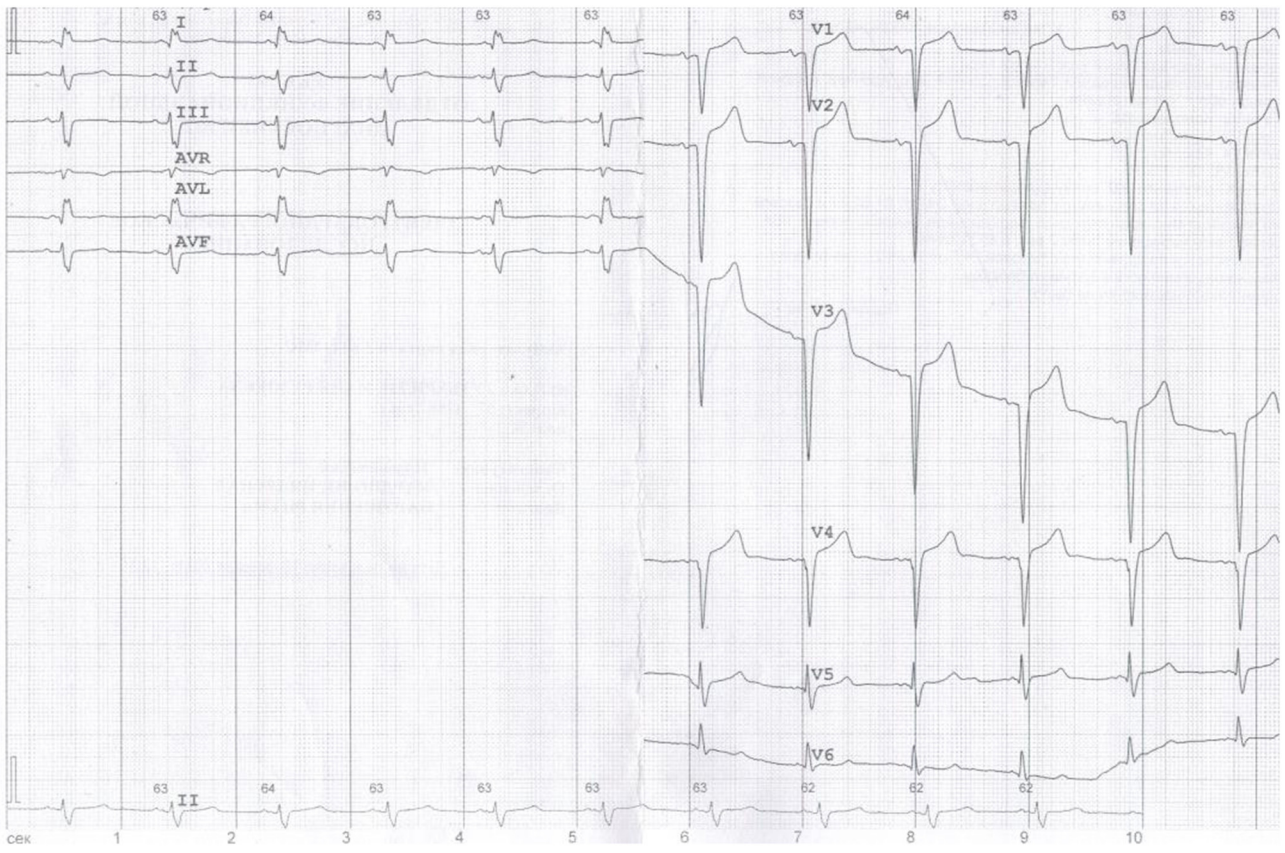

FIGURE 2 | ECG: Sinus tachycardia, heart rate (HR) 63 bpm, left anterior fascicular block, QS-pattern in leads V1–V3.

Whole-body low-dose CT scan revealed no foci of destruction.

Sternal puncture showed an increased amount of plasma cells up to 8%. Thus, according to the bone marrow cytological examination, no convincing data for MM were obtained (11, 15).

Subcutaneous fat and rectal mucosa biopsy with Congo red staining (for the diagnosis of specific amyloid lesions) was negative.

Trepanobiopsy (for final diagnosis verification) showed a morphological picture corresponding to the substrate of plasma cell myeloma; during an additional histochemical study, the amyloid-Congo-red-complex was found.

Thus, according to the European and National guidelines for the diagnosis and management of multiple myeloma and systemic AL amyloidosis, these diseases were confirmed (11, 15).

Additionally, due to the patient's cardiac complaints and signs of heart involvement, before starting potentially cardiotoxic cancer therapy, the patient was further examined (ECG, 24-h ECG, and 2D speckle tracking ECHO) (**Figures 2–4**) and consulted by a cardio-oncologist.

A 24-h ECG showed a sinus rhythm with average day HR 78 bpm, average night HR 77 bpm, SVEs—total 420, max per hour−39, six couplets, four paroxysms of SVT up to 2 s, VEs—total 227, max per hour−89, 15 couplets, paroxysms of unstable VT (3 paroxysms: 1 triplet, other consists of 4–5 beats), no inducible myocardial ischemia, no rhythm pauses longer than 2 s. 2D Speckle tracking ECHO: significant concentric LVH (average 17 mm, $N = 10$ mm) and RVH (7 mm, $N = 5$ mm), decreased LV systolic function [biplane EF = 48–50% (Simpson), dp/dt = 1,185 mmHg, GLS = −5% ($N > 18\%$)] and RV [TAPSE = 1 cm ($N > 1.7$)], motion abnormalities in LV basal and middle segments, restrictive type of LV diastolic dysfunction (E/A = 2.9), LA dilatation (4.2 cm, volume 75 ml, $N < 55$ ml), mitral/tricuspid valve leaflet thickening, moderate regurgitation, mild pulmonary hypertension (estimated sPAP = 37–40 mmHg, $N < 30$ mmHg), mild pericardial effusion (1–2 mm), typical depositional myocardium pattern ("luminescence"), and characteristic cardiac amyloidosis (**Figure 4**).

Consultation with a cardio-oncologist (ESC HFA/ICOS 2020) showed a baseline cardiovascular risk for development of CMT cardiovascular toxicity was very high (≥20%): previous cardiovascular disease (CVD; HF, cardiac amyloidosis, baseline LVEF < 50%, arrhythmia, LVH); elevated baseline NT-proBNP; CV risk factors (AH, dyslipidemia, high-dose dexamethasone) (16, 17). According to modern cardio-oncology guidelines, cardioprotective drugs should be administered to very high-risk patients (iACE/ARBs, BB, statins) (13, 17). In the case of congestive HF, treatment was carried out according to the ESC Guidelines for the diagnosis and treatment of heart failure 2016 (18).

Thus, based on the results of laboratory and instrumental examination, the diagnosis was verified: multiple myeloma, diffuse form, with the secretion of κ-type light chains; Bence-Jones κ-type proteinuria, stage III (ISS), Durie-Salmon stage I; secondary AL amyloidosis; restrictive cardiomyopathy; cardiac rhythm and conduction disorders—SVTs, VEs, paroxysms of unstable VT, and left anterior fascicular block; heart failure with mid-range EF, functional class III (NYHA); arterial hypertension stage II, degree 2, high CV-complication risk; dyslipidemia (treated by statins); atherosclerosis of the aorta, aortic, mitral, and tricuspid valves, CKD C2 (KDIGO). According to the ESC Position Paper on Diagnostic and Treatment of Cardiac Amyloidosis 2021, there was no doubt that cardiac amyloidosis was the main reason for the CV manifestation in this patient: LV wall thickness was ≥12mm + 1 "red flag" (hypotension, if previously hypertensive, proteinuria, carpal tunnel syndrome, subendocardial/transmural late gadolinium enhancement, reduced GLS, pseudo Q-waves on ECG) and the extracardiac biopsy was positive for amyloids. In this case, there were no indications for cardiac biopsy (6).

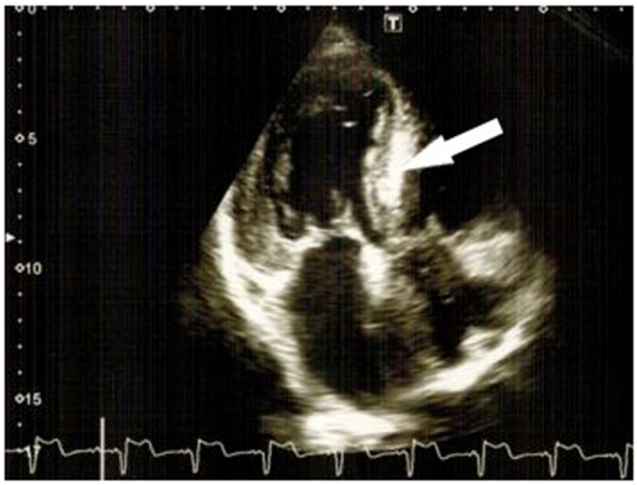

FIGURE 3 | "Characteristic luminescence" of the interventricular septum (bold arrows) and left ventricle hypertrophy.

Following the guidelines for the treatment of MM complicated by AL amyloidosis (15) and considering exceptionally high cardiotoxicity risk, the patient was scheduled for the VCD program [VELCADE (bortezomib), cyclophosphamide, dexamethasone]. In addition, recommended cardioprotective medications were prescribed: anti-arrhythmic: sotalol 60 mg daily, MRA: spironolactone 50 mg daily, diuretic: torasemide 10 mg daily, metabolic: trimetazidine 80 mg daily, hypolipidemic: atorvastatin 10 mg daily, and anticoagulant: apixaban 5 mg twice a day (according to hematological indications when using high doses of dexamethasone). Due to the high risk of hypotension, iACE/ARB administration was withdrawn until optimal BP levels were established. Class III antiarrhythmic drugs were chosen in order to prevent life-threatening arrhythmias (paroxysmal VT). There was an attempt to administer amiodarone, but it was not tolerated by the patient (extreme systemic hypotension, nausea, vomiting, and dizziness). Thus, sotalol was the only option in this case with regular ECG control (throughout the whole follow-up period no QTc prolongation was registered). There were no absolute indications for implanting a cardioverter-defibralator.

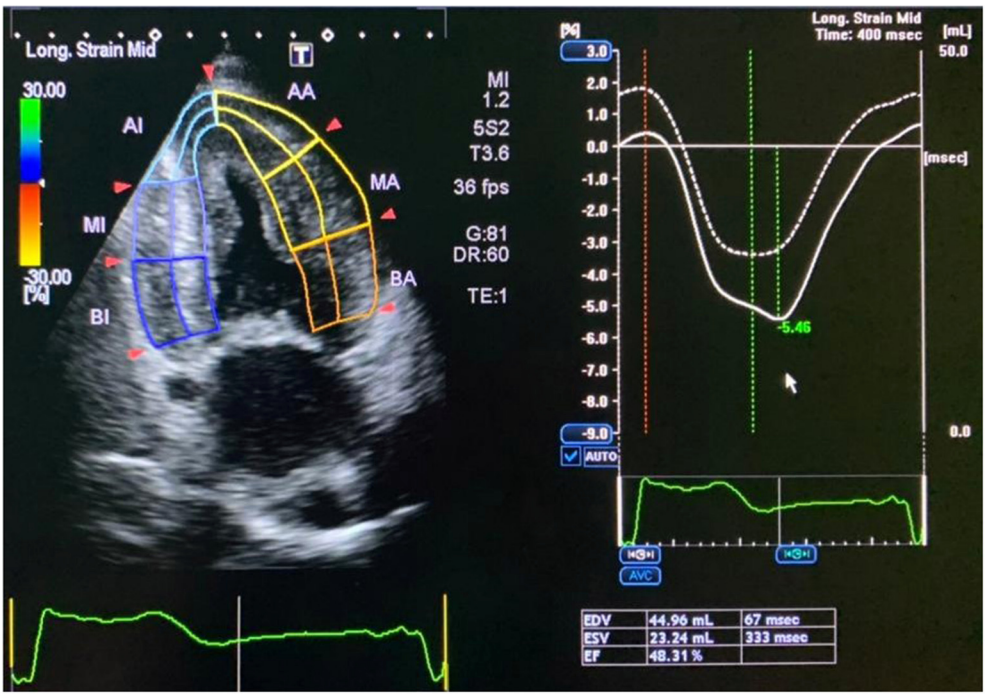

FIGURE 4 | Speckle tracking for two-chamber view.

The above management approach concerning specific anticancer and cardiac therapy allowed the patient to successfully receive the first CMT course without complications and/or intercurrent infections. In the control blood tests, cytopenia was not noted, and daily proteinuria was absent. The patient was discharged.

By June 2021, the patient had received four VCD chemotherapy courses at the same dosage. The second cycle was interrupted because of SARS-CoV infection, complicated by bilateral polysegmental viral pneumonia (CT-stage 2) with mild respiratory insufficiency and unilateral pleural infusion. Despite the underlying disease and because of COVID-19, the patient received tocilizumab 400 mg and glucocorticoids with a positive effect. Unfortunately, concerning CVD status, COVID-19 resulted in biventricular HF exacerbation, worsening of dyspnea and weakness, peripheral edema, exercise intolerance, NT-proBNP of 8,699. pg/ml, and LV EF = 49%. Correction of cardioprotective therapy was performed by a cardio-oncologist (transition to temporary intravenous diuretic therapy, increasing doses of spironolactone and sotalol) with a positive effect. There was still no opportunity to start iACE/ARBs due to the high hypotensive risk.

Control examination after four CMT courses showed a positive effect: no dysproteinemia with α-1/α-2 predominance, γ-globulin level within the normal range, CRP 6.9 mg/L (as post-COVID-19), NT-proBNP decreased to 4,623 pg/ml, M-gradient undetectable by blood immunoelectrophoresis, Bence-Jones daily proteinuria of only. One gram, 24-h ECG: sinus rhythm with average HR 82 bpm, SVEs: total 380, 13 couplets, 5 paroxysms of asymptomatic unstable SVT, VEs: total 725, 0 couplets, one episode of unstable VT (triplet), Speckle tracking ECHO:GLS increased up to −11%, and EF = 51%.

Such a multidisciplinary approach to patient management, active monitoring of the cardiovascular system's state, and in-time therapy correction made it possible to continue the effective and recommended CMT without delay/withholding.

In the future, re-inpatient examinations are planned for subsequent CMT courses, follow-up for MM, AL amyloidosis, and cardiac control.

DISCUSSION

This clinical case demonstrates the difficulties in verifying MM due to the lack of proven criteria for its diagnosis and the manifestation of the disease predominantly with cardiac complaints. The severity of the patient's condition is due to complications of MM, such as cardiac AL amyloidosis (restrictive cardiomyopathy, biventricular congestive HF, and life-threatening rhythm disorders). The presence of cardiac amyloidosis indicates a worse prognosis compared with amyloidosis damage to other organs. Predictors of an unfavorable outcome are congestive HF, arrhythmia, renal failure, and involvement of two

or more visceral organs in the pathological process. The median survival rate in patients with cardiac AL amyloidosis and HF does not exceed 67 months (8, 9). In addition, using CMT with known cardiovascular toxic effects may further worsen the prognosis in these patients. On the other hand, CMT is currently the only treatment option for MM complicated by AL amyloidosis. Reducing the risk of such therapy becomes possible only with a multidisciplinary approach to the management of these patients by oncologist/hematologist and cardiologist/cardio-oncologist.

Thus, high-quality examination (morphological, immunological, and immunohistochemical) makes it possible to verify the diagnosis and give in-time specific treatment. Moreover, patients with cancer and known CVD and/or cardiovascular risk factors are recommended to be assessed for baseline cardiovascular risk before initiating potentially cardiotoxic cancer therapies. All patients in high/very high-risk groups are needed to be consulted by a cardiologist/cardio-oncologist, and cardiological assessment should include ECG, speckle tracking ECHO, and cardiac biomarkers (hsTr, BNP/NT-proBNP) (17, 19, 20). In the case of a high/very high-risk patient, it is recommended to start cardioprotective drugs: iACE/ARBs, BB, or statins (13, 17). In recent publications, novel cardioprotective strategies were proposed based on SGLT-2 inhibition and interleukin-1 blockers (21, 22). However, for now, these are promising directions that need further investigation and a solid evidence base. Only this approach will help improve the quality of life and survival rate of these complex and prognostically unfavorable patients.

CONCLUSION

In this clinical case, we described a rare complication of multiple myeloma and severe restrictive cardiomyopathy due to secondary cardiac amyloidosis. The case's features were difficulties in verifying the underlying disease and its complications and the complexity of patient management according to modern principles of cardio-oncology.

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 the Local Ethics Committee of the Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov 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.

AUTHOR CONTRIBUTIONS

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

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REVIEW
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Prevention of Anthracycline-Induced Cardiotoxicity: The Good and Bad of Current and Alternative Therapies

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Doxorubicin (Dox)-induced cardiotoxicity (DIC) remains a serious health burden, especially in developing countries. Unfortunately, the high cost of current preventative strategies has marginalized numerous cancer patients because of socio-economic factors. In addition, the efficacy of these strategies, without reducing the chemotherapeutic properties of Dox, is frequently questioned. These limitations have widened the gap and necessity for alternative medicines, like flavonoids, to be investigated. However, new therapeutics may also present their own shortcomings, ruling out the idea of "natural is safe". The U.S. Food and Drug Administration (FDA) has stipulated that the concept of drug-safety be considered in all pre-clinical and clinical studies, to explore the pharmacokinetics and potential interactions of the drugs being investigated. As such our studies on flavonoids, as cardio-protectants against DIC, have been centered around cardiac and cancer models, to ensure that the efficacy of Dox is preserved. Our findings thus far suggest that flavonoids of *Galenia africana* could be suitable candidates for the prevention of DIC. However, this still requires further investigation, which would focus on drug-interactions as well as *in vivo* experimental models to determine the extent of cardioprotection.

Keywords: cardiotoxicity, doxorubicin, flavonoids, cardioprotection, drug-drug interaction

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INTRODUCTION

Over the years much effort has been placed on understanding the molecular and cellular biology of numerous cancers, which has led to rapid progressions in diagnostics, drug discovery and prevention of cancer-related morbidities and mortalities (1). In modern oncology, the introduction of chemotherapeutic regimens has been identified as a major contributing factor to the observed increased life expectancy of cancer patients. Notably, today, more than 67% of adult cancer patients will live up to 5 years after diagnosis, and over 75% of pediatric cancer patients will have a 10 year survival rate after diagnosis (2). Generally, chemotherapeutic drugs work by targeting cells at different phases of the cell cycle, which aids in predicting which drugs are likely to work well-together or be effective for a specific cancer (3). However, researchers have found that while chemotherapeutics were designed to target mutated and rapidly dividing cells, these drugs are unable to discern between cancer and healthy cells (4, 5). Therefore, normal cells, which can trigger

a self-initiated healing response, are also damaged, and eradicated during chemotherapy. Literature indicates that chemotherapeutic drugs, like anthracyclines (ATCs), are associated with a higher incidence of inducing cardiotoxic effects, which may progress into organ failure, relative to other cancer therapies (2, 6, 7). Therefore, the potent efficacy of ATCs is often overshadowed by their cardiotoxic side effects, which has limited their clinical use (8). Numerous studies investigating the mechanisms and risks associated with ATC-induced cardiotoxicity (AIC) have been conducted using doxorubicin (Dox) as a representative chemotherapeutic drug. Thus, the current review was formulated with a focus on Dox to discuss the incidence of cancer mortalities and risks associated with ATC-induced cardiotoxicity (AIC). Additionally, we also discuss the use of alternative therapies against AIC and how they may influence the pharmacology of Dox.

INCIDENCE OF CANCER-RELATED DEATHS

Approximately 10 million cancer-related deaths were recorded in 2020, making cancer the second leading cause of death worldwide (9). In Africa, the gruesome disparities that exist between public and private health sector's, which are driven by socio-economic factors, is directly reflected by the high cancer mortality rate vs. cancer incidence (**Figure 1**) (10). In contrast, most people in first-world regions, like America, have access to health insurance and therefore, present with a decreased cancer mortality rate vs. incidence when compared to the African and Asian communities (**Figure 1**) (10). To fully understand the global impact of cancer and progress made thereof, the American Cancer Society (ACS) reports that quantifying cancer-related mortalities, which are unlikely to be influenced by new diagnoses and survival outcomes within populations, can provide better insight into the disease burden (11).

In regions like South Africa platforms like the National Cancer Registry (NCR), which were formulated to provide data on the burden of cancer in both developing and developed countries with the aim of creating global awareness, remains poorly sourced and outdated (12). In this region, the registry was last updated in 2017 using data acquired from cancer deaths recorded in 2014. Such shortfalls and inconsistencies in data capturing make it difficult to efficiently track and manage the incidence of cancer and its co-morbidities in these demographics. Evidently, a planned population-based registry is clinically fundamental to drive decisions involving the screening and prevention of cancers, as well as the development of cancer treatment. Notably, since the early 1960's, the ACS has reported an increasing trend in the 5-year survival rate of cancer from 27 to 63%, for African patients, and from 39 to 68%, for Caucasian patients (11). In Southern Africa, an 86.9% survival increase in patients with the top eight cancers was reported from 2002 to 2020 (**Figure 2**) (10, 14). This improvement was largely driven by developments made against the top four cancers (breast, prostate, cervical and lung) in this demographic and the advances in chemotherapeutic agents, such as Dox.

PHARMACOLOGY OF DOXORUBICIN

The anticancer properties of Dox can be linked to the presence of flat aromatic moieties in Dox which allows it to form complexes with DNA by intercalating between the DNA base-pairs thereby, causing bidirectional transmission of positive torsion (15). The latter impedes topoisomerase II alpha (Top II-α) activity, which is needed for the regulation of DNA's super-helical state and unlinking intertwined DNA strands (16). Inhibition of Top II by Dox, stabilizes the DNA-Top II complex which disrupts the religation portion of the ligation-religation reaction (15). This results in DNA double-stranded breaks (DSBs) and fragmented nuclei with condensed chromatin, which triggers cancer cell death pathways such as apoptosis and necrosis (16). Also contributing to its tumoricidal and anti-carcinogenic properties, is Dox's ability to induce oxidative damage which is driven by the reduction of Dox to its secondary metabolites [doxorubicinol (Doxol), semiquinones (DSQ) and aglycones], a reaction catalyzed by NADPH cytochrome P450 (CYP) and carbonyl reductases (CBRs) (16–18). During its metabolism, the C-13 carbonyl group of Dox is reduced by CBR1 and CBR3 to Doxol, which then undergoes acid-catalyzed hydrolysis and then protonation at C-7 to form a double reduced C7-deoxyaglycone (16). C7-quinone-methide, which is a tautomer of C7-deoxyaglycone, generates reactive oxygen species (ROS) by covalently binding to DNA. Similarly, the production of Dox-semiquinones, *via* NADPH CYP enzymes, triggers oxidative stress-induced damage by generating superoxide's (O_2^-), hydroxyl radicals (•OH) and peroxides (H_2O_2), which causes further DNA damage (16) thereby, accelerating cancer apoptosis and aiding in combatting cancer (**Figure 3**). The metabolism of Dox can be further driven by mitochondrial and cytosolic NADPH dehydrogenases, xanthine oxidase (XO) or dehydrogenase (XDH), and nitric oxide synthases (NOS), to form more DSQs and aglycones. The increase in these metabolites in the circulatory system, has been associated with the occurrence of adverse reactions, like cardiotoxicity (19, 20). However, literature notes that while these metabolites induce cardiotoxicity more potently than their parent compound, Dox, these metabolites are not as effective at combatting cancer than Dox (21).

DIC: ARE TODAY'S CANCER SURVIVORS THE FUTURE CVD PATIENTS

Despite being a first-line anti-cancer drug, the clinical use of Dox has been surrounded by much controversy. On the one hand, the dramatic developments in chemotherapeutic drugs have increased the life expectancy of cancer patients, with the number of survivors projected to rise exponentially in the coming years (22). On the other hand, cancer survivors present with an increased cardiovascular disease (CVD) risk and are expected to develop cardiomyopathies and metabolic

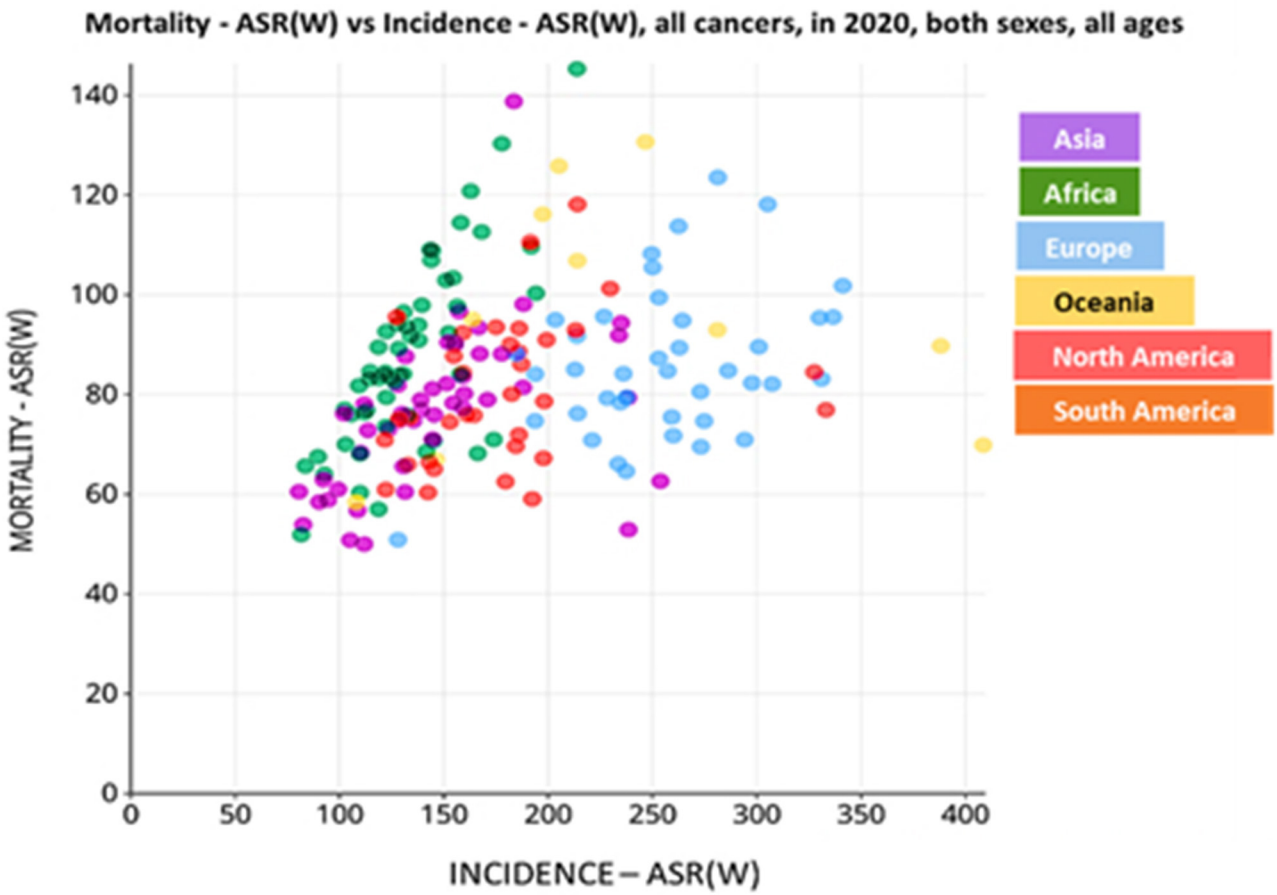

FIGURE 1 | Incidence of cancer-related deaths in the different continents, per 100,000 population. Data is based on the incidence vs. mortality rate of cancer patients in the year 2020. World age-standardized rate [ASR (W)]. Data source: Globocan 2020; World Health Organization (WHO) 2021.

toxicities within months or years after treatment cessation, consequently exacerbating the burden of CVDs (1). However, since chemotherapy has prolonged and enhanced the quality of life, the risk of cardiotoxicity is often outweighed by the overall benefit of cancer treatment. Generally, the risk of developing Dox-induced cardiomyopathy (DIC) is escalated as the cumulative dose of Dox increases: where a dose of 400 mg/m^2 increases the risk of DIC by 3–5% and that of 700 mg/m^2 effectuates an 18–48% DIC risk (**Table 1**) (8). As a result, oncologists caution that the cumulative dose of Dox should be limited to $\leq$ 550 mg/m^2 (2). However, even at relatively lower doses, the risk of developing cardiotoxicity is still present, especially amongst pediatric survivors (23). Evidently, children between the ages of 0–4 years old, who received lower ATC doses (1–249 mg/m^2) presented with an increased incidence of cardiomyopathy relative to children who were exposed to ATCs at an age of older than 13. In addition, higher ATC doses ($\geq$250 mg/m^2) led to an even higher risk of DIC in both 0–4 years old [relative rate (RR), 4.0; 95% cumulative incidence (CI), 2.5–6.4]and 4–13 years old kids (RR, 2.4; 95% CI, 1.7–3.5) at diagnosis when compared to kids older than 13 years (24). Thus, reiterating that younger individuals and patients receiving higher Dox doses have an increased predisposition to DIC.

Characteristics of Dox Cardiotoxicity

The cardiotoxicity in cancer patients is classified according to the time of onset, severity and characteristic, and may manifest as either acute, sub-chronic (early onset) or chronic (late stage) (**Table 2**). Often, these patients present with subclinical ventricular dysfunction leading to severe cardiomyopathy and eventually myocardial failure (23). Acute cardiotoxicity, which is the first type and is considered rare, manifests after a single dose or course of chemotherapy, and presents with reversible cardiac impairments during or immediately after treatment cessation administration (25). Sub-chronic cardiotoxicity, which is the second type and the principal form of cardiotoxicity, manifests within a year of chemotherapy and presents with irreversible dilated-hypokinetic cardiomyopathy leaning toward cardiac failure (8). The last and perhaps causing the highest burden is chronic cardiotoxicity, emerging years to decades after the last administered dose of Dox and causes irreversible left ventricular dysfunction (LVD) and eventual heart failure (HF)

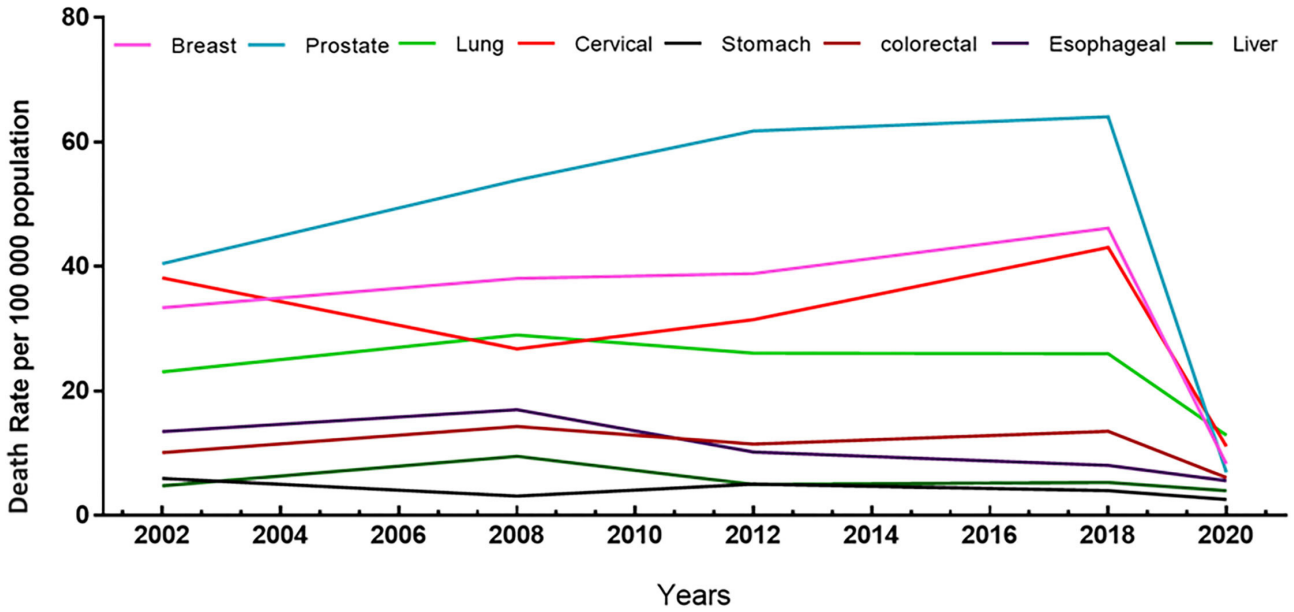

FIGURE 2 | Trends in cancer mortality in Southern Africa (2002–2020). Graph represents deaths associated with the top 8 cancers in South Africa for men and women, per 100,000 population, over the last decades. The data shows a sharp decline in the death rate of the top eight cancers in this region. Data source: Globocan 2021; Hamdi et al. (13).

(**Figure 4**) (7). On the contrary, Cardinale et al. (8) argued that classifying AIC into different categorizes might be primitive and biased, as these classifications were formulated in the 1980s around retrospective studies based on pediatric cancer survivors and their predisposition to cardiomyopathies (8). The authors explained that instead of being different entities, occurring at different times, AIC may be a continuous phenomenon that progresses from myocardial cellular injury to cardiac deformities which progress into asymptomatic cardiotoxicity, and eventually lead to overt HF. This view is supported by reports of increased cardiac troponin (cTn) levels with a concomitant reduction in global longitudinal strain (GLS) soon after the first administered dose of Dox. Beyond their ability to aid in diagnosing myocardial infarction, cTn often precede DIC and are commonly detectable in HF. Additionally, the assessment of GLS is more sensitive to LVD and a better predictor of cardiovascular outcomes when compared to LV ejection fraction (LVEF) (26). Therefore, it is not implausible that AIC might develop as early as after the first administered dose of Dox with clinical symptoms only being detected years after treatment cessation.

Mechanism of Dox-Induced Cardiotoxicity

Despite the extensive literature that is currently available on the pathophysiology of DIC, the exact mechanism by which Dox inflicts its adverse reactions remains inconclusive. Nonetheless, accumulating evidence suggests that the activation of cell death pathways during Dox administration may be the primary cause of DIC (27–29). These pathways are mediated by several biochemical processes namely, oxidative stress, inflammation, autophagy, DNA and mitochondrial damage (13, 29–32). The biochemical stimulation of these processes can be traced back to the pharmacology of Dox and its inability to distinguish between normal and cancerous cells, which allows metabolites such as Doxol, DSQ and Dox aglycones to accumulate in the myocardium whereby they inflict their adverse reactions (19, 20, 33). Considering cellular biology, several enzymes like NADPH oxidases (NOXs), NOSs, XOs and peroxisomes, which are located in the sarcoplasmic reticulum, mitochondria, and cytoplasm, account for a significant amount of ROS production (34–36). In the cardiac muscle, mitochondria-induced ROS production is driven by the reduction of Dox to DSQ, *via* NOXs. The infiltration of DSQ in the mitochondria disrupts the electron transport system (ETS), whereby DSQ displaces the antioxidant coenzyme Q10 to accept electrons from complex I and II and then donates them to molecular oxygen instead of transferring the electrons to complex III. This triggers the production of O_2^-, •OH and H_2O_2 (34). Additionally, the high affinity of DSQ to cardiolipin allows for its accumulation in the mitochondria resulting in excessive ROS production. By disrupting the ETS, which impairs bioenergetics and induces oxidative stress, DSQ are able to induce mitochondrial toxicity (34), thereby activating the intrinsic apoptotic pathway *via* the cytosolic translocation of caspase 3 to the nucleus (37). In addition, cardiac iron-overload, which is mediated by the enhanced expression of transferrin, an iron transferring glycoprotein, further accelerates Dox-induced oxidative stress by suppressing the activity of endogenous antioxidants [catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidases (GPXs)] which, consequently

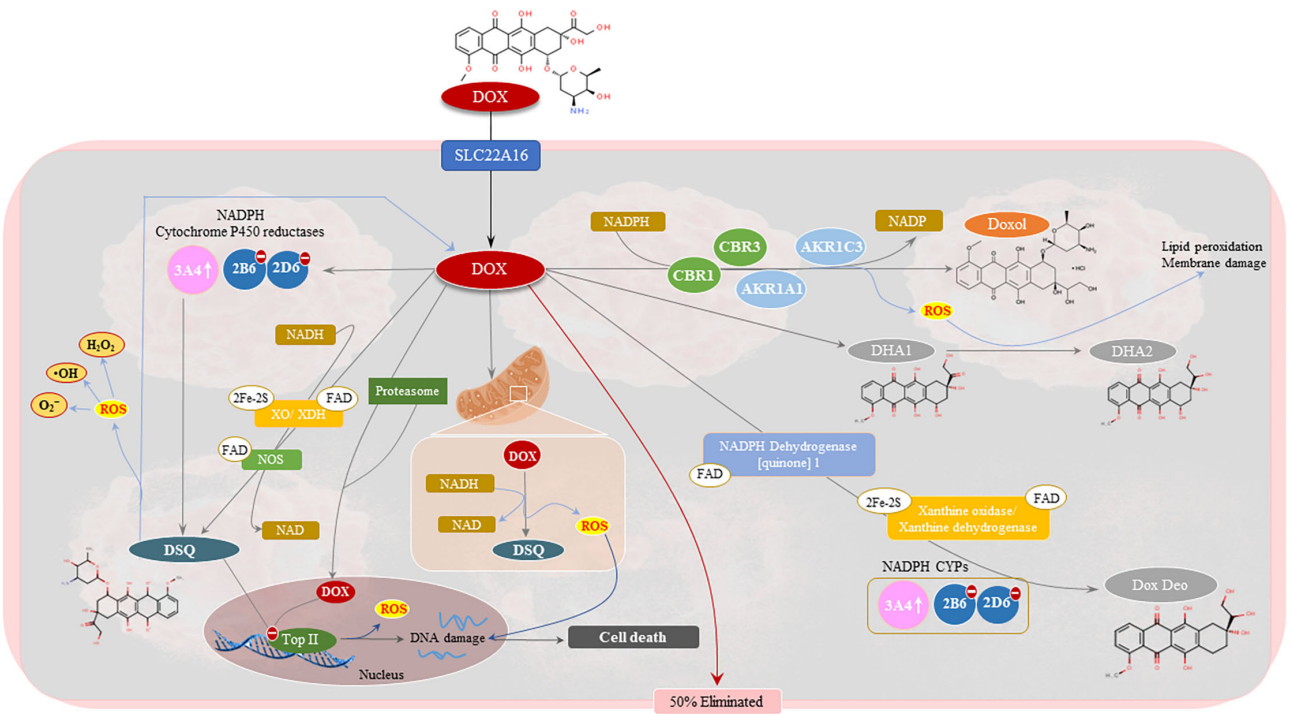

FIGURE 3 | Pharmacology of doxorubicin (Dox). Approximately 50% of Dox is eliminated from the body unchanged. The remaining Dox undergoes three metabolic processes to form doxorubicinol (Doxol), semiquinone radicals (DSQ), and 7-deoxyaglycone and hydroxyaglycone, respectively. The two-electron reduction of Dox forms Doxol *via* several oxidoreductases namely, carbonyl reductase 1 (CBR1) and 3 (CBR3), and aldo-keto reductases family 1 member (AKR1C3) and AKR1A1, in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). Semiquinone formation entails the one-electron reduction of Dox and is mediated by mitochondrial and cytosolic NADPH dehydrogenates, xanthine oxidase (XO)/dehydrogenase (XDH), NADPH cytochrome P450 (CYPs) reductases and nitric oxide synthases (NOS). Deglycosidation of Dox, in the presence of NADPH-CYPs, XO/XDH and NADPH dehydrogenase. The formation of these metabolites is associated with DNA damage, *via* topoisomerase II inhibition, and the production of hydroxyl radicals (•OH), superoxide anions (O_2^-) and peroxides (H_2O_2). This results in the activation of tumoricidal pathways which drive cancer cell death.

causes the peroxidation and rupture of membrane lipids and resultant ferroptosis (28). Much like cancer cells, which express Top IIα, cardiomyocytes also express nuclear and mitochondrial Top II, but in the β isoform. This makes the myocardium a suitable target of Dox toxicity, as Dox inhibits Top IIβ activity, to induce apoptosis *via* DNA damage (38). Dysregulated apoptosis is recognized as a necessary step for the onset of left ventricular (LV) remodeling, which is a hallmark of DIC. Another fundamental aspect to LV dysfunction is impaired inflammatory and autophagic response during Dox administration, which exacerbates myocardial cell death *via* the induction of pyroptosis and necroptosis, respectively (28, 39). The induction of Dox pyroptosis is mediated by the upregulation of interleukin 1β (IL-1β) and IL-18 in the presence of cytotoxic N-terminal of gasdermin D proteins, which are activated by NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasomes and caspases (1, 3, 4 and 11) (28). Contrary to the aforementioned biochemical processes, the effect of Dox on autophagy is controversial. This is because Dox either suppresses autophagy, resulting in the accumulation of damaged organelles which trigger oxidative stress and inflammation, or increases autophagic response to accelerate the removal of useful cellular components *via* apoptosis (13, 32, 40). Similarly, the upregulation of autophagy markers like light chain 3B (LC3B) has been previously shown to directly interact with receptor-interacting protein-1 (RIPK1) and RIPK3, which promote the formation of necrosomes in the presence of death receptors, such as Fas and tumor necrosis factor receptor 1 (TNFR1), thereby inducing necroptosis (28, 41, 42).

Prevention of DIC: Conventional Therapy and Limiting Dox Exposure

Often, cancer patients who are in complete remission lead very normal and healthy lives until they experience cardiovascular-related abnormalities, such as dyspnea and angina (20). Presumably, when this happens, irreversible signs of cardiotoxicity would have already manifested in these patients. In contrast, Cardinale et al. (8) challenged the irreversibility of AIC, arguing that close monitoring of cancer survivors could allow for early diagnosis and timely treatment initiation, which would likely reverse the cardiotoxicity and therefore, prevent the onset of DIC. The latter can also be mediated by two distinct approaches (**Figure 5**). The first approach entails the liposomal encapsulation and continuous infusion of Dox with the intension of reducing the plasma levels of Dox and its accumulation in the cardiomyocytes (2). The second approach

TABLE 1 | Factors associated with increased risk of DIC.

Cumulative dose and predictive risk		Other risks
150 mg/m^2	0.2%	Females
300 mg/m^2	1.6%	Children ≤ 4 years receiving low ATC dose Children ≥ 13 years old receiving high ATC dose
400 mg/m^2	3–5%	Adults > 65 years old
600 mg/m^2	8.7%	Pre-existing cardiac disease and hypertension
700 mg/m^2	18–48%	Combinational chemotherapy

ATC, anthracyclines.

TABLE 2 | Characteristics of DIC.

Type	Onset	Clinical features
Acute cardiotoxicity	During or immediately after chemotherapy Reversible	Cardiomyocyte injury Depression of myocardial contractility
Sub-chronic cardiotoxicity	Within 1 year after treatment cessation Irreversible Dose dependent	Asymptomatic cardiotoxicity Dilated cardiomyopathy
Chronic cardiotoxicity	More than a year after treatment cessation Irreversible Dose dependent	Overt cardiotoxicity Dilated cardiomyopathy Heart failure

involves the co-administrative use of Dox with dexrazoxane, which is the only U.S. Food and Drug Administration (FDA) approved cardioprotective drug used in chemotherapy.

Liposomal Encapsulation

Briefly, liposomes are miniature spheres that are spontaneously formed by singular or multiple hydrated phospholipid bilayers containing polar groups, which are oriented onto the inner and outer aqueous phase (43). The unique structure of liposomes allows for the encapsulation of bioactive amphipathic, lipophilic, and hydrophilic compounds within its aqueous or lipid compartments to improve drug efficacy. Contrary to standard Dox, liposomal encapsulation of Dox alters its pharmacokinetics. Literature demonstrates that liposomal Dox has a reduced plasma clearance rate which allows for much higher drug concentrations to be present in cancerous tissues than in normal tissues (44). This is because liposomal Dox effortlessly pierces through tumor vasculature, which is highly susceptible to penetration when compared to healthy tissue. With this in mind, the anticancer effect of Dox is preserved while the risk of developing cardiotoxicity is diminished (2). Unfortunately, the excessively high costs of liposomal Dox, which is currently priced at $1,727.18–2,480.54, for a 25-milliliter vial, has drastically limited its use, especially in developing countries. Another limitation is the selectivity of liposomal Dox which is currently FDA approved only for ovarian cancer, multiple myeloma and acquired immune deficiency syndrome–related Kaposi sarcoma (2). These apparent limitations along with the lack of long-term follow-up studies and the inconclusive evidence that exists on the efficacy of liposomal Dox in pediatric cancer patients has further limited its clinical application (45).

Continuous Infusion

As another preventative measure, administering Dox in divided doses has been shown to cause significantly lower adverse effects in cancer patients than those receiving bolus infusions of Dox (2, 46). The rationale is that consecutive daily doses of Dox builds patient tolerance and conditions the heart to be less susceptible to DIC, whereas single rapid infusions result in much higher Dox concentrations in the myocardium leading to more severe clinical cardiotoxicity in adult patients (2). In reality though pediatric cancer patients receiving continuous infusions have been shown to have no preserved or improved cardiovascular function when compared to children receiving bolus Dox doses (47). Instead, both participants presented with signs of deteriorating cardiovascular function at the 8-year follow up after the last dose of Dox had been administered, indicating that the continuous infusion had minimal preventative benefits (47). These disparities in treatment response, between adult and pediatric cancer patients, have since discredited the notion that administering Dox in continuous infusions can be considered as a preventative strategy.

Dexrazoxane: The Only FDA Approved Cardioprotective Drug

Currently, the only FDA approved cardioprotective drug and most reliable preventative option of DIC is the co-administrative use of Dox with Dexrazoxane (Dex). Briefly, Dex is an iron chelating agent that scavenges the pro-oxidants formed by Dox which drive oxidative stress and mitochondrial dysfunction (13, 48). Additionally, Dex alters the configuration of topoisomerase II beta (Top IIβ), to a closed-clamp structure by tightly binding to the ATP-binding sites of the topoisomerases. This inhibits the binding of Dox to Top IIβ thereby, preventing DNA damage and apoptosis. The cardioprotective benefits of this iron-chelating agent have been determined to be non-selective as the efficacy of Dex transcends numerous malignancies occurring in both adult and pediatric cancer patients (38, 49, 50). Evidently, in a clinical trial of advanced breast cancer, patients that were co-treated with Dex presented with significantly improved left ventricular ejection fraction (LVEF) when compared to patients receiving Dox alone (51). Similarly, Dex prevented AIC in pediatric cancer patients after a 5-year follow-up, where the mean LV fractional shortening and end-systolic dimension Z scores were determined to be noticeably better than those measured in children who had received Dox alone (49). This protection was further highlighted in Dex's ability to preserve the LV wall thickness and thickness-to-dimension ratio in cancer patients after 5-years of treatment cessation (49).

Although truly remarkable, the co-administrative use of Dex has been surrounded by considerable controversy. For instance,

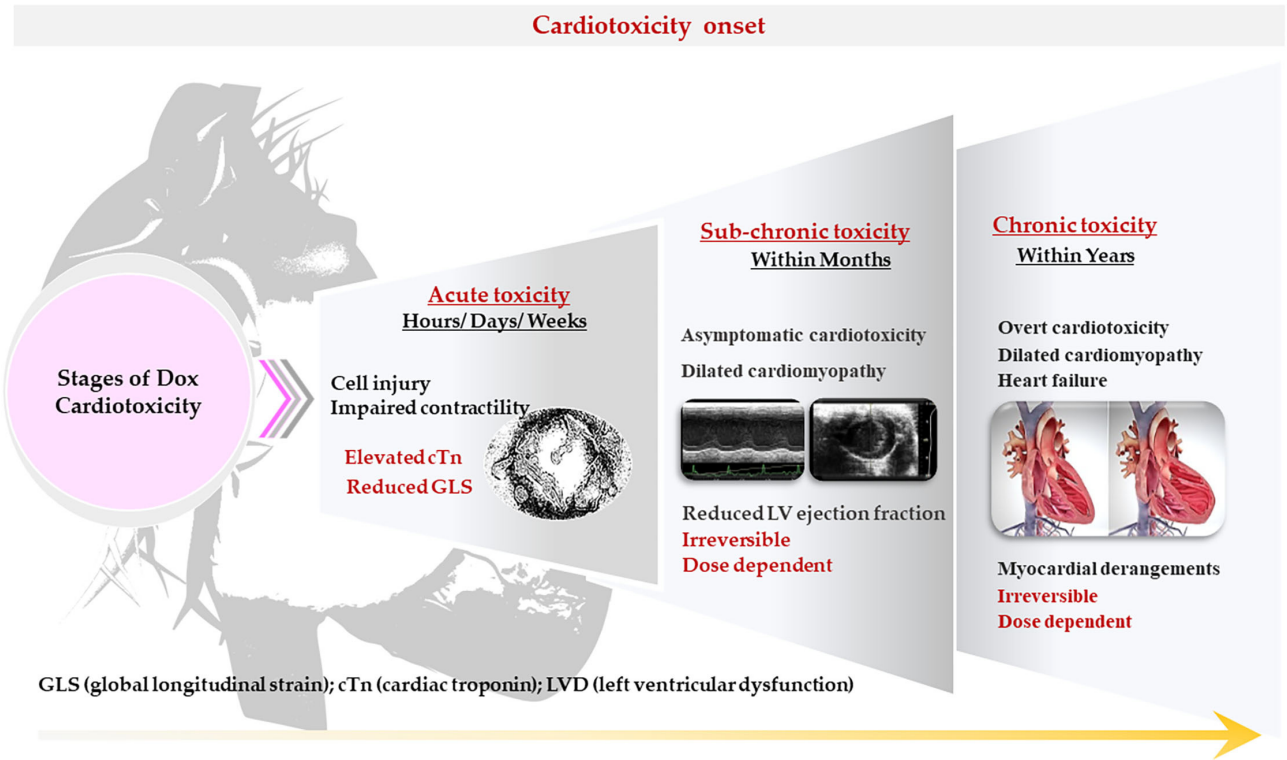

FIGURE 4 | Characteristics of doxorubicin (Dox)– induced cardiotoxicity. The cardiotoxicity in cancer patients is classified according to the time of onset, severity, and characteristics. It may manifest as either acute, sub-chronic (early onset) or chronic (late stage).

Dex is only FDA approved for females with metastatic breast cancer requiring an additional infusion of Dox to regulate tumor progression and eradicate the cancer, after they have received at least 300 mg/m^2 of chemotherapy (2). Furthermore, two clinical trials, on adult cancer patients, demonstrated an increased risk in the development of secondary malignant neoplasms when Dex was used (52, 53). Subsequently, in Europe and other jurisdictions, a ban in the use of Dex as a cardioprotectant in children was enforced to reduce the risk of developing secondary malignancies in these patients (52). While these claims have since been disputed, the American Society of Clinical Oncology (ASCO) guideline cautioned against the use of Dex in pediatric cancer patients due to the lack of conclusive evidence associated with the use of Dex (52). Another limitation of this drug is claiming that Dex offers greater cardioprotection to females than their male counterparts (2). Therefore, these limitations along with the high costs of Dex, which further limits its use in poorer communities, strongly advocates for the investigation of alternative therapies.

Other Cardiovascular Agents

Angiotensin Converting Enzyme Inhibitors

Although not FDA approved to be used concurrently with ATCs, several other cardioprotectants, aside from Dex, have been identified to have therapeutic benefits that may aid in alleviating the burden of DIC (23, 54). For starters angiotensin converting enzyme inhibitors (ACEI), which are historically known for their anti-hypertensive properties, are reported to mitigate heart failure by reducing cardiac afterload and systolic wall stress, decreasing aldosterone-induced fibrosis and apoptosis, whilst improving ventricular geometry (55, 56). These drugs are further said to curb the mortality rate in patients with asymptomatic LV dysfunction (55), which makes them suitable therapeutic options for the treatment of DIC. Indeed, a clinical study mimicking the prevention of chronic-cardiotoxicity revealed a gradual deterioration in cardiac function over a period of 12 months, as measured by an LVEF of 48% (56). However, the administration of enalapril, an ACEI, protected against myocardial damage by preserving LVEF, which was found to be 62% at the end of the study when compared to the 61.9% measured at baseline (56). In another study of acute-cardiotoxicity, cancer patients scheduled to undergo chemotherapy were co-treated with valsartan, which is an angiotensin receptor blocker (ARB), for 7 days with the aim (57). Findings from this study revealed the therapeutic ability of valsartan to improve ventricular function, which was indicated by a reduction in the serum levels of brain natriuretic peptide (BNP) and, LV end-diastolic diameter of the left ventricle (LVEDD) and corrected QT dispersion (QTcD) (57). However, although demonstrating promising prophylactic benefits against DIC, ACEI and ARB do not offer complete protection against DIC, but instead lowers the incidence of heart failure and premature death in cancer patients (58). Another area of concern

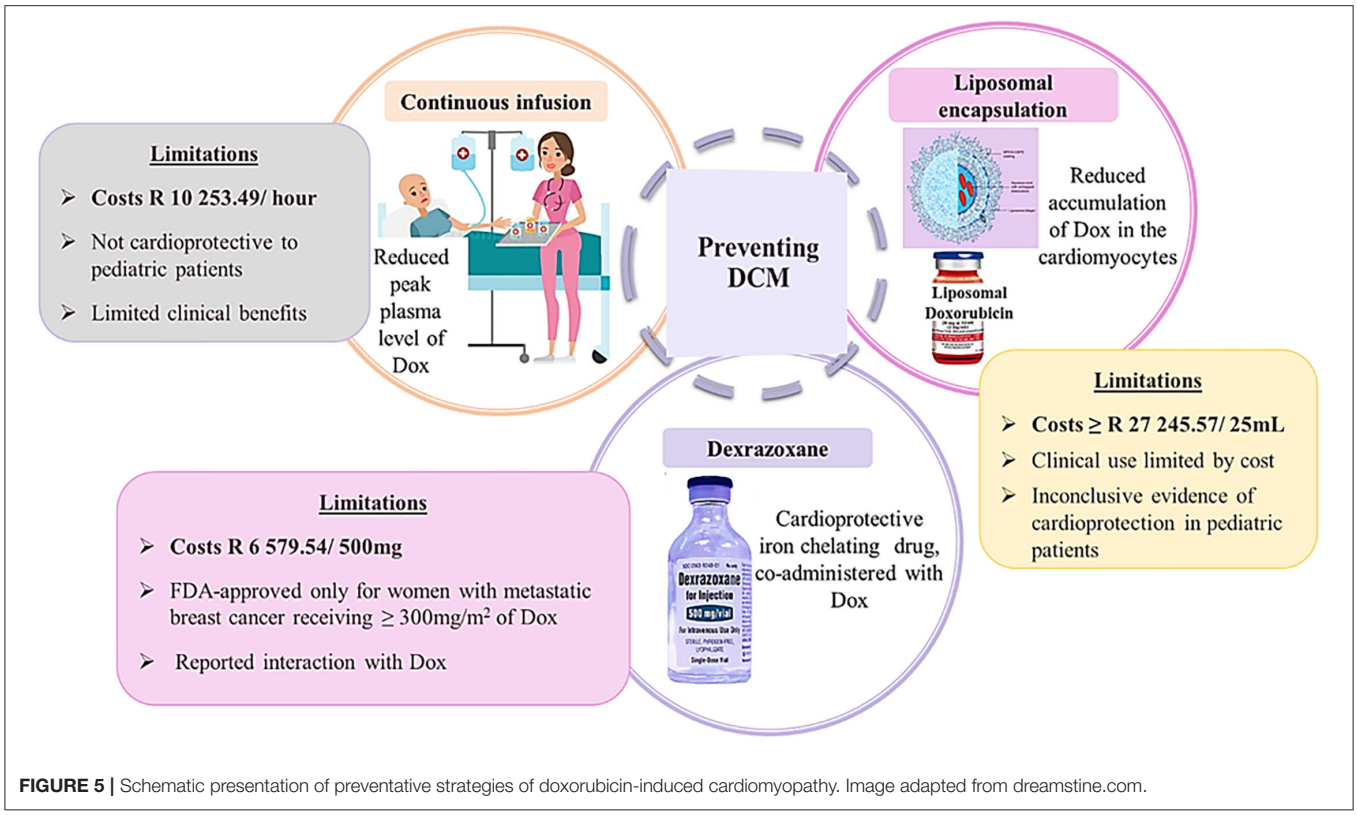

FIGURE 5 | Schematic presentation of preventative strategies of doxorubicin-induced cardiomyopathy. Image adapted from dreamstine.com.

is the high-cost-benefit ratio of administering ACEI (8), which may result in the inaccessibility of the drug to individuals from impoverished backgrounds.

Statins

Statins are best known for their ability to reduce low-density lipoprotein (LDL) cholesterol to aid in reversing atherosclerotic plaques, which alleviates the burden of coronary artery disease (59). In the context of DIC, the cardioprotective benefits of statins are associated with the drugs pleiotropic effects, which include their antioxidant and anti-inflammatory properties, as well as their ability to enhance endothelial function (8). This is especially important as one of the primary mechanisms of DIC involves the onset of cardiac oxidative damage (34, 38). In a mice model of DIC, atorvastatin ameliorated Dox-induced oxidative stress and DNA damage, which led to improved myocardial structural integrity (60). Similarly, LV systolic and diastolic function were significantly enhanced in rats co-treated with Dox plus rosuvastatin 4 weeks after treatment cessation (61). In newly diagnosed breast cancer patients, an observational clinical cohort study of, revealed that individuals that were co-treated with ATCs and statins had a lower risk of developing heart failure (HF) than patients that were only treated with ATCs (62). Lastly, pre-treatment with fluvastatin, in an acute model of DIC, demonstrated a significant reduction in oxidative stress, inflammation and apoptosis in the cardiac muscle (63). While these findings clearly highlight the beneficial properties of statins in cardiovascular health, it remains obscure whether these benefits can be sustained over a pro-longed period. Therefore, long-term studies are still needed to establish the long-term effects of statins in these patients.

Beta-Blockers

Literature also reports that the therapeutic properties exhibited by beta-blockers (β-blockers) may aid in alleviating the clinical burden of DIC (8, 58). Concisely, β-blockers have been FDA approved for the treatment of several CVDs and their comorbidities such as; hypertension, coronary artery disease, arrhythmias, myocardial infarction and congestive heart failure, just to name a few (64). A clinical study reported that carvedilol, a non-cardio selective β-blocker, prevents ventricular dysfunction in cancer patients receiving ATC treatment (65), while another demonstrated a significant reduction in myocardial strain impairments and troponin levels (66). In another clinical study involving HER2-negative breast cancer patients, receiving combinational chemotherapy including Dox, reported no apparent changes in LVEF and B-type natriuretic peptide between the placebo group and patients that had been treated co-treated with carvedilol (67). This study did, however, report a reduction in troponin I levels which they correlated to the reduced incidence of diastolic dysfunction in carvedilol treated patients (67). In another clinical study, the use of a selective β-blocker, nebivolol administered a week prior chemotherapy induction, preserved LV end-systolic (LVESD) and end-diastolic diameters (LVEDD) and serum levels of N-terminal (NT)-pro brain natriuretic peptide (NT-proBNP) (68). While nebivolol was

also found to sustain a 63% LVEF, the reduction in LVEF to 57% was still within normal range and not an indicator of cardiac dysfunction (68). In this study, cardiotoxicity was represented by the significant increase in proBNP levels in the placebo group (68). Seicean and colleagues revealed that the continuation of β-blockers, months after chemotherapy cessation, had a greater therapeutic outcome than administering β-blockers for only the duration of the chemotherapy cycles (69). This outcome was represented by the reduced HF incidence and new HF events (69). However, it appears that the usefulness of β-blockers against chemotherapy-related cardiotoxicity is controversial. For instance, an *in vitro* experimental study revealed the inability of metoprolol to prevent cardiotoxicity in C57Bl6 mice treated with Dox and trastuzumab (70). Similarly, Avila et al. (66) demonstrated carvedilol's inability to mitigate ATC-induced chronic cardiotoxicity in breast cancer patients (67).

Alternative Medicine: The Efficacy and Adverse Effects of Plant-Based Treatment

In recent decades a growing interest in alternative therapies, consisting of herbal extracts and plant-derived compounds, have been identified as a promising solution to combatting the burden of diseases like cancer and its associated side-effects namely cardiotoxicity, hepatotoxicity and nephrotoxicity, just to name a few (71–74). It is, therefore, no surprise that over 5,000 studies have investigated the therapeutic benefits of herbal-based treatments against DIC. To date, an excessive amount of research has been conducted on flavonoids to illuminate their pharmaceutical benefits as cardioprotective agents. Concisely, flavonoids are secondary metabolites of plants and have been shown to have anti-tumor and anti-oxidation properties in addition to improving cardiovascular outcomes by alleviating endothelial dysfunction and atherosclerosis (75–77). Accumulating evidence suggests that flavonoids can be very effective in attenuating DIC (78–80). For example, Apigenin, which is a non-mutagenic plant flavone, alleviated Dox-induced myocardial damage, in male rats, by preserving the hearts structural integrity and by improving its ejection fraction, fractional shortening, LV internal diameter end in diastole (LVIDd) and LVID end in systole (LVIDs) (81). These effects were associated with the flavonoid's ability to scavenge lipid peroxides through enhanced myocardial superoxide dismutase (SOD) activity and glutathione (GSH) content. Subsequently, a decrease in Dox-induced apoptosis *via* the reduction of BAX and Caspase-3 activity, and enhanced Bcl-2 expression, was observed (81). The reported benefits were further confirmed by the apparent reduction of myocardial injury biomarkers [cTn, lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB)], which explained the improved cardiac function in these animals.

Additionally, Latifolin, which is one of the major flavonoids found in *lignum dalbergiae odoriferae* and known for its anti-inflammatory and cardioprotective properties, was recently reported to have therapeutic benefits against DIC (80). In this study, Latifolin mitigated myocardial injury by decreasing macrophage expression of M1 [i.e., inducible nitric oxide synthase (iNOS) and Cluster of Differentiation 86 (CD86)] and M2 [i.e., CD206, interleukin-10 (IL-10) and IL-4R] polarization in Dox treated animals. As a result, a significant increase in LVEF and LV fractional shortening (LVFS), with a concomitant reduction in LDH levels, was observed (80). Similarly, Luteolin, a common flavonoid existing in numerous plants, mitigated Dox-induced cardiomyocyte contractile dysfunction by enhancing the cells peak shortening amplitude and maximal velocity of re-lengthening and shortening (79). Luteolin, further attenuated cardiomyocyte injury by preserving mitochondrial membrane potential and autophagy, *via* the Drp1/mTOR/TFEB signaling pathway, as well as preventing mitochondrial-induced ROS activity and apoptosis. While these flavonoids have proven to be highly effective at preventing or alleviating the burden of DIC, it remains unknown what effect these flavonoids will have on the anti-carcinogenic properties of Dox. Evidently, this is a major concern in cardio-oncology research as novel therapeutic agent may potentiate the progression of cancer by inhibiting the efficacy of chemotherapeutic agents. The fact that flavonoids naturally possess high antioxidant properties could potentially benefit the cancer cells, as they may redirect some of these antioxidants to enhance the activity of their own antioxidants thereby preventing Dox-induced oxidative stress and apoptosis, which may potentiate the cancer. For this reason, it is not only important that the search for novel cardioprotective agents continues, but that their effect in cancer models be investigated, especially when used with other chemotherapeutic drugs.

Cardioprotective Potential of Quercetin Against DIC

Quercetin is an important bioflavonoid, belonging to the class of flavanols, found in numerous plants and plant products such as, *Camellia sinensis*, grapes and *Nasturtium officinale* (**Figure 6**) (82). The therapeutic benefits of quercetin are primarily attributed to its anti-inflammatory, antioxidative, anti-proliferative and anti-histamine properties (83). These pharmacological benefits have been reported in experimental models of cardiovascular disease, hepatopathy and anti-cancer studies (84–86). Previously, Chen and colleagues (82) hypothesized that the cardioprotective properties of quercetin were driven by its effect on mitochondrial function *via* the activity of 14-3-3γ, a protein belonging to the highly conserved multifunctional 30 kDa acidic protein family. In this study, quercetin mitigated DIC by enhancing the expression of 14-3-3γ. This was demonstrated by an increase in the levels of endogenous antioxidants, GSH, SOD, CAT and GPx, in the cardiomyocytes (84). Subsequently, the cardiac cells were protected from Dox-induced oxidative damage, as shown by the significant reduction in lactate dehydrogenase (LDH), lipid peroxidation and ROS activity. Additionally, quercetin decreased the activity of the apoptotic markers, caspase 3 activity, mitochondrial permeability transition pore and annexin v and propidium iodide (84). In another study, quercetin was shown to preserve the structural integrity of the cardiomyocytes by decreasing Dox-induced expression of proteins involved in modulating protein folding (83). The downregulation of these proteins led to a reduction in ROS activity which reduced the degree of incorrectly folded proteins thereby, attenuating the expression of 60 kDa heat shock protein and heat shock protein beta-1, alpha-crystallin

FIGURE 6 | Plant sources of quercetin, a bioflavonoid with numerous medicinal properties.

B, stress-induced-phosphoprotein 1 and T-complex protein 1 (83). Dong et al. (87) then showed a significant reduction in DNA damage and mitochondrial ROS production following the co-administrative use of Dox with quercetin. These findings were supported by another report demonstrating how quercetin mitigates Dox-induced myocardial dysfunction by improving LVEF, LVFS, LVEDD and LVESD in C57/BL6 mice (87). Consequently, an increased survival outcome in the quercetin plus Dox treated mice vs. those treated with Dox alone was observed (87). While the cardioprotective benefits of quercetin are undeniable and transcend DIC, it is imperative that we establish the effect of quercetin on cancer cells and its effect on the anti-carcinogenic properties of Dox.

Therapeutic Benefits of Quercetin Against Cancer

A study by Wang et al. (85) assessed the anti-cancer properties of quercetin on human hepatocellular carcinoma cells HepG2 and Hep3B, triple negative MDA-MB-231 breast cancer cells, and colorectal cancer cells HCT116. In this study, quercetin was shown to trigger autophagy in the cancer cells by increasing the expression of autophagy markers, ATG7, LC3-II and p62 (85). These findings promoted lysosomal activation and nuclear translocation of transcription factor EB in the cancer cells, which enhanced quercetin-induced cell death, independent of p53 expression. The former was attributed to the degradation of ferritin light chain (FTL) and ferritin heavy chain (FTH), and the induction of lipid peroxidation, which resulted in cancer cell toxicity (85). These finding are especially important as the release of iron from ferritin storage has been shown to cause iron-overload, which may trigger the activation of tumor suppressor genes initiating ferroptosis (88). Additionally, quercetin also enhanced the expression of the pro-apoptotic proteins, Bid, cytochrome C expression in the cytoplasm and cleavage of caspase 9, which accelerated cancer cell death *via* apoptosis (85). The anti-cancer properties of quercetin have also been reported in tumor bearing mice, mimicking a breast cancer model (89). Here, quercetin stimulated rapid tumor regression and significantly increased animal survival when compared to the untreated mice (89). Similarly, the anti-carcinogenic properties of quercetin also prevented angiogenesis *via* enhanced expression of thrombospondin-1, which is an endogenous anti-angiogenic factor protein that inhibits tumorigenesis (90).

The Effect of Quercetin on the Pharmacokinetic Profile of Dox

In the context of co-administering Dox with quercetin, data obtained from previous studies and PubChem indicate that quercetin is a strong inhibitor of CYP2D6 ($0.65 \pm 0.13\,\mu M$) and CYP3A4 ($5.5 \pm 0.7\,\mu M$) (91, 92). The inhibitory effect of quercetin on CYP3A4 ($1.97\,\mu M$) was also confirmed by Choi et al. (21), who additionally showed an inhibition of

P-glycoprotein (P-gp), a Dox transporter, in rats and MCF-7/ADR cells that were co-treated with Dox plus quercetin. As a general rule, drugs that are inhibitors or substrates of the same drug should not be administered simultaneously. However, considering that Dox is a known potent inhibitor of CYP2D6 and a substrate of CYP3A4, these findings suggest that quercetin may influence the pharmacokinetic profile of Dox. It is well-documented that the metabolism of Dox to its secondary metabolites is facilitated by the induction of CYP3A4, which consequently increases the accumulation of its cardiotoxic metabolites in the myocardium (33). Therefore, the inhibition of CYP3A4, as well as other metabolizing enzymes, could be an alternative therapeutic target that may aid in alleviating the burden of DIC and further explains the cardioprotective benefits of quercetin against DIC. The former may also aid in enhancing the efficacy of Dox through increased plasma levels of the unmetabolized Dox in the absence of CYP3A4 and CYP2D6 activity. Indeed, Choi et al. (21) revealed that co-administering Dox with quercetin enhanced the peak plasma concentrations and bioavailability of Dox. This increase was attributed to the inhibition of P-gp which decreased phase I metabolism of Dox resulting in an increased absorption of Dox in the gastrointestinal tract (21). Therefore, these findings strongly suggest that the use of quercetin as a cardioprotective alternative against DIC is unlikely to reduce the chemotherapeutic benefits of Dox.

Pinocembrin, a Diversely Therapeutic Flavonoid, Attenuates DIC

Another flavonoid of interest is pinocembrin (Pin), which possesses potent cardioprotective benefits against DIC (93). Briefly, Pin is a pharmacologically active flavonoid found abundantly in propolis and may also be isolated in numerous plants such as *Galenia africana* and *Asteraceae* families, to name just a few (94, 95) (**Figure 7**). Our laboratory was the first to investigate the co-administrative effects of Pin with Dox in an *in vitro* cardiomyocyte and cancer cell model. Since Dox accumulates in cardiac mitochondria, it disrupts the transfer of electrons across the electron transport chain (ETC) where it re-directs the electrons to generate ROS and trigger mitochondrial outer membrane permeabilization (MOMP) whilst impairing mitochondrial bioenergetics (13). Generally, MOMP is considered an irreversible process that drives end-stage cell death, such as apoptosis and necrosis, *via* the activation of caspases and autophagy, by diffusing the presence of several proteins that are usually situated between the outer (OMM) and inner (IMM) mitochondrial membranes inside the cytosol. In our study, we mimicked an *in vitro* model of chronic DIC by exposing cardiomyocytes to Dox for 6 days. We then showed that Pin, as an adjunct to Dox, alleviated mitochondrial-induced ROS and lipid peroxidation by enhancing the antioxidant capacity (GSH and SOD) of the cardiomyocytes when compared to cells that were treated with Dox alone. Consequently, cardiac mitochondrial function was significantly ameliorated after co-treatment with Pin, as could be seen by increased mitochondrial flux ratios, ATP-linked respiration, ATP turnover and maximal respiration in the cells, as well as an increase in cells' spare respiratory capacity. With this improvement, the cardiomyocytes mitochondrial membrane integrity was preserved, which led to a noticeable reduction in caspase 3/7 activity and resultant apoptosis (93).

These findings were recently confirmed in an *in vivo* study conducted by Gu et al. (96). In this study, co-treatment with Pin attenuated Dox-induced cardiac dysfunction by improving the LVEF, LVFS, LVIDd and LVIDs of male C57BL/6 mice. Likewise, co-treatment with Pin decreased myocardial fibrosis and injury, as determined by histological analysis and reduced serum LDH and CK-MB levels (96). Since Dox triggers numerous cell death pathways, other than apoptosis, the authors studied the effect of Pin on Dox-induced pyroptosis, which is initiated by an impaired inflammatory response. As a co-treatment, Pin attenuated pyroptosis-mediated cell death by reducing caspase-1 activity, protein and serum expression of the inflammatory cytokines, IL-1β and IL-16, as well as inflammasomes, NOD-like receptor protein 3 (NLRP3) and gasdermin-D (GSDMD). This reduction could be attributed to Pin's ability to activate the Nrf2/ Sirtuin 3 (Sirt3) pathway, which suppresses cell death and DIC (96).

The Effect of Pinocembrin on the Efficacy of Doxorubicin as a Chemotherapeutic

While alternative therapies have proven to be quite effective at protecting against the onset and progression of DIC in *in vitro* and *in vivo* cardiac experimental models, most of these therapies have not been investigated in cancer models to determine their effect on the anti-carcinogenic properties of chemotherapeutic drugs. The latter is an ongoing problem in cardio-oncology research as most plant-derived cardioprotective agents have high antioxidant and anti-apoptotic properties, which could benefit cancer cells by boosting their endogenous antioxidant levels and in turn, protects them against Dox-induced cytotoxicity. This outcome would be quite detrimental, especially for cancer patients, as this would not only potentiate cancer progression but, could very well increase cancer-related mortalities. For this reason, it is crucial that when investigating novel cardioprotective agents, in models of DIC, the risks associated with these agents be simultaneously assessed in cancer models to ensure that the efficacy of chemotherapeutic drugs is preserved and not inhibited by new cardioprotectants (97). In this context, our group investigated the efficacy of Dox when used in combination with Pin in human estrogen receptor positive breast cancer cells (93). In this study, Pin as an adjunct to Dox, had no significant effect on the antioxidant profile of breast cancer cells which was demonstrated by the comparable GSH and GSSG levels between these cells and those treated with Dox alone. In this way Dox was still able to induce oxidative stress by channeling electrons away from the ETS which was confirmed by the observed reduction in the cancer cells metabolic status. Consequently, the efficacy of the mitochondria was compromised, which facilitated Dox-mediated mitochondrial damage and in turn triggered cell death pathways. We further found that while co-treatment with Dox plus Pin induced relatively lower apoptosis when compared to cells treated with Dox alone, Pin, as an adjunct to Dox, led to a significantly higher degree of necrosis in the breast cancer cells.

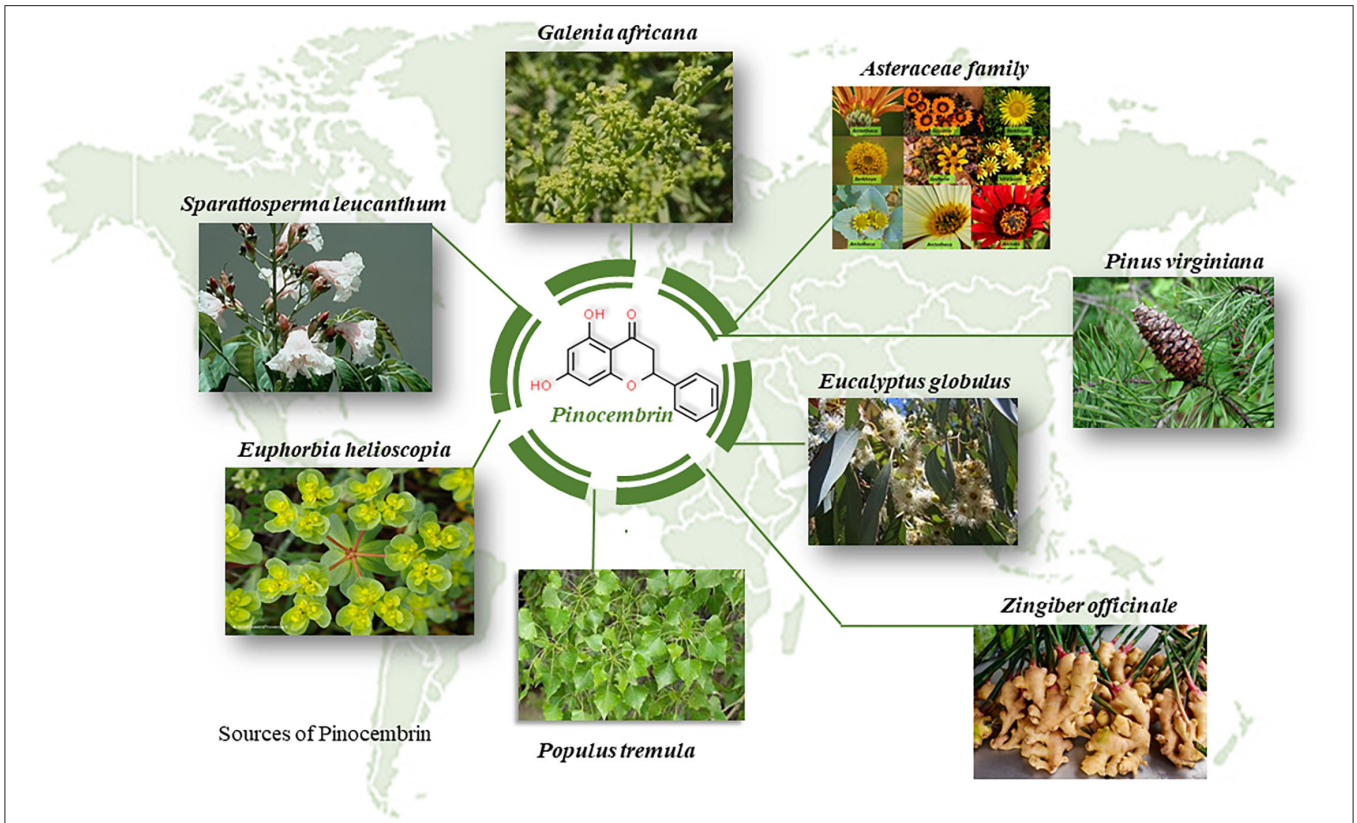

FIGURE 7 | Plant sources of pinocembrin all over the globe. Pinocembrin is a plant derived flavonoid with numerous therapeutic benefits.

These findings suggest that the co-administration of Dox plus Pin might synergistically aid in eradicating the cancer.

The Potential Effect of Pinocembrin on the Pharmacokinetic Profile of Dox

While only two studies have reported on the prophylactic benefits of Pin against DIC, the extensive pharmacological properties of Pin have led to its approval as a novel therapeutic drug by the Chinese Food and Drug Administration (CFDA), and its safety and pharmaceutical benefits are currently being studied in phase II clinical trials (94). Previous clinical and pre-clinical experimental studies have demonstrated the good pharmacokinetic profile of Pin, which is highlighted by its rapid absorption and wide distribution with negligible residue accumulation (94, 98, 99). In the context of Dox, Pin being a known inhibitor of CYP3A4 and also being implicated in the inhibition of CYP2D6, suggests that co-administering Dox with Pin might give rise to herb-drug interactions (100). Depending on how potently Pin inhibits CYP2D6, in comparison to Dox, may either increase the bioavailability of Dox plasma concentration or reduce it. Since Pin also inhibits CYP3A4 the metabolism of Dox *via* NADPH CYP reductases may be impaired resulting in a reduction in Doxol, semiquinones and aglycones in the circulatory system, and in this manner reduce the severity of cardiotoxicity. Indeed the use of Pin as an adjunct to Dox has already been shown to mitigate DIC therefore, the effect of Pin on CYP3A4 potentially explains how Pin influences the pharmacokinetic profile of Dox to offer cardioprotection (93, 96). Furthermore, the reduction in the biotransformation of Dox to its secondary metabolites could also be considered beneficial in eradicating cancer since Dox, in its unmetabolized form, is reported to have a more potent tumoricidal effect than its metabolites, which are suggested to suppress the anti-cancer properties of Dox (33, 101, 102). These observations are in line with our previous results on the MCF-7 breast cancer cells which revealed no significant reduction in the apoptotic effects of Dox when co-administered with Pin. Nonetheless, the validity of these claims still requires further investigation.

Risks of Drug-Interactions Between Flavonoids and Chemotherapeutics

Considering the characteristics of DIC and the combinational use of novel cardio-protectants with Dox, the risk of inducing drug-drug interactions, that may present with clinically significant reactions, is quite high. This view is supported by the U.S Food and Drug Administration (FDA) who stipulated that due diligence must be done when introducing new therapeutics, by conducting pre-clinical and clinical studies before these drugs are marketed to be used by the public (103). The initial screening of pharmacokinetic profiles of new therapeutics

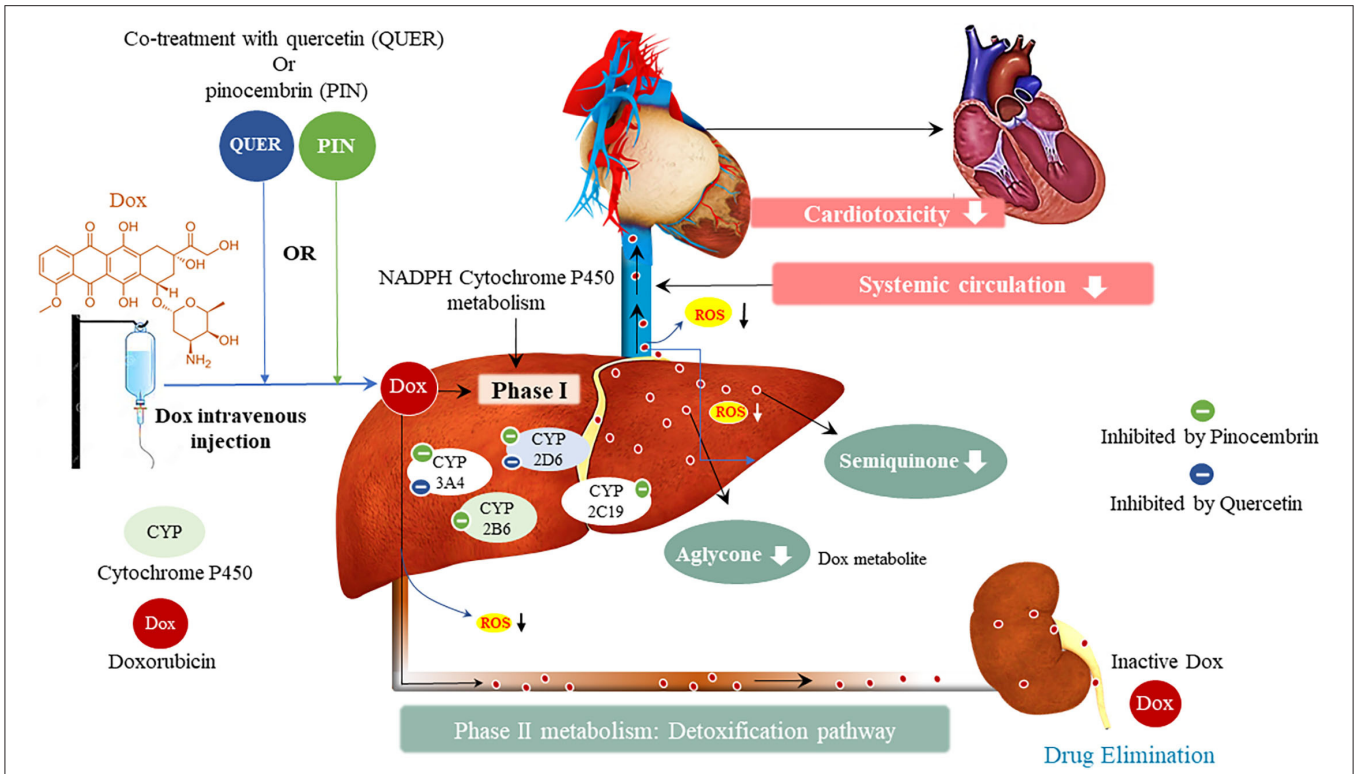

FIGURE 8 | Metabolism of doxorubicin (Dox) and resultant Dox-induced cardiotoxicity, and the potential effect of pinocembrin and quercetin on Dox metabolism. Cytochrome P450 (CYPs) enzymes allow us to predict the pharmacokinetic and pharmacodynamic variability that may exist in novel therapeutics. Doxorubicin inhibits CYP2D6, CYP2C19, and CYP2B6, whilst inducing CYP3A4. The interaction of Dox with these metabolizing enzymes formulates the toxic metabolites semiquinone and aglycone, which induce cardiotoxic side effects.

is done by performing *in vitro* experiments using Vivid® recombinant CYP450 enzymes, which measure the activity of drug metabolizing enzymes. This highlights the importance of *in vitro* studies as it is not feasible to study unanticipated drug-drug interactions in human subjects (104). Briefly, CYPs are metabolizing enzymes that drive the phase I metabolism of most drugs and lipophilic xenobiotics, which make them relevant entities in clinical pharmacology (**Figure 8**) (105). The CYP enzymes, that may be associated with chemotherapy, are categorized into two classes, namely class I and class II. Class I enzymes (CYP1A1, CYP1A2, CYP2E1 and CYP3A4) lack functional polymorphic relevance and are active in the metabolism of pre-carcinogens and other drugs (106). Class II CYPs (CYP2C9, CYP2C19 and CYP2D6) are highly polymorphic and are responsible for the phase I metabolism of various drugs, but not pre-carcinogens (106).

In the context of combinational treatment, using Dox with other CYP2D6 and CYP3A4 inhibitors, has been demonstrated to cause clinically significant interactions, which are likely to enhance Dox plasma concentrations thereby, increasing the severity and incidence of adverse reactions even at lower doses (107, 108). The opposite is also true, the concurrent use of Dox with other CYP2D6 and CYP3A4 inducers, may accelerate drug clearance which would reduce the efficacy of the drug thereby, potentiating the disease state (107). In this view, it is important to note that the drug-drug interactions can have minor, mild or fatal effects depending on the type of inhibition, i.e., reversible or irreversible (109). These type of inhibitions are based on the inactivation of the CYP enzyme *via* metabolic intermediates that bind reversibly or irreversibly to the enzyme. The clinical implications of the irreversible inhibition are expected to last longer than those of the reversible inhibitor after multiple treatment doses (100). This enables clinicians to plan for the appropriate scheduling of sequential regimens that either both inhibit or induce CYP2D6 and CYP3A4. The use of combinational therapy is further supported by the large and flexible active sites of the CYP enzymes, which readily adapt to concurrently accommodate several substrates with distinct structures, without inducing any adverse reactions (100, 109). This might explain how Pin, which is known to cause irreversible CYP3A4 inhibition (100), was able to mitigate Dox-induced cardiotoxicity without reducing the anti-carcinogenic properties of Dox (93).

Although CYP2C19 and CYP2B6 have not been closely associated with Dox, they have been reported to influence cardiovascular outcomes and are involved in the metabolism of other chemotherapeutic agents (106). The inhibition of CYP2C19, in patients with acute coronary syndrome, has been implicated in the occurrence of stent thrombosis and myocardial death (110). In addition, CYP2C19 inhibition in a clinical

study involving readmitted patients with myocardial infarction, demonstrated an increased risk of reinfarction (111). In contrast, another clinical study, of patients receiving dual antiplatelet therapy, revealed no significant effect on platelet aggregation following CYP2C19 inhibition (112). In essence these variations in the clinical outcome of CYP2C19 activity highlight the intricacy of pharmacokinetics in treatment and disease response.

CONCLUSION

In essence the major issue with the prevalence of DIC is the efficacy of Dox, as an anti-carcinogen. Due to its contribution to the overall improvement in the survival of cancer patients, Dox has been kept in clinical practice whilst the risk of developing cardiovascular dysfunction accumulates. It is, therefore, no surprise that a plethora of work has focused on finding alternative therapies, by using natural compounds like flavonoids, to prevent DIC. Since flavonoids have been continuously reported to mitigate Dox-induced cardiac oxidative damage and cardiomyocyte loss, suggests their suitability as cardioprotective agents against DIC. However, considering the risk of drug interactions, adopting the concept of drug-safety at the initial screening of novel cardioprotectants may aid in preventing unanticipated drug-interactions that may present with clinically significant reactions. In this way, the discovery and development of new alternative therapies, as adjuvants to Dox, can be fast-tracked.

AUTHOR CONTRIBUTIONS

NS conceptualized and wrote the manuscript. NS, RJ, SN, LM, BH, KG, DV, and RB edited and approved the final draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Time-Dependent Effect of Anthracycline-Based Chemotherapy on Central Arterial Stiffness: A Systematic Review and Meta-Analysis

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Background and Aims: Anthracycline-based chemotherapy (ANTH-BC) has been proposed to increase arterial stiffness, however, the time-dependency of these effects remain unclear. This systematic review and meta-analysis aimed to investigate the time-dependent effect of ANTH-BC on markers of central aortic stiffness, namely aortic distensibility (AD) and pulse-wave-velocity (PWV) in cancer patients.

Methods: An extensive literature search without language restrictions was performed to identify all studies presenting longitudinal data on the effect of ANTH-BC on either AD and/or central PWV in cancer patients of all ages. An inverse-variance weighted random-effect model was performed with differences from before to after chemotherapy, as well as for short vs. mid-term effects.

Results: Of 2,130 articles identified, 9 observational studies with a total of 535 patients (mean age 52 ± 11; 73% women) were included, of which four studies measured AD and seven PWV. Short-term (2–4 months), there was a clinically meaningful increase in arterial stiffness, namely an increase in PWV of 2.05 m/s (95% CI 0.68–3.43) and a decrease in AD (albeit non-significant) of −1.49 mmHg-1 (−3.25 to 0.27) but a smaller effect was observed mid-term (6–12 months) for PWV of 0.88 m/s (−0.25 to 2.02) and AD of −0.37 mmHg-1 (−1.13 to 0.39). There was considerable heterogeneity among the studies.

Conclusions: Results from this analysis suggest that in the short-term, ANTH-BC increases arterial stiffness, but that these changes may partly be reversible after therapy termination. Future studies need to elucidate the long-term consequences of ANTH-BC on arterial stiffness, by performing repeated follow-up measurements after ANTH-BC termination.

Systematic Review Registration: [www.crd.york.ac.uk/prospero/], identifier [CRD42019141837].

Keywords: vasculotoxicity, aortic distensibility, pulse-wave-velocity, breast cancer, lymphoma

Abbreviations: AD, aortic distensibility, ANTH-BC, anthracycline-based chemotherapy, CFPWV, carotid-femoral pulse-wave velocity, CI, confidence interval, CMR, cardiac magnetic resonance, PR, phase- contrast, PWV, pulse-wave-velocity, CVD, cardiovascular disease, CV, cardiovascular.

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HIGHLIGHTS

- Besides myocardial dysfunction, vascular toxicity has been recognized as a potential side effect of ANTH-BC that can be quantified by measurement of arterial stiffness, a robust surrogate marker of cardiovascular disease.
- Results from this analysis suggest that in the short-term, ANTH-BC increases arterial stiffness, but that these changes may (partly) be reversible after therapy termination.
- This is a novel finding and different from the permanent negative effects of ANTH-BC on myocardial function.
- However, given the high heterogeneity among studies included in this meta-analysis, additional studies will have to address the limitations, including measurement of confounders, and performing repeated and standardized follow-up measurements of arterial stiffness after ANTH-BC termination.
- Assessment of arterial stiffness may have the potential to contribute to risk prediction and clinical decision making in patients with ANTH-BC.

INTRODUCTION

Heart disease and cancer are the leading causes of mortality worldwide (1). Due to remarkable improvements in screening, diagnosis, and treatment of many cancers, the number of cancer survivors is steadily increasing (2). However, cancer survivors have an increased risk for cardiovascular disease (CVD), either as a result from shared cardiovascular risk factors and suboptimal lifestyle choices or from toxicities of cancer treatment (3–5). A retrospective cohort study has shown that 10 years after cancer diagnosis the risk for death from CVD exceeds the risk of death from cancer (3).

Anthracyclines are very effective chemotherapeutic agents used for treatment of solid tumors and hematologic malignancies. However, due to their dose-dependent cardiotoxic effects, such as systolic and/or diastolic left ventricular (LV) dysfunction and heart failure (6–10), their repetitive administration is limited. Hence, monitoring of LV function by echocardiography before and after treatment is recommended (11, 12). Additionally, many anticancer drugs also have adverse effects on the vascular endothelium (13, 14), It has been proposed that anthracycline-based chemotherapy (ANTH-BC) may increase arterial stiffness (15) *via* generating reactive oxygen species and promoting oxidative stress (16, 17). This in turn leads to structural changes within the vascular matrix and thus interferes with the regulation of vascular smooth muscle tone (14). Both, *in vitro* and *in vivo* studies found that ANTH-BC also causes apoptosis of vascular endothelial cells, which may impair vasodilatory and contractile responses and lead to endothelial dysfunction (18, 19).

The most established non-invasive methods to assess central arterial stiffness are central pulse wave velocity (PWV) (20) and aortic distensibility (AD) by cardiac magnetic resonance (CMR) or echocardiography (20, 21). Both methods have been shown to predict CV events and CV mortality in various populations (22, 23).

Previous studies on the vasculotoxic effects of chemotherapies have mainly focused on anti-angiogenic drugs and some of the newer anticancer signaling inhibitors (24, 25). A recent review and meta-analysis has summarized effects of various vasculotoxic chemotherapies, including anthracyclines, on arterial stiffness from longitudinal and cross-sectional studies (26). Due to often various successive treatments in cancer patients, these cross-sectional studies do not allow the identification of the vasculotoxic effect of isolated ANTH-BC. ANTH-BC–induced vasculotoxicity may further be aggravated by the individual CV risk factor profile (i.e., current smoking, obesity, etc.), which are difficult to fully control for in cross-sectional studies. To date, several small longitudinal studies have assessed arterial stiffness before and after ANTH-BC, but the vascular effects of ANTH-BC over time remain unclear. An evidence synthesis is important because long-term vascular dysfunction may increase the risk for cardiovascular events and mortality (22, 27–29). Therefore, we have conducted a systematic review to appraise the literature regarding the time-dependent effect of ANTH-BC on markers of central aortic stiffness, namely PWV and AD measured before and after ANTH-BC in cancer patients.

METHODS

Study Design

The search was conducted according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) recommendations. The original study protocol was registered prospectively in PROSPERO (CRD42019141837).

Study Eligibility

Studies were eligible if they met all of the following criteria: (a) experimental or observational studies (prospective or retrospective); (b) reporting on the effect of ANTH-BC on either AD and/or central [carotid-femoral (cf)/aortic arch/carotid artery] PWV in cancer patients of all ages; (c) longitudinal assessment with baseline measurement before administration of anthracyclines and at least one measurement during or after ANTH-BC; (d) based on human data. We did not include studies which provided PWV from peripheral arteries or derived from pulse wave analysis, due to the fact that PWV is not directly measured in pulse wave analysis but calculated based on the estimated distance of assumed reflection sites (30).

Database Search

The MEDLINE, Embase, Web of Science and the Cochrane Library databases were searched for eligible studies from database inception to February 18, 2021. The search strategy was built based on the PICO strategy. A combination of free textwords and MeSH subheadings were used, including the terms *cardiotoxicity, aortic distensibility, central pulse wave velocity*, *anthracycline*, *doxorubicin*, *daunorubicin*, *adriamycin*, *idarubicin*, *epirubicin*, appropriately linked with the Boolean operators AND or OR. Case reports, comments, and editorials were excluded. No language restrictions were applied. The full search algorithm for

each database can be found in the **Supplementary Appendix** (**Supplementary Table 1**).

Study Selection and Data Extraction

Upon removal of duplicate publications, the title and abstract of the selected studies were screened by 3 independent reviewers (C.S., P.E. N.G.). For each potentially eligible study, two reviewers (C.S., P.E.) independently assessed the full manuscripts. In cases of disagreement, a decision was made by consensus or the third reviewer was consulted. The reference lists of selected publications were also manually searched to identify additional eligible studies. For data extraction, a template was used including information on study size and design, baseline population, location, age at baseline, anthracycline-dose, duration of follow-up, type of outcome assessment, type and numbers of outcomes, concomitant treatment, comorbidities of population and the reported degree of adjustment.

Risk of Bias Assessment

Risk of bias was assessed using the validated National Institute of Health (NIH) assessment tool for Before-After (Pre-Post) studies without control group (31). Cut-offs were used to judge overall risk of bias with 8–12 points indicating low risk, 5–7 points indicating moderate and 1–4 indicating high risk of bias. In addition, we used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) method to assess the quality of evidence in the current systematic review (32). The GRADE method evaluates each outcome separately based on the quality of evidence (including the risk of bias, study design, consistency and directness of findings) and further considers the magnitude of effect. The evidence is categorized as either high, moderate, low or very low.

Statistical Analysis

Mean differences were calculated from the differences between group means at different time points. Standard deviations (SD) of the mean differences (MD) were derived by using reported *p*-values from repeated measure analyses using the following formula $SD = MD \times \sqrt{(n)}/t$ (33), with n being the number of patients, and t the t-value for the given *p*-value and degrees of freedom according to the table on critical values of the Student's t distribution.

Measurement units were converted where appropriate. An inverse variance weighted random-effect model was used to obtain the pooled mean difference with 95% CI for the change in outcome from before to after ANTH-BC treatment, separated by time-point of assessment into short-term (2–4 months) and mid-term effects (6–12 months).

We constructed forest plots, and assessed heterogeneity using the I^2 statistic, with $I^2 \leq 25\%$ considered low, $25\% < I^2 < 75\%$ moderate, and $I^2 \geq 75\%$ high (34).

Sensitivity analyses were performed to assess the impact of age ($< / \geq 50$ years), cumulative ANTH-BC dose ($< / \geq 200$ mg/m^2), and assessment method (CMR vs. echocardiography for AD and CMR vs. Doppler echography for PWV) on vasculotoxicity. Results of all studies (AD and PWV data) were collated by expressing the mean change relative to mean baseline. Dose-response relationship was assessed by linear regression between arterial stiffness ratio relative to baseline and cumulative mean dose (if only range of dose was indicated, the central value was used). Statistical analyses were performed using Rev Manager [Version 5.3, The Cochrane Collaboration] and R [Version 4.1.2, R Core Team].

RESULTS

Study Selection and Characteristics

Of the 2,130 studies identified, 9 studies met the inclusion criteria for this review (**Figure 1**), with clinical characteristics shown in **Table 1**.

All studies were published between 2010 and 2021 and included patients with solid tumors, such as breast cancer or sarcoma (35–40), or hematologic malignancies, such as lymphoma and leukemia (35, 36, 39, 41–43), or a combination thereof (35, 36, 39). All studies were prospective with data provided from before treatment as well as after a follow-up period between 1 and 14 months (**Figure 2**). Three studies included a control group consisting of healthy, age-matched volunteers (35, 38), or a cancer group without ANTH-BC (39). Studies were based on 10–133 patients, with mean age 52 (SD 11) years, and 73% women. Two of the studies included in this analysis excluded patients with CV comorbidities (37, 41). Concomitant treatments mostly included cyclophosphamide, trastuzumab, taxanes and/or radiotherapy. Based on available data, we decided to perform meta-analyses on short-term effects at 2–4 months, which coincided with conclusion of ANTH-BC in breast cancer and some lymphoma patients, and at 6–12 months, at which time point also all lymphoma patients had concluded their treatment (44, 45), while some patients were likely to have terminated ANTH-BC several months previously. Mean cumulative dose of Doxorubicin delivered was 310 mg/m^2 (range 215–436 mg/m^2, range for individual patients 50–436 mg/m^2). Dose-response relationship showed a non-significant regression between arterial stiffness ratio and administered ANTH-BC-dose ($r = 0.06$, $p = 0.594$). Other anthracyclines included were Daunorubicin and Epirubicin, which have comparable or lower cardiotoxic effects compared to Doxorubicin (12, 46).

Four studies provided data on AD and seven on PWV (AD and PWV were concomitantly reported in two studies, **Figure 2**). Three studies measured AD by cardiovascular magnetic resonance imaging (CMR) (35, 38, 39) and one by echocardiography (37, 41). PWV was assessed using CMR (35, 36, 38), echocardiography (37, 42), SphygmoCor (43) or carotid artery ultrasound (40) and was reported in m/s by all studies.

Risk of Bias Analysis and Quality of Evidence

Risk of bias was moderate in most included studies (5–8 points, **Table 2**). Only four studies provided sufficient information on eligibility criteria (37, 38, 41). None of the nine included studies provided a study flow. Results of PVW and AD were of very low certainty. The evidence is based solely on observational studies

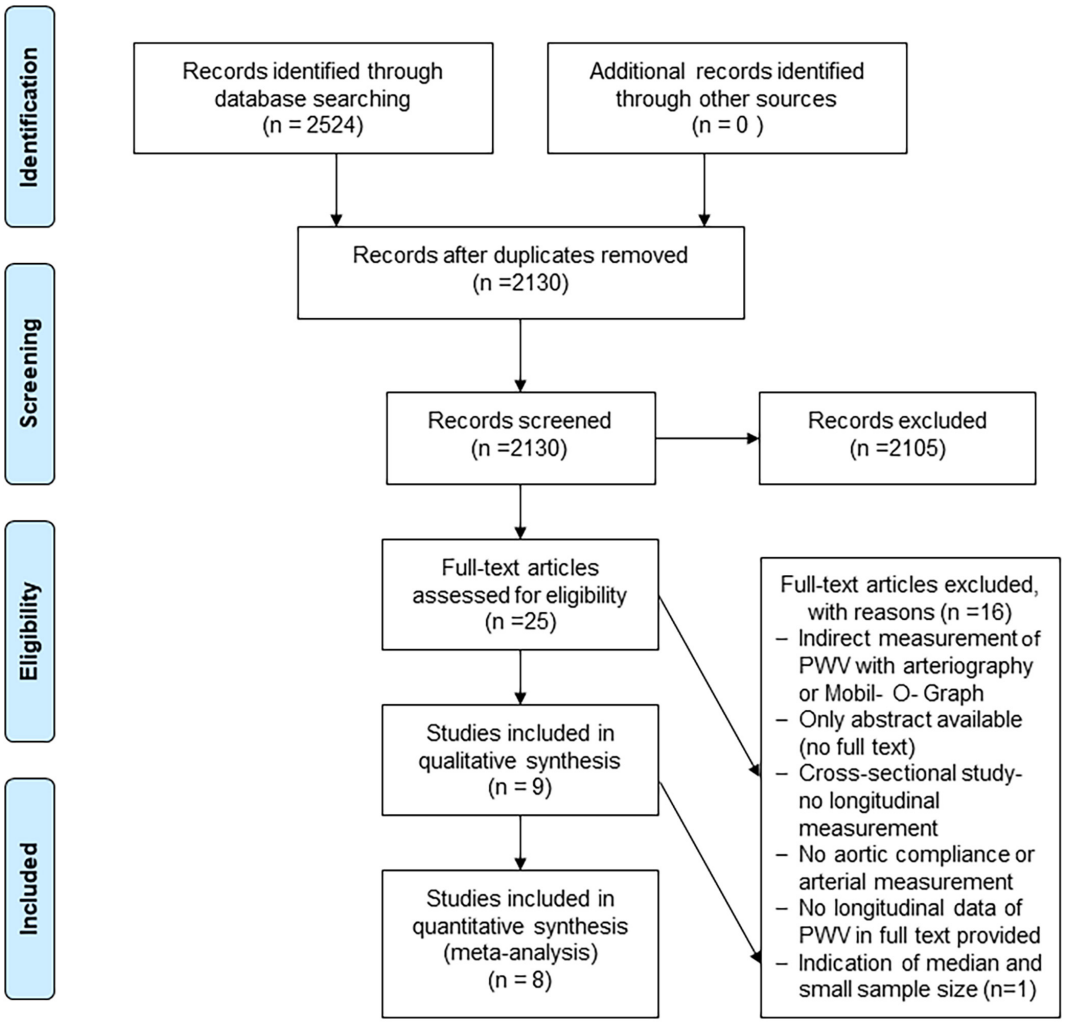

FIGURE 1 | Flowchart for study inclusion, adapted from the PRISMA statement. Flow chart illustrating the study search, screening and selection processes.

and despite good generalizability regarding the study population and each outcome, we found some unexplained heterogeneity. Due to the small number of studies, publication bias was not assessed. Imprecision, inconsistency and risk of bias were a serious concern for both outcomes. **Supplementary Table 1** summarizes the assessment of evidence quality.

Aortic Distensibility

Meta-analyses for short- and mid-term reporting on AD are summarized in **Figure 3**. AD was reported in $mmHg^{-1}$ in all except one study (41), which we converted as 1 $dyne/cm^2 = 0.00075$ mmHg.

Short-term analysis of studies assessing AD after 3 or 4 months, coinciding with termination of ANTH-BC in breast cancer and lymphoma patients who had 4 and 6 chemotherapy cycles, showed an effect of -1.49 $mmHg^{-1}$ (95%CI -3.25; 0.27). There was considerable heterogeneity amongst these studies ($Chi^2 = 29.03$, df = 2, $p < 0.00001$, $I^2 = 93\%$, **Figure 3A**). In the subgroup analysis for measuring method, heterogeneity disappeared in the CMR studies where AD was reduced significantly by -2.28 $mmHg^{-1}$ (95%CI -3.06; -1.49, $I^2 = 0\%$) (**Supplementary Figure 1A**). This sub-group analysis corresponded to the sensitivity analysis for age and anthracycline dose since the study which used echocardiography was also the study with younger mean age (44 ± 19 years) and lower anthracycline dose (< 200 mg/m^2) (41).

The mean weighted change in AD for the studies with follow-up at 6–12 months was -0.37 $mmHg^{-1}$ (95% CI -1.13; 0.39, $I^2 = 82\%$, **Figure 3B**). Heterogeneity persisted in the subgroup analysis for assessment method in CMR studies (-0.95 $mmHg^{-1}$; 95%CI -3.59; 1.69, $I^2 = 91\%$, **Supplementary Figure 1B**).

Pulse Wave Velocity

Four studies reporting on PWV presented means with standard deviations (SD) at each time point, whereas two studies presented the median (40, 43). Since the study by Turan et al. (43) reported the median (range) it was included in the systematic review only (43). In the study by Novo and colleagues, PWV was indicated

TABLE 1 | Description of the included studies.

Source	Cancer type (%)	Age	Cumulative dose (mg/m^2)	Sample size n (% female)	Outcome assessment	Baseline PWV [m/s]	Follow-up PWV [m/s]	Baseline AD [$mmHg^{-1}$]	Follow-up AD [$mmHg^{-1}$]	CV risk factors
Novo et al. (43)	Breast cancer	56 ± 12	NA 4 cycles of Anthracycline treatment (every 21 days)	133 (100%)	Carotid arteries ultrasound	Median (IQR): 5.5 (5.15–6.4)	Median (IQR): 3 months: 6.7 (5.6–7.2) ($p > 0.004$) 6 months: 5.75 (5.2–6.7) ($p > 0.05$) 12 months: 5.7 (5.15–6.6) ($p > 0.05$)			Diabetes (13.5%) Hypertension (22%) Dyslipidemia (22%) Smoking (13.5%) Family history of CVD (18%) **Inclusion criteria**: LVEF > 50% Absence of: Coronary artery disease Hemodynamically relevant valvular heart disease Carotid atherosclerotic plaque **Exclusion criteria:** Pre-existing LV dysfunction before start of chemotherapy Severe liver or renal dysfunction
Mihalcea et al. (42)	Lymphoma (non-Hodgkin)	58 ± 11	8 ± 2 cycles of Doxorubicin at 50 mg/m^2 = 429 ± 61 after 3rd cycle: ∼150	110 (54%)	Echo right common carotid artery,	6.7 ± 1.1	3rd cycle 7.2 ± 1.2 ($p < 0.05$) Final 7.8 ± 1.5 ($p < 0.05$)			Diabetes (4%) Hypertension (17%) Dyslipidemia (8%) Smoking (9%) **Exclusion criteria:** History of CV disease History of radiotherapy
Turan et al. (43)	Lymphoma (non-Hodgkin)	52 (36–68)	6 cycles of Doxorubicin 436 ± 94	10 (80%)	SphygmoCor system (AtCor Medical, Sydney, Australia)	Median (min-max): 9.08 (8.12–9.76)	First cycle: Median (min-max) 10.31 (8.22–12.62) Sixth cycle 9.64 (8.22–12.62)			Hypertension (20%) Dyslipidemia, (20%) Smoking (10%) **Exclusion criteria**: History of coronary artery disease and heart failure
Chaosuwannakit et al. (35)	Breast cancer (48%) Lymphoma (28%) Leukemia (25%)	52 ± 11 (24–65)	Doxorubicin 215; 60–320 Daunorubicin 265; 100–600	Cancer: 40 (70%) Healthy controls: 13	CMR PC-CMR	6.9 ± 2.3	3.6 months: 13.5 ± 4.7 ($p < 0.0001$)	4.1 ± 1.6	3.6 months: 1.9 ± 1.2 ($p < 0.0001$)	Diabetes (13%) Hypertension (33%) Hyperlipidemia (23%)
Grover et al. (38)	Breast cancer (100%)	54 ± 11	3–6 cycles of Epirubicin at 100 mg/m^2 = 300–600 3–6 cycles of Doxorubicin at 50 mg/m^2 = 150–300	27 (100%) ANTH-BC: 15 TZM: 12 Healthy: 12	CMR PC-CMR	6.8 ± 3.2	1 month: 7.8 ± 4.3 ($p > 0.05$) 4 months: 8.9 ± 6.4 ($p < 0.05$) 12 months: 8.2 ± 4.2 ($p < 0.05$)	Anth-group only: 9.2 ± 2.8 All patients 8.1 ± 3.6	All patients, 4 months: 5.7 ± 3.2 ($p < 0.001$) 12 months: 6.9 ± 2.3 ($p > 0.05$) Anth-group only, 12 months: 6.8 ± 2.5 (p = 0.009)	Diabetes (15%) Hypertension (19%) Hypercholesterolemia (37%) Smokers: current (7%) Smokers: ex/41%) Family history of CAD (26%)

(Continued)

TABLE 1 | (Continued)

Source	Cancer type (%)	Age	Cumulative dose (mg/m^2)	Sample size n (% female)	Outcome assessment	Baseline PWV [m/s]	Follow-up PWV [m/s]	Baseline AD [$mmHg^{-1}$]	Follow-up AD [$mmHg^{-1}$]	CV risk factors
Drafts et al. (36)	Breast cancer (42%) Lymphoma (32%) Leukemia (24%) Myelodysplastic syndrome (2%)	50 ± 2 (19- 80)	Doxorubicin in 37 patients: 240; 50- 375 Daunorubicin in 16 patients: 180; 26–500	53 (58%)	PC-CMR	6.7 ± 0.5	6 months: 10.1 ± 1 ($p = 0.0006$)			Diabetes (13%) Hypertension (40%) Hyperlipidemia (25%) Smoking (45%) Coronary artery disease (8%)
Mizia- Stec et al. (37)	Breast cancer (100%)	50 ± 9 (35–68)	Doxorubicin: 278 ± 55; 100–300 Epirubicin: 414; 150–630	31 (100%)	Echo	16.7 ± 11.8	9–12 months: 14.9 ± 8.4 ($p > 0.05$)			Controlled hypertension: 52% **Exclusion criteria:** -Heart failure -Uncontrolled hypertension -Diabetes -CAD -Left side chest wall radiation -Currently smoking
Daskalaki et al. (41)	Lymphoma Non-Hodgkin 45 (62%) Hodgkin 25 (386%)	44 ± 19 Non-Hodgkin 52 ± 17 Hodgkin 28 ± 9	Doxorubicin 3 months: 150–200 End of treatment: 300–400	70 (47%)	Echo			3.31 ± 0.27 (2.48 ± 0.2 $10^{-6} \times dyn^{-1} \times cm^2$)	3 months: 3.21 ± 0.24 ($p = 0.059$) *(2.41 ± 0.18† $10^{-6} \times dyn^{-1} \times cm^2$)* End of treatment: 3.15 ± 0.31 ($p < 0.0001$) *(2.36 ± 0.23‡ $10^{-6} \times dyn^{-1} \times cm^2$*	Currently smoking: 11% **Exclusion criteria:** -History of myocardial infarction -Heart failure -Diabetes mellitus -Renal failure -Treatment with beta blockers, ARBs or ACE inhibitors
Jordan et al.* (39)	ANTH-BC: Breast cancer (44%) Leukemia (18%) Lymphoma (31%) Sarcoma (7%)	51 ± 12	Doxorubicin: 232 ± 103	ANTH-BC: 61 (69%) Non-ANTH-BC*:15 Healthy: 24	PC-CMR			1.68 ± 1.31	6 months: 1.98 ± 1.70 ($p = 0.28$)	ANTH-BC patients: Diabetes: 18% Hypertension: 38% Hyperlipidemia 26% Known CAD: 5%

**Non ANTH-BC group: breast cancer patients treated with trastuzumab regimen with either Docetaxel or Taxol (n=13) and patients treated for a hematologic malignancy with either all Transretinoic acid (n=1) or Bendamustine/Rituxan therapy (n=1) ACE, angiotensin-converting–enzyme; AD, aortic distensibility; ANTH-BC, anthracycline-based chemotherapy; ARBs, angiotensin-receptor blockers; CAD, coronary artery disease; CV, cardiovascular PC-CMR, phase-contrast cardiovascular magnetic resonance; PWV, pulse- wave- velocity; TZM, trastuzumab.*

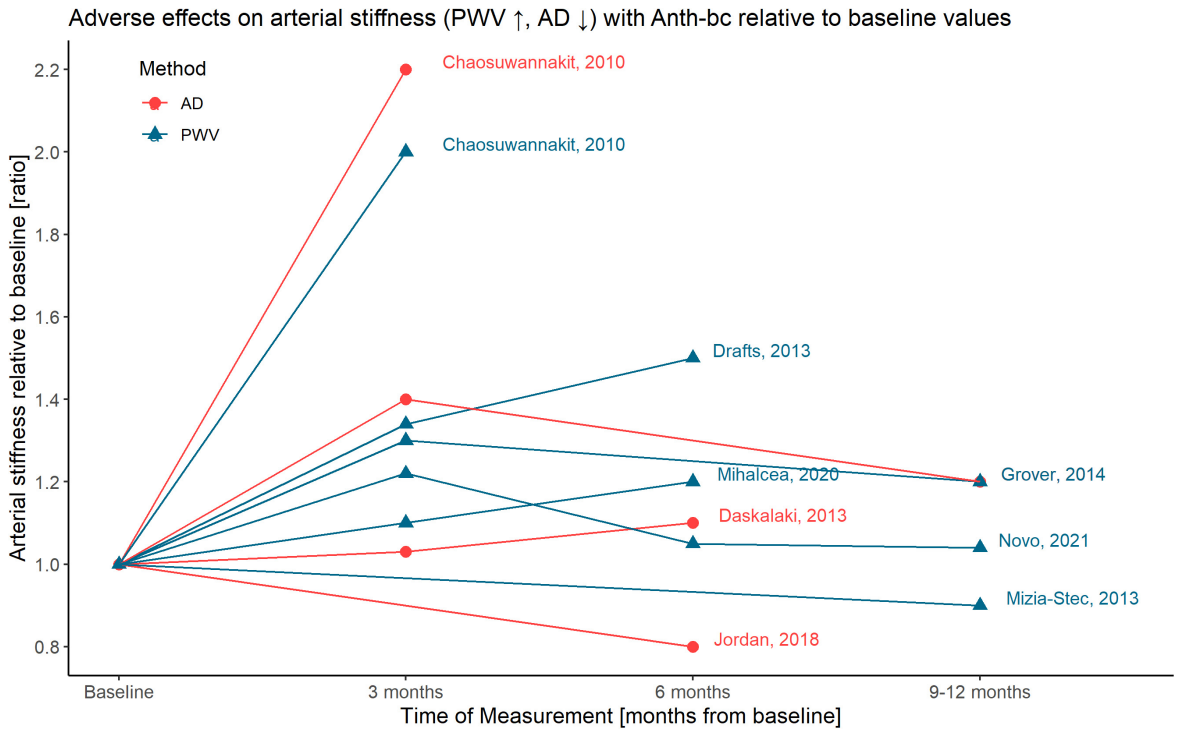

FIGURE 2 | Graphical illustration of time-dependent adverse effects on arterial stiffness with ANTH-BC relative to baseline values; AD, aortic distensibility; PWV, pulse-wave-velocity.

as median (interquartile range) (40). Due to the relatively large sample size of this study (n = 133), we included it into our meta-analysis by using the median as mean and approximated the standard deviation according to the following Cochrane formula: width of the interquartile range = 1.35 standard deviations (33). Two studies did not provide an exact p-value for PWV change but only indicated that it was non-significant (37, 40). Since the SD of the change could not be calculated for these studies, it was approximated by taking the mean SD of the other three studies who provided exact p-values. Another study stated that p was < 0.05. Using a conservative estimation, we calculate the SD of the mean change based on p = 0.049 (42). In a third study, PWV data at 4 months was only provided in a graph from which data was estimated visually (36).

Meta-analysis of the five studies who provided data at 2–4 months showed an increase in PWV of 2.05 m/s (95%CI 0.0.68; 3.43) from before to after ANTH-BC (**Figure 4A**) with considerable heterogeneity among the studies (Chi^2 = 21.89, df = 3, p < 0.0001, I^2 = 82%). Subgroup analysis for CMR-studies only showed an increase in PWV of 3.34 m/s (95%CI 1.10; 5.58, **Supplementary Figure 2A**) with considerable heterogeneity amongst the studies (Chi^2 = 7.22, df = 2, p = 0.03, I^2 = 72%).

For studies with follow-up at 6–12 months, mean weighted change in PWV was 0.88 m/s (95% CI −0.25; 2.02, I^2 = 64%, **Figure 4B**). Subgroup analysis for assessment method showed a significant effect of 2.16 m/s (95% CI 0.26; 4.07) in CMR studies with reduced heterogeneity (I^2 = 53%, **Supplementary Figure 2B**).

DISCUSSION

This systematic review summarized the current evidence of the time-dependent effect of ANTH-BC on central aortic stiffness, assessed as AD or central PWV. Results from this meta-analysis suggest that in the short term (at termination of ANTH-BC), moderate dose ANTH-BC has a clinically meaningful effect on increasing arterial stiffness, presenting as an increase in PWV and a decrease in AD, albeit non-significant for AD. Findings from this study are in line with the results of a recent meta-analysis on this topic (26). However, as a novel finding, we observed smaller effects when measurements were performed at 6–12 months (**Figure 2**), suggesting at least partial recovery, which was supported by two out of the three studies who provided repeat measurements at short- and mid-term time points. This suggest that ANTH-BC vascular toxicity may at least in part be reversible, in contrast to myocardial toxicity. The risk of bias of the included studies was moderate. The quality of the studies included in this review was limited mainly by study design and methodology.

Comparison With Other Studies

Over the past 10–15 years, an extensive body of literature has been published identifying increased arterial stiffness as a predictor of cardiovascular events and mortality.(22, 23, 47) AD has been found a sensitive parameter of arterial stiffness in patients younger than 50 years, while PWV is the more sensitive parameter after the age of 50.(21) According to a meta-analysis

of general population studies, a 1 m/s increase in PWV, as found in our study in the long-term, corresponds to an age-, sex-, and risk factor-adjusted risk increase of approximately 14% in total CV events, CV mortality, and all-cause mortality,(48) underlining the clinical importance of this finding. According to a study by Redheuil et al. who assessed the predictive value of AD

TABLE 2 | Quality assessment of included studies using the NIH.

Criteria	Chaosu-wannakit	Drafts	Grover	Jordan	Daska-laki	Mizia-Stec	Mihalcea	Novo
1. Was the study question or objective clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Were eligibility/selection criteria for the study population prespecified and clearly described?	No	No	Yes	No	Yes	Yes	Yes	Yes
3. Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Yes	Yes	Yes	Yes	No	No	Yes	Yes
4. Were all eligible participants that met the prespecified entry criteria enrolled?	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
5. Was the sample size sufficiently large to provide confidence in the findings?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6. Was the intervention (ANTH-BC) clearly described and delivered consistently across the study population?	Yes	Yes	Yes	Yes	n.r.	Yes	Yes	n.r.
7. Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8. Were the people assessing the outcomes blinded to the participants' exposures/interventions?	Yes	Yes	n.r.	Yes	Yes	n.r.	n.r.	n.r.
9. Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	n.r.	n.r.	Yes	n.r.	Yes	Yes	No (loss more than 20%, baseline: 147, final assessment 110)	n.r.
10. Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	Yes, but method n.r.	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11. Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	No	No	No	No	No	No	No (once before intervention, but twice after (3rd and last cycle)	No
12. If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	NA	NA	NA	NA	NA	NA	NA	NA
Overall rating	7/12	7/12	8/12	7/12	7/12	7/12	7/12	6/12

Quality assessment tool for before-after (pre-post) studies with no control group.
The colours represent the quality of the studies included in this meta-analysis with red for high risk, yellow for uncertain and green for low risk of bias.

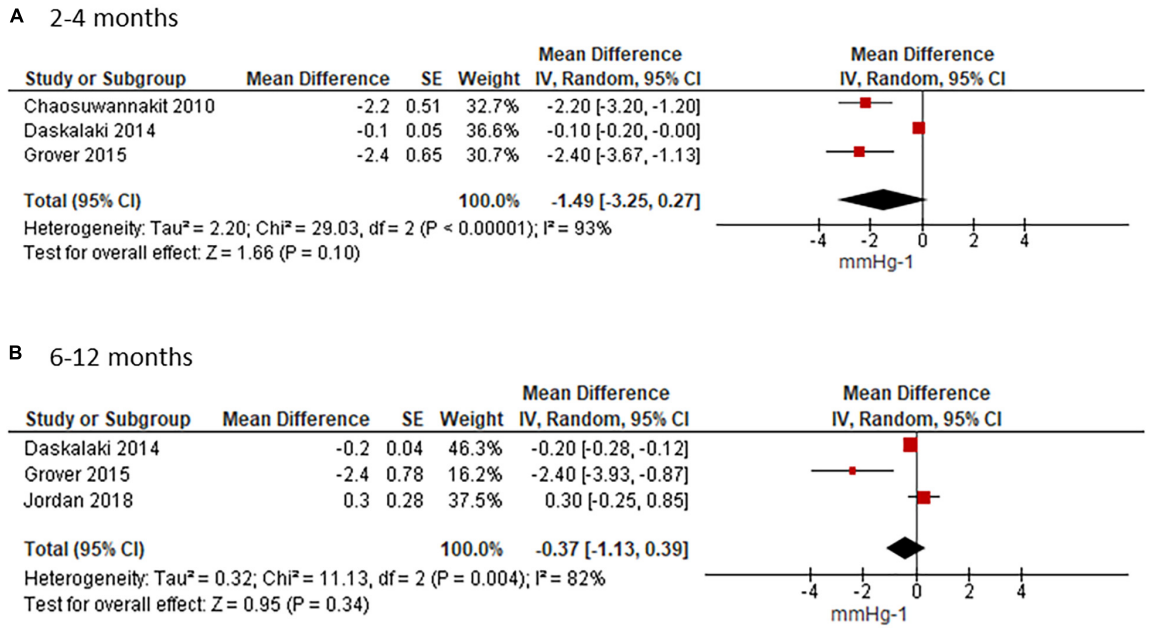

FIGURE 3 | Effect of ANTH-BC on AD. Forest plots illustrating the effect of ANTH-BC on AD divided by time-point of assessment into **(A)** short-term (3–4 months) and **(B)** mid-term (6–12 months) effects.

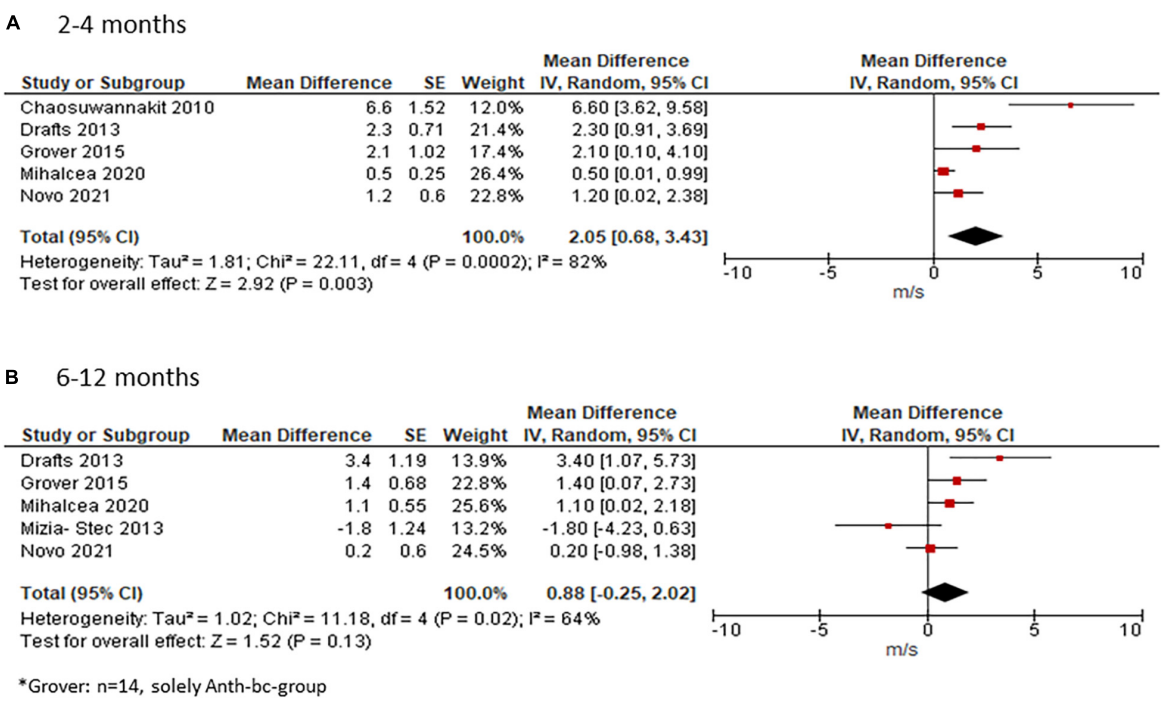

FIGURE 4 | Effect of ANTH-BC on PWV. Forest plot illustrating the effect of ANTH-BC on PWV divided by time-point of assessment into **(A)** short-term (2–4 months, corresponding to subgroup analysis for CMR) and **(B)** mid-term (6–12 months) effects.

for mortality, hard CV events and HF events in 3,675 patients without clinical CVD (mean age 61 ± 10 years) (23), patients included in our meta-analysis had either a not elevated (35, 39) to twofold increased risk (38, 41) for CV events.

Our meta-analysis suggests that adverse effects of ANTH-BC on arterial stiffness may partially be reversible after ANTH-BC termination. Of the five studies that performed two follow-up measurements, one at 3 months and one at 6 months

(36, 40–42) or 12 months (38, 40), three studies found a further worsening (36, 41, 42), while the study by Grover and Novo and colleagues found a recovery toward baseline values (**Figure 2**). Even though arterial stiffness parameters may partially recover from acute ANTH-BC exposure, this may not mean that long-term vasculotoxic effects will not be present. Nevertheless, at 5 or 10 years after treatment termination it will be difficult to ascribe increased arterial stiffness to certain chemotherapies, as other treatments, advanced age, cancer itself, or cardiovascular risk factors are known to also play a role. The largest study that was included in this meta-analysis showed a clear recovery of arterial stiffness after the initial decline at 3 months (40). Since both follow-up measurements at 6 and 12 months showed values equal to pre-anthracycline measurements despite further treatment with other chemo- or radiotherapies, this study added considerably to the conclusion that the adverse effect of ANTH-BC to arterial stiffness may be reversible. The hypothesis of partial recovery of adverse effects over time will need to be confirmed in longitudinal studies which measure before, at completion of ANTH-BC and at a later follow-up time. Further, it is clinically important to assess whether partial recovery may be due to cardioprotective treatment of diagnosed cardiotoxic side-effects following cancer therapy.

In our meta-analysis, baseline AD values of three studies were within the range of 1.7 ± 1.3 to 4.1 ± 1.6 $mmHg^{-1}$ (35, 39, 41), and in the range of reference values in the literature for age-matched, healthy individuals (3.1 ± 1.8 to 4.0 ± 1.6 $mmHg^{-1}$) (49). However, baseline AD in the study by Grover et al. was markedly higher (8.1 ± 3.6 $mmHg^{-1}$). Similarly, values for baseline PWV from the study by Mizia-Stec and colleagues, who measured cfPWV by Doppler echography were noticeably higher (16.7 ± 11.8 m/s) compared to those assessed in the other studies (6.7 ± 0.5 to 6.9 ± 2.3 m/s), which measured aortic arch PWV by CMR (35, 36, 38). Surface cfPWV has been found to overestimate true aortic PWV by 2–3 m/s (21), however, this methodological difference cannot explain the almost 10 m/s higher values. However, the unusually high SD of 11 m/s in the study by Mizia-Stec and colleagues raises some doubt about the reliability of their PWV data.

Sources of Heterogeneity

Overall, we found high heterogeneity amongst the studies included in the random-effect analyses for AD and PWV that persisted when performing sensitivity and subgroup analyses. Possible reason for the observed heterogeneity could be the clinical diversity of the study populations with various degrees of cardiovascular risk, bias from patient drop-out, or lack of blinding. None of the studies could be found in a trial registry for verification of reported results with study protocol, and none presented a patient flow. In addition, publication bias may be present.

Potential Modulators of Vasculotoxicity

Vasculotoxicity is likely to be modulated by age, the effect of cumulative ANTH-BC dose, the individual cardiovascular risk factor profile, additional chemo- and radiotherapies, and cardioprotective medication. It is well established that cumulative ANTH-BC dose plays an important role in the development of cardiotoxicity (12). While Chaosuwannakit et al. found an association between cumulative ANTH-BC dose and worsening of AD ($r = 0.34$, $p = 0.02$), Drafts et al. could not confirm these findings ($p = 0.6$). In this meta-analysis, studies with moderate ANTH-BC-dose (between 200 and 450 mg/m^2) show either a much (ratio of 2.2) or somewhat increased arterial stiffness (ratio of 1.1–1.4) or a decrease (ratio of 0.8–1.0, see **Figure 2**) leading to non-significant regression ($r = 0.06$, $p = 0.594$). However, this may not be interpreted as a non-existing dose-response relationship but rather be a consequence of the large heterogeneity between the included studies.

Most of our studies investigated the relation between blood pressure and vascular injury (35–38, 41). Grover et al. found a higher increase in arterial stiffness in patients with higher systolic BP. A higher PWV at baseline and greater increase over time with higher systolic BP was also found by Drafts et al., and Daskalaki et al. found decreased AD to be associated with higher systolic BP. Contrarily, Mizia-Stec and colleagues did not find any relationship between the diagnosis of systemic hypertension and ANTH-BC induced changes in PWV. However, none of the studies adjusted changes in arterial stiffness for changes in BP, which has a direct impact on PWV (50). As blood pressure tends to be decreased with ANTH-BC (51), the increase in arterial stiffness measured by PWV found in this and the previous meta-analysis (26) may be underestimated (50).

None of our studies found a significant effect of additional chemotherapies (35, 36), however, the small sample sizes may have precluded the detection of such associations. Future studies are warranted to gain more insight into the effect of age, cumulative ANTH-BC dose, the presence of cardiovascular risk factors and the addition of co-medication on vascular function.

Strengths and Limitations

Subgroup analyses of different time points has allowed the detection of a potential (partial) reversibility of adverse effects by ANTH-BC on arterial stiffness. Another strength of this meta-analysis is the inclusion of studies assessing central arterial stiffness only. This is important since central (i.e., aorta and carotid arteries) and peripheral (i.e., brachial or femoral) arteries differ in their passive and active contractile properties (52). In contrast to a recent meta-analysis on the same topic, using p-values of repeat measure analyses provided us with a higher power to detect significant results due to a more efficient adjustment for confounders. GRADE assessment allowed an in-depth rating of the evidence for each outcome.

A limitation of our study was that all included studies were observational and expectedly did not include a truly comparable control group of cancer patients. This greatly limits the value of a meta-analysis (33). Therefore, the effect of cancer itself, presence of CV risk factors or other confounding treatments and comorbidities could not be identified. Secondly, except for two studies (40, 42), they were based on small numbers of participants, which explains the large CIs of some of the studies. Another limitation of this meta-analysis is that the assumption

of a normal distribution has been made for PWV in the study by Novo et al., (40) even though data was indicated as median (IQR). Studies did not report ANTH-BC duration, making it difficult to estimate the follow-up time after ANTH-BC termination for the various cancer patients. Unfortunately, none of included studies were able to provide individual patient data.

Conclusions and Clinical Implications

Results from this analysis suggest that in the short-term, ANTH-BC increases arterial stiffness, but that these changes may (partly) be reversible after therapy termination. Future studies need to elucidate the long-term consequences of ANTH-BC on arterial stiffness, by performing repeated and standardized follow-up measurements after ANTH-BC termination to confirm or challenge the findings of reversibility of arterial stiffness put forward by the study of Novo and colleagues. Reporting of data needs to be improved and availability of individual patient data in repositories is highly desirable. The adverse effect of ANTH-BC on arterial stiffness likely applies to the whole vasculature and expands beyond the myocardium. Several reviews highlighted the importance of arterial stiffness in the prediction of all-cause cardiovascular outcomes (22, 27–29). Therefore, non-invasive assessment of arterial stiffness may be used for detection of early cardiovascular injury in asymptomatic patients at risk during treatment and effects of cardio-/vasculo-protective treatments.

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.

AUTHOR CONTRIBUTIONS

CS, PE, and MW were involved in the conception and design. CS, TM, and PE performed the analysis and interpretation of this meta-analysis and drafted the manuscript. NG-J assisted with screening of potential studies and was further involved in the design of this analysis. AB was involved in the analysis and interpretation of data and revised the manuscript. KC and TS revised the manuscript critically to provide intellectual content. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.873898/full#supplementary-material

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Beta-Adrenergic Antagonist Tolerance in Amyloid Cardiomyopathy

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Background: Beta-adrenergic antagonists or blockers (BB) are a cornerstone of cardiac therapy for multiple indications. However, BB are considered relatively contraindicated in amyloid cardiomyopathy due to poor tolerance. This intolerance is hypothesized to be due to concomitant neuropathy and significant restrictive cardiomyopathy. This study analyzes the incidence and characteristics of BB tolerance in patients with amyloid cardiomyopathy.

Methods: Through a single-center retrospective chart review, patients with amyloid cardiomyopathy, confirmed by endomyocardial biopsy or technetium-99 pyrophosphate scan, were identified and clinical data was collected. Statistical methods included Chi-square test and two sample *t*-tests.

Results: Of 135 cardiac amyloidosis patients, 27 patients (20.0%) had no BB use, 56 patients (41.5%) were current BB users, and 52 patients (38.5%) were prior BB users. The most frequent indications for BB use were heart failure, hypertension, coronary artery disease, and arrhythmia. The most common reason for stopping BB therapy was hypotension (62.8%) followed by fatigue, bradycardia, and orthostasis. Neurologic symptoms at the initial BB prescription or most recent evaluation were not significantly different between current and prior BB users. Their cardiovascular profiles were similar by ejection fraction, wall thickness, troponin I, and brain natriuretic peptide. There was no association for BB discontinuation based on amyloid subtype, sex, or race.

Conclusion: The majority of patients with amyloid cardiomyopathy were prescribed BB, and over half of these patients still tolerated BB therapy. Current and prior BB users had similar profiles from a cardiovascular and neurologic perspective, with no association identified to predict BB discontinuation.

Keywords: amyloidosis, heart failure, light chain, pharmacology, transthyretin

INTRODUCTION

Amyloid cardiomyopathy is increasingly being recognized as an under-diagnosed cause of heart failure. Through extracellular deposition of amyloid fibrils, cardiac amyloidosis produces a non-ischemic, restrictive cardiomyopathy, which initially manifests as diastolic heart failure and may progress to systolic dysfunction in later stages. Recent screening studies have highlighted the need for higher clinical suspicion for amyloidosis in the setting of heart failure due to a higher prevalence of amyloid cardiomyopathy in patients with heart failure than previously thought (1–3). Studies showing higher prevalence have helped to drive the development of new treatment modalities for amyloid cardiomyopathy and, more generally, for various systemic amyloidosis causes (4–7). New amyloidosis treatments have imparted increased importance in effectively managing organ-specific amyloid manifestations—such as amyloid cardiomyopathy—in order to extend survival (8).

Guideline-recommended medical management of cardiac amyloidosis sequela—such as heart failure, conduction system disease, and arrhythmias—can be difficult. Due to systemic amyloid involvement in peripheral and autonomic nerves, neuropathy can limit tolerance of neurohormonal medications, such as beta-adrenergic antagonists or blockers (BBs) (9). In addition to their utility in preventing adrenergic receptor downregulation in systolic heart failure, BBs also help prevent cardiac arrhythmias and are used for atrial fibrillation rate control (10). Arrhythmia is a frequent complication of amyloid cardiomyopathy with a prevalence as high as 40% in this disease population—including a 25% prevalence of atrial fibrillation—and correlates to poorer hospital outcomes, increased hospital length-of-stay, and increased hospitalization cost (11).

Currently, BBs are considered to be relatively contraindicated in the management of cardiac amyloidosis (3, 9, 12). BBs face hemodynamic intolerance and bradycardia risk in a heart that is prone to conduction system disease and may be relying on compensatory tachycardia for adequate cardiac output (13–15). BB tolerance has been associated with improved all-cause mortality in transthyretin amyloidosis (ATTR) and light chain amyloidosis (AL) in some studies (16), but not in others (17).

Novel amyloid treatment can slow disease progression and enhance survival for cardiac amyloidosis patients (9). These developments highlight the importance of exploring patient tolerance to and utility of guideline directed medical therapy (GDMT) in cardiac amyloidosis. For BB therapy's effect on the long-term clinical trajectory of cardiac amyloid patients to be investigated, these medications must be hemodynamically tolerated in the short-term. Through a retrospective observational study, we define the incidence of BB tolerance and the characteristics that may be associated with BB tolerance in patients with amyloid cardiomyopathy seen at our institution between 2008 and 2020.

MATERIALS AND METHODS

Ethical Approval

The Office of Responsible Research Practices determined this study (2020E0998) exempt from institutional review board (IRB) review. In addition, the Ohio State University HIPAA Privacy Board granted the project a full waiver of HIPAA authorization by expedited review, according to 45 CFR 164.512.

Participants

Patients with suspected cardiac amyloidosis were identified based on ICD-9 or ICD-10 codes between 2008 and 2020 at The Ohio State University Wexner Medical Center (OSU). Inclusion criteria were diagnosis with wild type transthyretin amyloidosis (ATTRwt) (E85.82), hereditary or variant transthyretin amyloidosis (ATTRv) (E85.2), or light chain amyloidosis (AL) (E85.81) with known cardiac involvement (E85.4); or diagnosis of heart failure (ICD-9: 428.*; ICD-10:150.*) plus diagnosis of amyloidosis (ICD-9: 277.3*; ICD-10 E85.*). Exclusion criteria were clinically unconfirmed disease. Additionally, a single patient was excluded due to a diagnosis of secondary amyloidosis (AA). Endomyocardial biopsy-derived pathology specimens or technetium-99 pyrophosphate scans were used to confirm disease for AL and ATTR amyloidosis. To increase internal validity and decrease selection bias, all cardiac amyloidosis patients seen at OSU during the study timeframe were evaluated for inclusion and exclusion criteria.

Upon meeting study criteria, patients were stratified to current, prior, and no BB use groups for analysis. Patients were grouped in this manner to facilitate comparison of the current-use and prior-use categories. Patients currently on BB therapy must be reasonably tolerating therapy, whereas prior BB users required discontinuation.

Variables

Demographic variables collected were age, sex, and race. Amyloid type, subtype, and diagnosis date were collected to characterize disease. BB use was characterized by BB type, initiation and discontinuation dates, indication, and reason for discontinuation. Cardiac profiles included laboratory data (troponin and brain natriuretic peptide) and imaging data (ejection fraction, stroke volume, and septal wall thickness *via* echocardiogram). Neurologic involvement was assessed by collecting reported neurologic symptoms.

Neurologic and cardiac data were obtained at two separate timepoints when available. Specific timing of the two data collection points was based on the category of BB use pattern. For current BB users, data was collected at or near initial BB prescription date and at the most recently available datapoint. For prior BB users, data was collected at or near initial BB prescription date and at or near BB discontinuation date. For non-BB users, data was collected at or near initial amyloid diagnosis and at the most recently available datapoint. These time points were chosen for their ability to represent clinical change over the course of BB use and/or disease course. When possible, data was obtained on the exact relevant date (i.e., vital

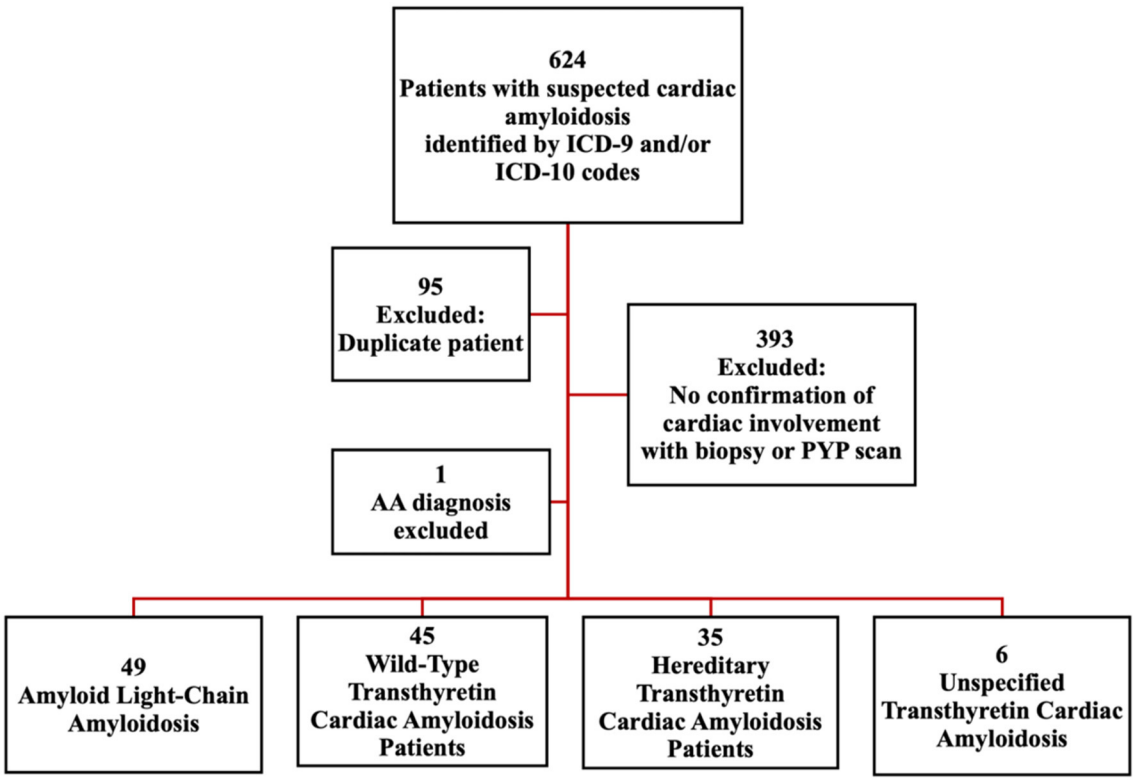

FIGURE 1 | Schematic to identify cardiac amyloidosis patients. Identification of cardiac amyloidosis patients based on ICD codes: Criterion 1, Diagnosis with wild type Wild-Type Transthyretin Amyloidosis (wtATTR), Light Chain Amyloidosis (AL) or Hereditary Transthyretin Amyloidosis (hATTR), with known cardiac involvement. OR Criterion 2, Diagnosis of heart failure (ICD-9: 428.*; ICD-10: I50.*) plus diagnosis of amyloidosis (ICD-9: 277.3*; ICD-10 E85.*). AA, Secondary Amyloidosis; PYP scan, Technetium-99 Pyrophosphate Scintigraphy.

TABLE 1 | Demographic data by beta blocker use pattern.

	Current beta-blocker use	Prior beta-blocker use	No beta-blocker use
Patients (n)	56	52	27
Mean age (years ± SD)	71.80 ± 11.28	72.17 ± 10.02	70.73 ± 11.23
Age range (years)	42–96	48–93	44–91
Female (%)	23.20	26.90	25.90
Caucasian (%)	62.50	67.31	85.20

Standard deviation (SD).

signs obtained from clinician note for an appointment in which a BB was prescribed). This proved difficult with some variables. Specifically, imaging and lab data were frequently gathered from the available date in nearest proximity to the desired data collection date.

All patients prescribed a BB were included in the current or prior category regardless of length of therapy. Indication data was collected at the point of initial BB prescription, and not subsequent BB medication changes or additions. BB discontinuation data was collected only for prior BB users at the time of final BB discontinuation, and not for BB that were switched to other BB or only temporarily discontinued. Data collection was reviewed by two investigators for accuracy.

Analysis

Continuous variables were reported as mean (standard deviation) and differences between groups were assessed *via* unpaired Student's *T*-test. Categorical variables were reported as percentage (number) and differences between groups were assessed *via* chi-square test. Statistical significance was considered with a P-value <0.05. Stata software was used for all statistical calculations.

RESULTS

A total of 624 patients were identified to have suspected cardiac amyloidosis based on ICD-9 or ICD-10 codes between 2008 and 2020. Of these, 95 records were duplicate and 393 patients were excluded due to lack of confirmed cardiac involvement *via* endomyocardial biopsy (for either AL or ATTR amyloidosis) or technetium-99 pyrophosphate scan (for ATTR amyloidosis). Additionally, one secondary amyloidosis (AA) patient was excluded. We identified 135 patients with confirmed amyloid cardiomyopathy meeting inclusion and exclusion criteria. Of these, 49 had AL amyloidosis, 45 had ATTRwt amyloidosis, 35 had ATTRm amyloidosis, and 6 had unspecified ATTR amyloidosis (**Figure 1**).

Patients were stratified to current, prior, and no BB use groups for comparison and analysis (**Table 1**). In the current BB use category, there were 56 participants with a mean age of 72 years (SD = 11.28), 23.2% female, and 62.5% were Caucasian. In the prior BB use category, there were 52 patients with a mean age of 72 years (SD = 10.02), 26.9% female, and 67.3% were Caucasian. In the no BB use category, there were 27 patients with a mean age of 71 years (SD =11.23), 25.9% female and 85.2% Caucasian.

The most frequent BB indications were heart failure (46.4% vs. 38.5%), hypertension (28.6% vs. 34.6%), coronary artery disease (10.6% vs. 4.8%), and arrhythmias (8.9% vs. 5.8%), for current and prior BB use, respectively (**Figure 2**). For current and prior BB use group, there was no statistical difference in indication for BB initiation ($\chi^2 = 3.09$, p-value $= 0.54$), the proportion of patients initially placed on BBs before vs. after amyloid diagnosis, and the BB type (**Table 2**). The most common reasons for stopping BB therapy were hypotension

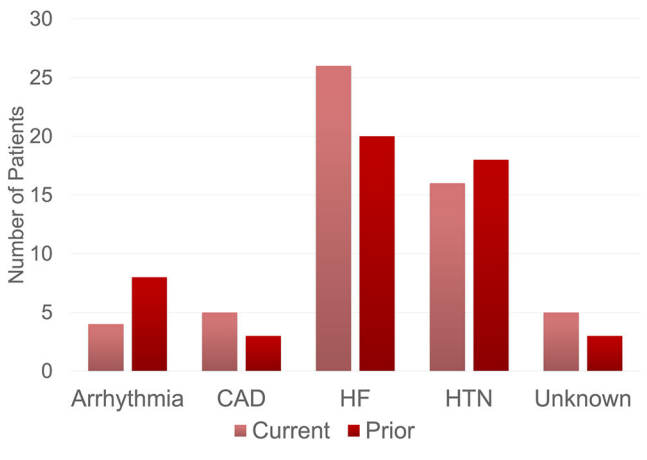

FIGURE 2 | Beta-blocker indications. Indication for beta-blocker use among patients receiving current and prior beta-blocker therapy. The current group includes cardiac amyloid patients who were on beta-blocker therapy at time of data collection. The prior group includes cardiac amyloid patients who were previously on beta-blocker therapy but were no longer using beta-blockers at the time of data collection. The most common reason for beta-blocker therapy in both groups included heart failure followed by hypertension. CAD, Coronary Artery Disease, HF, Heart Failure; HTN, Hypertension.

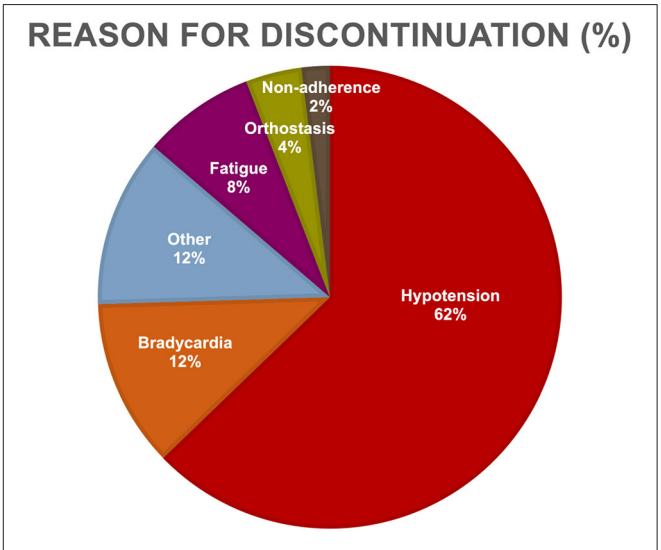

FIGURE 3 | Reasons for beta-blocker discontinuation. Listed are the reasons for discontinuation of behhta-blocker therapy among cardiac amyloid patients. The most common reasons for stopping beta-blocker therapy were hypotension, bradycardia, fatigue, and orthostasis.

TABLE 2 | Beta-blocker (BB) prescription type and timing in relationship to amyloidosis diagnosis.

	Current BB use % (*N*)	Prior BB use % (*N*)	Test statistic	*P*-value
BB type[a]				
Atenolol	8.9 (5)	7.7 (4)	$X^2 = 0.0539$	0.816
Carvedilol	46.4 (26)	42.3 (22)	$X^2 = 0.1854$	0.667
Metoprolol	69.6 (39)	80.8 (42)	$X^2 = 1.7802$	0.182
Timing of initial BB prescription				
Prior to amyloid diagnosis	73.2 (41)	77 (40)	$X^2 = 0.1978$	0.657
After amyloid diagnosis	26.8 (15)	23 (12)		

[a]*only BB used by at least 5 patients were included.*

TABLE 3 | Comparison of demographic variables compared between patients with current vs prior beta blocker categories.

Variable	Current BB use % (*N*)	Prior BB use % (*N*)	Test statistic	*P*-value
Amyloid subtype	(56)	(52)	$X^2 = 3.76$	0.288
AL	30 (17)	34 (18)		
ATTRv	30 (17)	25 (13)		
ATTRwt	38 (21)	31 (16)		
ATTR unspecified	2 (1)	10 (5)		
Sex	(56)	(52)	$X^2 = 0.20$	0.657
Male	77 (43)	73 (38)		
Female	23 (13)	27 (14)		
Race	(56)	(52)	$X^2 = 1.53$	0.465
Caucasian	62 (35)	67 (35)		
Black	38 (21)	31 (16)		
Other	(0)	2 (1)		

Beta-blocker (BB), Light chain amyloidosis (AL), Variant or hereditary transthyretin amyloidosis (ATTRv), wild-type transthyretin amyloidosis (ATTRwt).

TABLE 4 | Cardiac and neurologic variables by current and prior beta-blocker (BB) use.

Variable	Current BB use Mean ± SD (N)	Prior BB use Mean ± SD (N)	Test statistic	*P*-value
Ejection fraction (%)				
Initial on BB	45.36 ± 13.74 (48)	50.10 ± 11.92 (46)	$t = 1.78$	0.078
Ejection (%)				
Most recent on BB	43.10 ± 14.39 (41)	43.20 ± 13.65 (47)	$t = 0.03$	0.972
Stroke volume (cm/ml)				
Initial on BB	39.40 ± 14.03 (30)	49.11 ± 25.95 (31)	$t = 1.81$	0.075
Stroke volume (cm/ml)				
Most recent on BB	43.86 ± 21.73 (24)	40.25 ± 16.88 (43)	$t = -0.76$	0.451
Septal wall thickness (cm)				
Initial on BB	1.50 ± 0.43 (38)	1.47 ± 0.42 (40)	$t = -0.36$	0.721
Septal wall thickness (cm)				
Most recent on BB	1.56 ± 0.39 (33)	1.64 ± 0.60 (45)	$t = 0.62$	0.540
Troponin I (ng/mL)				
Initial on BB	0.18 ± 0.18 (53)	0.24 ± 0.36 (42)	$t = 1.22$	0.227
Troponin I (ng/mL)				
Most recent on BB	0.79 ± 3.22 (48)	0.44 ± 0.95 (49)	$t = -0.73$	0.468
Brain natriuretic peptide (pg/mL)				
Initial on BB	551.79 ± 469.40 (48)	593.06 ± 755.30 (41)	$t = 0.31$	0.754
Brain natriuretic peptide (pg/mL)				
Most recent on BB	864.27 ± 857.18 (48)	785.71 ± 632.39 (49)	$t = -0.51$	0.608
Neurological symptoms at initial BB prescription % (*N*)	52% (29)	52% (27)	$X^2 = 0.0002$	0.989

(62.8%), bradycardia (11.8%), fatigue (7.8%), and orthostasis (3.9%) (**Figure 3**).

Multiple parameters were assessed to determine whether demographic or disease parameters could account for discontinuation of BB therapy. No difference was found between current and prior BB use groups in regard to for amyloid subtype, sex, and race (**Table 3**). Current and prior BB users' cardiovascular profiles were similar by echocardiogram parameters including ejection fraction (45% vs. 50%) and wall thickness (1.50 vs. 1.47 cm) and by cardiac biomarkers including troponin I (0.175 vs. 0.244 ng/mL), and brain natriuretic peptide (552 vs. 593 pg/mL). The presence of neurological symptoms at initial BB prescription was not associated with BB tolerance (**Table 4**).

DISCUSSION

In this study of 135 patients at a US tertiary referral center with confirmed amyloid cardiomyopathy, the majority of patients (80%) were prescribed BBs with 41.5% of study patients as current BB users. Our study reports a much higher baseline prescription rate of BB than previously described, which may reflect differences in US practice patterns as well as differences in subtypes of amyloidosis, including ATTRv. In a retrospective

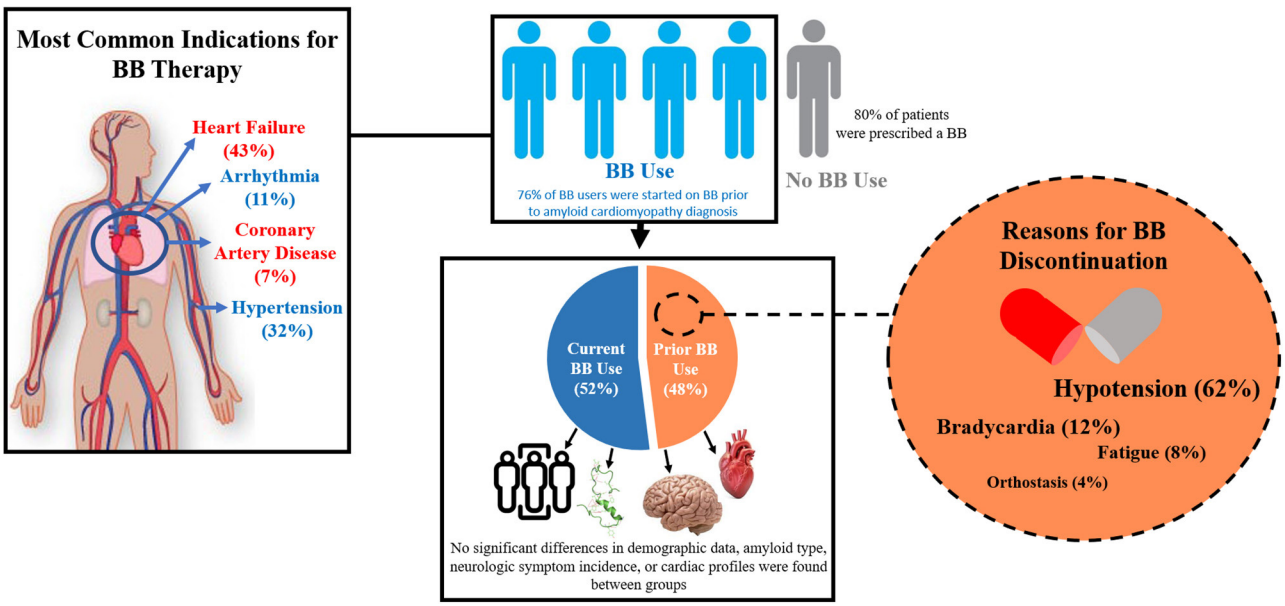

FIGURE 4 | Summary Figure. Beta-blocker use and tolerance were analyzed in a cohort of patients with confirmed amyloid cardiomyopathy in a large amyloidosis referral center in the United States. The most common indications for beta blocker (BB) therapy in our cohort included heart failure, hypertension, arrhythmia, and coronary artery disease. Most patients in our study cohort were prescribed a beta blocker (BB). Of these, over half of them were tolerating the therapy enough to remain on the medication. Between patient groups tolerating (Current BB Use) and not tolerating (Prior BB Use) BB therapy, no significant differences in cardiac profiles, neurologic symptom incidence, amyloid type, or demographic data exist. The most common reasons for BB discontinuation include hypotension, bradycardia, fatigue, and orthostasis.

study of Italian patients with ATTR cardiac amyloidosis, only 57% of patients were prescribed BB and 33% of patients continued on BB therapy (18). A Spanish study of 128 ATTR cardiomyopathy patients found 50.8% on BB therapy with an only 25% discontinuation rate (16). A Greek study of 53 patients with AL amyloid cardiomyopathy on BB therapy found that 47% discontinued therapy. (19) Intentional prescription of BB was able to increase patients on BB therapy from 61 to 87% without an increase in adverse events in a recent Italian study (20). The prevalence of BB use at final data collection point in our study correlates with other real-world, non-trial analyses of BB use among amyloid cardiomyopathy patients (21).

Investigation into factors associated with BB tolerance among amyloid cardiomyopathy patients has been limited (22). In our study, heart failure and hypertension comprised the majority of documented reasons for BB initiation. An Italian study of 642 patients with cardiac amyloidosis found BB prescription was driven primarily by atrial fibrillation or ventricular arrhythmias (18). This striking difference in initiation reasons may reflect higher prevalence of underlying hypertension in the US population. Left ventricular ejection fraction was also higher overall in the Italian study. Consistent with other clinical observations, hypotension was the most common reason for BB discontinuation in this study.

Between patients who were discontinued or continued on BB therapy, no significant differences in cardiac profiles, neurologic symptom incidence, amyloid type (AL or ATTR), BB type, or demographic data were found in our study. One study did find increased BB intolerance in patients with more advanced AL disease with higher NYHA class and Mayo stage (19). Nevertheless, few analyses have found significant association in BB intolerance—in part because the numbers are small.

Although our study did not find any significant differences between groups tolerant and intolerant to BB therapy, it does build on a prevalent tolerance of BB therapy in amyloid cardiomyopathy seen in the above studies. The number of patients both prescribed and tolerating BB therapy in our study demonstrates the clinical complexity formed by competing considerations of GDMT heart failure strategies and the specific conduction system and neurohormonal concerns in the amyloid population. Determining whether the long-term efficacy of BB in amyloid cardiomyopathy is equivalent to the GDMT benefit in other forms of heart failure remains unknown and is an imperative future study.

Limitations to this study include the retrospective nature at a single institution, which introduces the potential for measurement bias in relation to clinical care data. Though appropriate steps were taken to limit this, some data points were either missing or unable to be collected at the exact appropriate time. Missing laboratory data prevented reporting of clinical staging data, which may have proved useful to investigate relationships between disease severity and BB tolerance. Further,

the impact of BB on outcomes could not be appropriately judged in this analysis. Although our study is relatively large in this underrecognized disease, our study lacks appropriate numbers to power subgroup analyses and investigate more specific types of amyloid patients who may best tolerate BBs.

This study shows that in a cohort of 135 amyloid cardiomyopathy patients receiving care at an amyloid referral center in the US, the majority of patients were prescribed a BB (**Figure 4**). Furthermore, over half of patients prescribed a BB were tolerating the therapy enough to remain on the medication. Between patient groups tolerating and not tolerating BB therapy, no significant differences in cardiac profiles, neurologic symptom incidence, amyloid type, or demographic data exist. The most common reason for BB discontinuation was hypotension. In the context of amyloid cardiomyopathy, further study is needed to better understand which characteristics may be predictive of BB tolerance, ideal BB regimens for patients tolerating therapy, and the effects of BB therapy on cardiac disease progression.

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 the Office of Responsible Research Practices. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CC and RK conceived this study. SR, CA, BR, CT, and CC designed, analyzed, and interpreted the data. SR and CA wrote the first draft of the manuscript. All authors have contributed significantly to this work, manuscript revision, read, and approved the submitted version.

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Women at heart: Introducing gender cardio-oncology

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As cardio-oncology imposed itself as the reference specialty for a comprehensive cardiovascular approach to all patients with cancer, a more specific and careful cardiac evaluation of women entering their journey into cancer care is needed. Gender medicine refers to the study of how sex-based biological and gender-based socioeconomic and cultural differences influence people's health. Gender-related aspects could account for differences in the development, progression, and clinical signs of diseases as well as in the treatment of adverse events. Gender also accounts for major differences in access to healthcare. As for medicine and healthcare in general, gender-related characteristics have gained significance in cardio-oncology and should no longer be neglected in both clinical practice and research. We aimed to review the most relevant cardiovascular issues in women related to the cardio-oncology approach to offer a specific gender-related point of view for clinicians involved in the care process for both cancer and cardiovascular disease.

KEYWORDS

cardio-oncology, gender medicine, radiotherapy, anthracyclines, immunotherapy

Introduction: The need for gender cardio-oncology

Cardio-oncology (CO) now leads the cardiology care pathway for patients with cancer and provides guidance for clinicians involved in this challenging management. European and American cardiology and medical oncology scientific societies released guidelines and recommendations (1–3) on CO and an increasing number of national cardiology societies have published CO reports (4–6). Bearing in mind the well-established role of CO in clinical practice, a step ahead toward a more focused CO approach on women entering their journey into cancer care is needed.

In truth, there are differences between men and women in the frequency, symptomatology, and severity of many diseases, as well as in the responsiveness to therapies and adverse drug responses (7, 8). In clinical practice, a sex-based approach promotes the appropriateness and personalization of care with the goal to improve quality of life (9). It advocates for a new approach to medicine, recommending policies targeted at establishing new preventive, diagnostic, prognostic, and therapeutic health measures that take gender variations into consideration. Biological and clinical parameters, as well as cultural and socio-psychological factors, should all be taken into account. Despite the fact that there are known biochemical and sex-related factors that influence the risk of disease in women, the connections between various diseases in women are still understudied (10). Understanding the temporal pattern of the illness network may assist promote a life-course approach to women's health and uncover crucial indicators to decrease the risk of future bad outcomes, which is critical for providing cost-effective and improved healthcare for women (7).

This CO sex and gender-oriented paradigm shift will try to fill the gap in offering a more tailored clinical approach.

We analyzed the most important cardiovascular issues in women related to CO approaches to provide a gender-specific perspective for doctors working in cancer and cardiovascular disease care (**Figure 1**, panel A).

Sex differences in heart failure

Heart failure (HF) is a complex syndrome characterized by structural and functional impairment of left ventricle. It can be considered a significant public health issue, as its prevalence is rising (about 1–2% of adults in western countries) with high morbidity and mortality rates (11). Important sex differences are represented in HF: Etiology, clinical characteristic, and prognosis are different between men and women. Of note, women are underrepresented in HF clinical trials (12). Prevalence data show no difference between men and women; however, women are more likely to be affected by heart failure with preserved ejection fraction (HFpEF), while heart failure with reduced ejection fraction (HFrEF), where ischemic component is predominant, is more represented in men (13–15). Peripartum cardiomyopathy and certain genetic X-linked cardiomyopathies such as Duchenne or Becker dystrophies are special clinical HF scenarios of women (16) as is chemotherapy-related cardiomyopathy due to anthracycline or Her-2 therapy cardiotoxicity in breast cancer (17). Takotsubo syndrome is predominant in women; its etiopathology is not completely clear, but it seems that a decrease in estrogen levels during the menopausal period could increase the sensitivity of the heart in catecholamine circulation and be responsible for this clinical manifestation (18).

Traditional cardiovascular risk factors (CVRFs) have a different impact in male and female risk of developing HF. It is quite difficult to generalize as the prevalence of traditional CVRF differs greatly around the world, but the impact of cigarette smoking, diabetes, hypertension, and obesity in causing HF seems to be greater in women (19–24). Women have also sex-specific risk factors: Gestational diabetes and hypertension are predisposing conditions to develop HF in the following years (25–27).

Anthracycline cardiotoxicity in women

Anthracyclines represent the cornerstone for the treatment of many solid and hematological cancers; their cardiac toxicity is known from decades and ranges from asymptomatic reduction of left ventricle ejection fraction (LVEF) to symptomatic heart failure (28). Several treatment and patient-related items are described as risk factors for anthracycline cardiotoxicity. Among those patient-related, female sex and age deserve special consideration. Moreover, there is a significant clinical difference between pediatric and adult doxorubicin-induced cardiotoxicity.

For young girls who survived cancer in pediatric age, cardiotoxicity risk is approximately four times greater than the risk for male childhood cancer survivors treated with anthracyclines (29). Lipshultz et al. reported that left ventricular contractility of female childhood cancer survivors 8 years after completing doxorubicin treatment was significantly worse than that of their male counterparts and the female sex was an independent factor for cardiac dysfunction (30). In Mulrooney's study, the relative hazard of congestive heart failure was 40% higher in female survivors than in male survivors after childhood cancer (31). Of note, not all clinical studies or registries on cancer survival identify the female sex as a risk factor for cardiotoxicity. In a large cohort of Danish childhood cancer survivors, no evidence of the female sex as a risk factor for HF was found (32, 33).

On the contrary, studies that analyze sex-related differences in cardiac side effects in adult cancer population showed that

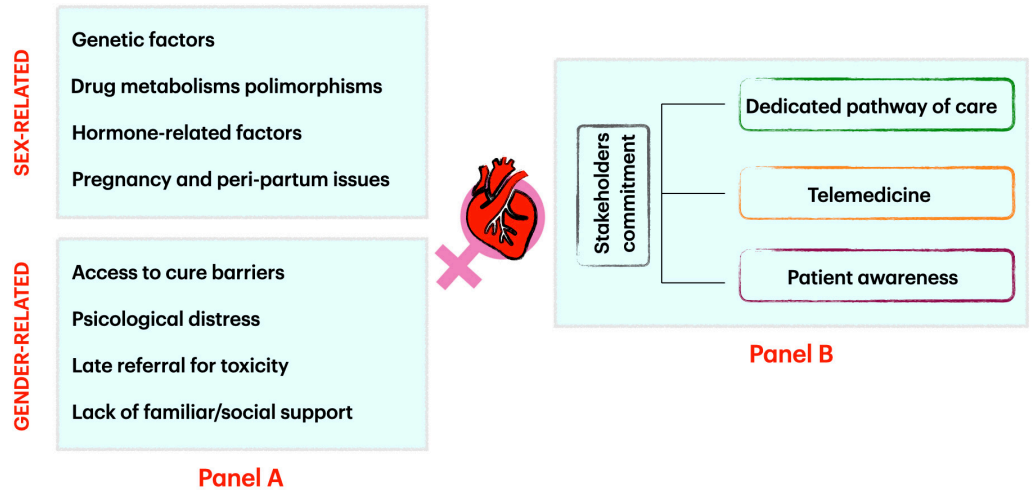

FIGURE 1
Factors influencing sex and gender-related cardio-oncology issues **(Panel A)** and how to address them **(Panel B)**.

the male sex has an increased risk for cardiovascular events and these differences could be explained (at least in part) by the presence of cardiac pre-existent disease, although post-menopausal women seem to be as susceptible to cardiotoxicity as men. In a population-based cohort study of chemotherapy-treated Hodgkin lymphoma with pre-existing cardiac heart disease, the male sex was a significant risk factor (34).

Another issue to explain this difference is due to the fact that most of the research on anthracycline cardiotoxicity in an adult cancer population are conducted in breast cancer, that is, primarily a female-related cancer. Similarly, in another large cohort study, patients with cancer who developed cardiac events (heart failure and cardiac death) were significantly older, predominantly men with pre-existing cardiac risk factors and history (35).

Very few pre-clinical studies with juvenile animal models can help to understand the sex difference in anthracycline cardiotoxicity. In pre-clinical studies, with adult animal models, the female sex is protective against anthracycline cardiotoxicity compared to the male sex both in the acute and chronic setting (36, 37).

Reasons to clearly explain sex differences in cardiac toxicity from anthracyclines are lacking. Some hypotheses have been proposed as the role of female hormones in oxidative stress and in mitochondrial dysfunction (both pathways are believed to be involved in the genesis of cardiac damage from doxorubicin) (38–41). Last, the role of pharmacokinetics differences between male patient and female patient cannot be excluded (42).

It could be concluded that the female sex is a risk factor for anthracycline cardiotoxicity in patients with childhood cancer, while it seems to be protective in adult fertile women. Post-menopausal patients with cancer have the same cardiac risk of the elderly men.

Cardiac issues of cancer treatment during pregnancy

Cancer diagnosis during pregnancy should be considered as a rare situation in oncology with an estimated incidence of one case every 1000 pregnancies (43). An increase in incidence in the next decades can be expected, in particular in Western countries, due to an older age at first pregnancy (44) and to the wider use of non-invasive prenatal testing that may identify early-stage non-symptomatic malignancies (45). Breast cancer represents the most common cancer type found during pregnancy, but the incidence of other types (as cervical cancer, lymphoma, ovarian cancer, and leukemia) is not negligible (46). When a diagnosis of pregnancy-associated cancer is made, the patient should be referred to a center with specific expertise and managed by a multidisciplinary expert team (47). Breast surgery is feasible throughout the pregnancy, while radiotherapy should be postponed until after delivery due to the high risk of fetal abnormalities (48, 49).

The use of chemotherapy during pregnancy should be avoided during the first trimester due to the high risk of fetal malformations, but it is considered safe during the second and third trimesters. In the first 12 weeks of pregnancy, the placenta does not effectively protect the fetus against the effects of cytotoxic drugs, so anticancer agents could interfere with organogenesis leading to an increased risk of miscarriage and congenital malformations (50, 51). After the first trimester, chemotherapy can be safely administered because the incidence of fetal malformations overlaps with that of the general population (52). Anthracyclines, cyclophosphamide, and taxane-based regimens are widely used for the treatment of patients with breast cancer (53, 54). The cardiotoxic effects of

anthracyclines during pregnancy (second and third trimester) on women do not differ from those of the general cancer population, and the same precautionary rules to reduce the risk of cardiac side effects should be followed (2). Although chemotherapy is generally considered safe after the first 12 weeks of pregnancy, an increased risk of prematurity and rupture of membranes was reported in a large population study on 11 milion births (55); hence, caution is required. To explain this effect, a direct anthracycline-related vascular damage of placenta has been proposed. Doxorubicin-exposed pregnant mice showed a vascular-derived placental toxicity with a reduced blood flow and a lower birth weight (56).

Population data focused on the long-term cardiac and general safety outcome of children with *in utero* exposure to chemotherapy. Overall, retrospective cohort studies are reassuring with no evidence of after-birth cardiac issues compared to babies born from healthy women (57). A prospective case–control study compared 129 children with *in utero* exposure to anticancer agents in the second or third trimester with 129 matched control children without exposure. The authors did not report any clear adverse effects on growth, cognitive, and cardiac function in early childhood even if the incidence of preterm birth and small gestational age was higher among the exposed group (48).

The second drug group historically related to cardiac toxicity is anti-HER2 agents. The use of trastuzumab during pregnancy is contraindicated in relation to the increased risk of developing oligo- and/or anhydramnios. A meta-analysis on 30 patients recently reported a total of 32 fetuses in trastuzumab-exposed women mainly in the metastatic setting. Oligohydramnios or anhydramnios was the most common (58.1%) adverse event reported. There was a statistically significant decrease in its incidence in patients receiving trastuzumab only during the first trimester. In 43.3% of cases, a completely healthy neonate was born. About 41.7% of fetuses exposed to trastuzumab during the second and/or third trimester were born completely healthy versus 75.0% of fetuses exposed exclusively in the first trimester (58). Few data are available for newer anti-HER2 agents. A recent report focused on pregnancy issues in ALTTO and NeoALTTO trials, both testing trastuzumab and lapatinib in patients with early breast cancer. Despite both protocols, as usual, required active contraception for women with childbearing potential, 12 women exposed to anti-HER2 therapy or immediately after treatment completion became pregnant. Seven patients opted for an induced abortion, while five completed the pregnancy. All pregnancies and deliveries had no complications, and no congenital anomalies were reported (59). Given the strong recommendation against the use of anti-HER2 agents, no data are available for other anti-HER2 agents such as pertuzumab, trastuzumab emtansine, and neratinib, and thus their administration in pregnant women is contraindicated.

Cardiovascular adverse events during pregnancy after exposure to cardiotoxic therapies in survivors of childhood, adolescent, and young adult cancers

Improvements in anticancer global strategy resulted in better outcomes for a large number of patients with cancer, with many of them experiencing definitive cure or long-term survival. In particular, the survival rate for childhood, adolescent, and young adult (CAYA) cancers peaked near 85% with a consequent steadily growing population of long-term survivors (60). As a consequence, more than 1,000,000 survivors of CAYA cancer can be identified across North America and Europe (61, 62). Survivors of CAYA cancers are at risk for late toxicities from anticancer therapies as well as psychological and social issues, and an increased incidence of comorbidities has been reported (63–65). Late cardiovascular sequelae are a major concern for this group of patients and are mainly related to chest radiation therapy and anthracycline exposure (66, 67). On these grounds, it is not surprising that cardiovascular safety of pregnant women previously exposed to cardiotoxic anticancer treatments requires special attention.

Hines and colleagues described the outcome of 1554 pregnancies among 847 female cancer survivors. They reported an overall very low incidence rate of cardiomyopathy during pregnancy (0.3%), slightly increased taking into account postpartum and pre-pregnancy cardiomyopathy. The only risk factor for pregnancy-related cardiomyopathy was a higher median dose of anthracyclines received (68). As a consequence, the authors stated the general cardiac safety of pregnancy in CAYA cancer survivors but highlighted the need for a careful evaluation and follow-up during pregnancy (and later on) in women with a history of anthracycline exposure and/or a documented previous or current subclinical or symptomatic cardiomyopathy. Similar results have been reported in a Canadian series of 78 women (94 pregnancies) treated with cancer therapy as CAYAs. The majority of cases received anthracyclines, while around one-third received non-anthracycline-based chemotherapy and/or radiation therapy. The observed risk of developing heart failure during pregnancy was very low in female CAYA cancer survivors without a history of cardiotoxicity, while those with a history of cardiotoxicity have approximately 30% chance of developing heart failure and so should be offered a close cardiac monitoring program by an expert multidisciplinary team (69). A previous report on a small population of female survivors of childhood cancers pointed out the safety of pregnancy from a cardiac point of view but, once more, those women presenting with left ventricular dysfunction before pregnancy were at risk for worse outcome during and after pregnancy (70). M.D. Anderson Cancer Center Experience on this topic has been reported few years ago.

Compared to a matched control group of female survivors of CAYA cancers, pregnancy represented a risk factor for adverse cardiac events as well as a higher anthracyclines cumulative dose and a longer time from cancer treatment to first pregnancy (71). Van Dalen et al. reviewed 53 childhood cancer survivors with a total of 100 deliveries. Two of these patients had a history of acute congestive heart failure related to anthracyclines therapy. No heart failure event occurred during pregnancy leading to a 0% incidence rate but, as the authors stated, larger cohort studies with adequate power and long-term follow-up are needed (72). A recently published retrospective analysis on 64 women and 110 pregnancies reported a slightly higher incidence of cardiac events in female CAYA cancer survivors. A total of five women (7.8%) had peripartum cardiac events (symptomatic and subclinical). Symptomatic dysfunction without prior cardiac dysfunction incidence was lower (1.8%), but represented a 55-fold increased risk compared to the general population. Risk factors were younger age at cancer diagnosis and higher anthracyclines dose. Of note, in a total of five cases, cardiac function recovery after delivery occurred in one case only (73) (**Table 1**).

A recent meta-analysis of six studies consisting of 2,016 pregnancies, predominantly in childhood cancer survivors, clearly highlighted the very low rate of pregnancy-related cardiac events in the general population. Only 33 cardiac events were reported leading to an overall weighted incidence of left ventricular dysfunction or heart failure of 1.7%. A sharp increase in incidence was noticed in patients with a history of cardiac toxicity from previous anticancer therapy. While the incidence of cardiac adverse events was 0.24% in cases without previous cardiac toxicity, it peaked to 28.4% in women with a history of cardiac side effects translating into an odds ratio of 47.4 for the increase in the risk of heart failure and left ventricular dysfunction (74). A population-based cohort analysis on obstetrical and perinatal outcomes in CAYA cancer survivors showed that female survivors had an increased risk for maternal cardiac morbidity (75).

Sex influence in radiation-associated cardiac disease

Unintended irradiation of healthy tissues surrounding tumor can elicit endothelial dysfunction that leads to inflammatory responses and subsequent vascular damage (76, 77). These phenomena cause the so-called radiation-associated cardiac disease (RACD), an umbrella term that encompasses myocardial fibrosis with a possible evolution in myocardial dysfunction and congestive heart failure, pericarditis, valvular heart disease, conduction abnormalities, and vascular disease including coronary artery disease (CAD). The vascular damage can occur in the carotid and intracranial arteries when head and neck tumors are irradiated, in the coronary arteries when lymphomas, breast, lung, esophageal, and gastric cancers are irradiated, and in the aorta, renal, intestinal, and peripheral arteries in lymphoma, intestinal, and testicular cancers (78, 79). The hallmark of radiotherapy(RT)-induced vascular damage is media disruption, fibrosis and atrophy, and adventitial thickening and fibrosis; intimal plaques are not different from those observed in non-irradiated patients, with a fibrocalcific component more prominent than a proliferative component (80, 81). Patients surviving for many decades after treatment showed late cardiotoxic effects of the radiation therapy, mostly CAD events. Modern techniques have banned extended fields and have modified delivery techniques to reduce cardiac exposure, but a mean heart dose > 10Gy can still be needed and can significantly increase cardiovascular disease mortality risk (82).

Coronary artery disease is the most frequent cardiotoxic phenotype after thoracic RT, and this is the point where sex becomes an issue. We know that women have different clinical presentations of CAD if compared with men and that genetic, anatomic, physiologic, psychosocial, cultural, and economic factors account for the different clinical phenotypes. CAD in male patients affects mainly epicardial coronary arteries, whereas in female patients the microvascular circulation has the greatest impact. These differences will be translated in the CAD phenotype of RACD (83–86). In female patients, traditional cardiovascular risk factors such as tobacco use, obesity, type 2 diabetes mellitus, depression, and psychosocial stress have a more powerful impact on CVD compared to male patients (87). In more than 2,000 female patients treated with RT for breast cancer from 1958 to 2001, baseline risk factors accounted for a 2-fold increased risk of major cardiovascular events and a history of CAD for a 6-fold increased risk (88). Sex-related differences in RACD can be studied mostly in patients with lymphoma and in patients with pulmonary malignancies. A reliable comparison of cardiotoxicity between male patients and female patients cannot be done in breast cancer, a malignancy studied almost exclusively in the female gender.

A recent pre-clinical study investigated the molecular basis of sex-specific differences in toxicity following localized radiotherapy in male and female mice exposed to 19Gy cardiac irradiation; female mice showed increased tolerance to radiotherapy, and this cardio-protective effect was proven to be dependent on estrogens via a Rho-B-activated estrogen pathway (89). Unfortunately, in the clinical setting, very few studies have made a comparison of RACD in male patients and female patients. In a study performed with old radiotherapeutic techniques (between 1969 and 1998), 1279 patients with clinical Stage IA-IVB Hodgkin lymphoma were treated with mediastinal RT and followed up for a median time of almost 15 years; in these patients, old age and male sex predicted the occurrence of cardiac events and this fact was supposed to be linked to a higher proportion of cardiovascular risk factors in male patients

TABLE 1 Summary of published reports on cardiac outcome during and after pregnancy in survivor women of childhood, adolescent, and young adult cancers.

Authors	Year	Type	Population/Pregnancies	Cardiac outcome
Hines et al. (68)	2016	Retrospective	847/1554	Overall very low incidence (0.3%) of cardiomyopathy but warning in case of previous cardiac toxicity, Anthra exposure or documented cardiomyopathy
Liu et al. (69)	2018	Retrospective	78/94	Low incidence (5.3%) of heart failure in general population. All cases occurred in women with a history of cardiotoxicity
Bar et al. (70)	2003	Prospective	37/72	Overall favorable outcome but warning in those patients with left ventricle disfunction before pregnancy
Thompson et al. (71)	2017	Retrospective	337/86	Increased incidence of adverse cardiac events in pregnant vs. non-pregnant survivors. Higher Anthra cumulative dose and longer time to first pregnancy were risk factors for adverse cardiac events.
van Dalen et al. (72)	2006	Retrospective	53/100	No heart failure event reported
Chait-Rubinek et al. (73)	2019	Retrospective	64/110	Peripartum cardiac events were uncommon but incidence was not negligible. Younger age at cancer diagnosis and a higher Anthra cumulative dose were risk factors.

Anthra = anthracyclines.

(90). In a more recent review of 10 studies (four prospective and six retrospective), with a population of 13,975 patients (41% female patients and 59% male patients), a 4-fold increased rate of cardiovascular events and a 2-fold increase on all-cause mortality in women were observed following radiation therapy for Hodgkin lymphoma (91). Moreover, even though both male patients and female patients had higher mortality rates with advancing age, this effect was higher in female patients. The reason for this disadvantage of female patients in RACD has not received a full explanation. It could be due to the reduced presence of women in these clinical trials, to the higher doses of radiation needed to treat Hodgkin lymphoma in women, and to the more frequent microvascular phenotype of CAD in women. Overall the higher risk of radiation therapy is independent from cardiovascular traditional risk factors (88). There are also female-specific risk factors associated with an increased risk of cardiovascular issues that need to be addressed when evaluating global cardiovascular risk of women in which a thoracic radiotherapy is planned, especially young and middle-aged women in the adjuvant setting: a history of adverse pregnancy outcomes (e.g., preeclampsia and gestational hypertension, gestational diabetes, and preterm delivery), early-onset menopause, polycystic ovarian syndrome, breast or ovarian cancer, and inflammatory disorders such as rheumatoid arthritis, psoriasis, and systemic erythematous lupus. When chest radiotherapy is planned for patients with pre-existing traditional and/or female-specific risk factors, a tailored pre-treatment evaluation, an aggressive treatment of risk factors, and a personalized monitoring are mandatory. Even though a sex specificity for adjuvant RT in breast cancer cannot be assessed, it is important to be aware of the importance of a careful history in female patients with breast cancer. **Table 2** summarizes most significant published evidence on RACD.

As far as survivors of childhood malignancies are concerned, the female sex is considered a risk factor for cardiotoxicity, but the impact of RT alone has not been investigated (92). Concomitant chemotherapy (especially if anthracycline-based) increases the risk of cardiovascular disease (93). Other manifestations of RACD such as valvular heart disease, pericarditis, and conduction abnormalities are well-known and diffusely described, but there is no clear evidence of a sex effect.

Sex-related differences of cardiac toxicity of immunotherapy

Immunotherapies have revolutionized the treatment of a variety of solid and hematologic cancers, but they come with their own set of side effects that vary depending on the kind of immunotherapy and are linked to the mechanism of action (94). Disinhibition of T-cell function by immune checkpoint inhibitors (ICIs) can lead to a spectrum of inflammatory side effects, or immune-related adverse events (irAEs). Although the specific pathophysiology of irAEs is unknown, multiple pathways have been hypothesized to account for their formation (95).

Sex-related differences in toxicity of ICIs have been described. Women treated with anti-programmed cell death protein 1 treatment are more likely to experience irAEs

TABLE 2 Summary of published evidence on cardiac toxicity of radiotherapy.

Authors	Year	Type	Population	Cardiac outcome
Darby et al. (88)	2013	Population-based case-control study. Follow-up 0–20 years	2168 women treated with RT for breast cancer in the years between 1958 and 2001 in Sweden and Denmark. Estimation of the mean radiation dose to the whole heart (MHD) and to the left anterior descending artery was performed.	963 major coronary events were documented; the incidence of major coronary events started within 5 years after RT, increased linearly with the mean dose to the heart and continued for at least two decades. A greater absolute risk was observed in those with pre-existing CVRF.
Galper et al. (90)	2011	Retrospective Median follow-up: 14.7 years	1279 Hodgkin lymphoma patients treated with mediastinal irradiation between 1969 and 1998 in Harvard-affiliated hospitals.	636 cardiac events in 187 patients, cardiac procedures in 89 patients. Absolute excess risk of irradiated patients was 18.2 for CABG, 19.3 for PCI, 9.4 for implantation of an ICD or a PM, 14.1 for pericardial surgery. Older age at diagnosis and male gender predicted cardiac events.
Khalid et al. (91)	2020	systematic review and network meta-analysis of 10 studies (4 prospective, 6 retrospective).	13,975 Hodgkin's Lymphoma patients (41% females, 59% males)	CV events/mortality significantly higher in women compared to men. All-cause mortality was also higher in women compared to men. Elderly populations showed a higher rate of mortality, which was even higher for women than men
Van Nimwegen et al. (93)	2015	case-control study	2617 five-year survivors of Hodgkin lymphoma diagnosed before age 51 years and treated with radiotherapy and/or chemotherapy between 1965 and 1995. Estimation of MHD and MLVD was performed.	91 cases of moderate to severe HF. HF rates increased at MHD greater than 25 Gy or MLVD greater than 15 Gy. Anthracycline-containing chemotherapy induced an almost 3-fold increase in HF rate.

RT, radiotherapy; CVRF, cardiovascular risk factors; CABG, coronary artery by-pass graft; PCI, percutaneous coronary intervention; ICD, implantable cardioverter defibrillator; PM, pacemaker; HF, heart failure; MHD, median heart dose; MLVD, median left ventricle dose.

than male patients. In addition, specific irAEs, such as endocrinopathies and pneumonitis, were more common in women (96), but not all observations confirm these sex-related differences in toxicity (97).

Due to the minimal participation of female patients in relevant clinical trials, evaluation of sex differences in cardiotoxicity associated with immune treatment is limited. Female patients may be at higher risk of ICIs-related myocarditis, according to certain research, albeit this has not been proven consistently (98). A study on a pharmacovigilance database seems to identify the female sex (as older age) to be risk factors for ICIs-associated myocarditis but the results could be biased by various confounding factors as the tendency to report unusual or more serious adverse events only and the aforementioned reduced number of women treated for non-small-cell lung cancer representing the principal setting for immunotherapy (99).

Some feelings about a difference between male and female toxicity profile of immunotherapy appear, but a clear conclusion cannot be drawn as a more focused sex and gender-oriented research is needed.

Older women treatment with anthracyclines

Treatment of old people (age $\geq$ 65 years) is very challenging, and geriatric patients may be undertreated and exposed to a higher mortality or overtreated and exposed to higher toxicity. Older women are no exception to this rule; furthermore, female patients have their peculiar phenotypes of cardiac disease (already described in the previous chapter) and are underrepresented in clinical trials. Anthracycline cardiotoxicity is dose-related but in the last two decades age has emerged as a relevant risk factor for anthracycline-related HF. Older patients (age > 65 years) showed a greater incidence of HF when compared to younger patients after a cumulative dose of 400 mg/m^2 (100). In a population of more than 30,000 women with early breast cancer, anthracycline was administered to 18% of patients with the more favorable cardiovascular profile, but still the hazard ratio for cardiomyopathy, HF, and heart disease was 2.48, 1.38, and 1.35, respectively, and this risk was still elevated 5 years after the diagnosis (101). In a population of more than 40,000 patients with breast cancer of which 11% were treated with adjuvant anthracyclines, women aged 66 to 70 years showed an increased risk for HF, whereas women aged 71 to 80 did not (102). Another study of almost 20,000 women documented an increased risk of cardiomyopathy (hazard ratio 1.95), HF, and cardiac dysrhythmias, whereas the association with CAD or conduction disorders was not significantly increased (103).

There are many vulnerabilities linked to older age, and the aging process induces loss of cardiomyocytes, alteration of pharmacokinetics, and the frequent development of comorbidities enhancing chemotherapy-related cardiotoxicity. Among cancer-related risk factors, drug–drug interactions due

to the common polypharmacy and lifestyle-dependent risk factors such as physical inactivity and obesity increase the risk of chemotherapy-induced cardiotoxicity, along with a frequent deterioration of renal function as a result of dehydration and/or hypovolemia (104). As far as the sex issue is concerned, the higher risk of anthracycline-induced cardiotoxicity observed in young female patients when compared to young male patients has not been clearly documented in post-menopausal women (105, 106).

Many mechanisms have been proposed to explain enhanced anthracycline-related cardiotoxicity in advanced age. Doxorubicin seems to induce cellular senescence with release of pro-inflammatory cytokines and telomere dysfunction that impairs mitochondrial biogenesis leading to the production of reactive oxygen species (107–109). This effect may be amplified in older female patients, but in the near future this toxic "senescent status" of cells may be targeted and reversed and this fact could reduce the burden of anthracycline-induced cardiotoxicity (110, 111). In last years the immunity system has gained a pivotal role in many diseases, and aging of the immune system (the so-called immunosenescence) has a contributing effect on morbidity and mortality in the elderly (112, 113).

In conclusion, aging of the population will lead to an increasing number of breast cancers in the elderly female patients; these patients are at high risk of cardiotoxicity, but they should not be denied the best treatment. Every effort should be made to reduce the burden of modifiable risk factors and to plan a careful monitoring and follow-up process. This is the point where cardio-oncologists come on stage to help these patients to get their best option care.

How to manage healthcare sex and gender disparities in cardio-oncology

Disparities related to sex and gender could affect the possibility of female individuals to access to healthcare CO facilities leading to mis- or late diagnosis, un-appropriate early anti-cancer treatment discontinuation, or late referral for cardiovascular toxicity management.

A strong commitment of all CO stakeholders is needed to provide a safe, reliable, and balanced approach to sex and gender issues. First, a sex and gender-focused CO pathway should be available in all CO services. Physicians, nurses, and CO service staff should be warned about the possibility of sex and gender issues and undergo specific training.

Telemedicine could offer the possibility to reduce some of the patients' concerns about physical, social, racial, and sex and gender issues when referring to a medical facility for a CO consultation. Virtual platforms have proved useful instruments for multidisciplinary discussion and video consultation with staff involved in patient care, with the patient himself or caregiver in family environment (if needed).

Lastly, patient awareness is crucial. All possible efforts shall be made to let patients know that CO programs are familiar with sex- and gender-related issues and that they can find help and tailored solutions into CO services (**Figure 1**, panel B).

Conclusion

We are just at the dawning of sex- and gender-related issues in the field of CO. While for anthracyclines and RACD some more robust evidence pointed out the role of sex in predicting side effects of anticancer treatments, for all new drug classes in oncology (in particular immunotherapy) gender-CO is a story to be written. Last but not least, a focused approach on CO social as well as on the quality of life issues of women should be implemented to guarantee a comprehensive care.

Author contributions

MLC, IB, GG, GR, and AC wrote sections of the manuscript. All authors contributed to the conception of the work, manuscript revision, 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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Association of QT interval-prolonging drugs with clinical trial eligibility in patients with advanced cancer

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Introduction: Drug-induced prolongation of the heart rate-corrected QT interval (QTc) is associated with increased risk for the potentially fatal arrhythmia torsades de pointes. Due to arrhythmia risk, clinical trials with cancer therapeutics often exclude patients based on thresholds for QTc prolongation. Our objective was to assess associations between prescriptions for QT-prolonging drugs and the odds of meeting cancer trial exclusionary QTc thresholds in a cohort of adults with advanced cancer.

Methods: Electronic health records were retrospectively reviewed for 271 patients seen at our institutional molecular solid tumor clinic. Collected data included demographics, QTc measurements, ventricular arrhythmia-related diagnoses, and all inpatient and outpatient prescriptions. Potential associations were assessed between demographic and clinical variables, including prescriptions for QT-prolonging drugs, and QTc measurements.

Results: Women had longer median QTc measurements than men (p = 0.030) and were prescribed more QT-prolonging drugs during the study (p = 0.010). In all patients, prescriptions for QT-prolonging drugs were associated with longer median and maximum QTc measurements at multiple assessed time points (i.e., for QT-prolonging drugs prescribed within 10, 30, 60, and 90 days of QTc measurements). Similarly, the number of QT-prolonging drugs prescribed was correlated with longer median and maximum QTc measurements at multiple time points. Common QTc-related exclusionary criteria were collected from a review of ClinicalTrials.gov for recent cancer clinical trials. Based on common

exclusion criteria, prescriptions for QT-prolonging drugs increased the odds of trial exclusion.

Conclusion: This study demonstrates that prescriptions for QT-prolonging drugs were associated with longer QTc measurements and increased odds of being excluded from cancer clinical trials.

KEYWORDS

cancer, clinical trial eligibility, clinical trial exclusion, QT interval, QT-prolonging drugs, QTc

Introduction

Drug-induced prolongation of the QT interval on the surface electrocardiogram (ECG), which corresponds to the period in which cardiac ventricular depolarization and repolarization occur, is associated with an increased risk of potentially fatal ventricular arrhythmias, including torsades de pointes (TdP) (1). QT interval length reflects a balance between depolarizing and repolarizing ionic currents in the ventricle, and drugs that prolong the QT interval do so by affecting the function of ventricular currents, most commonly *via* inhibition of the rapid component of the delayed rectifier potassium current (I_{Kr}) (2). The heart rate-corrected QT interval (QTc) is an established monitoring parameter to assess the risk of drug-induced TdP both in the clinical setting (3) and during development and regulatory approval of new medications (4).

The QTc interval is also frequently used as a criterion for clinical trial eligibility, including in cancer, where a number of efficacious treatment options have been demonstrated to prolong QTc (5). A multitude of ongoing cancer trials in the United States (US) have exclusion criteria based on QTc thresholds (as listed on ClinicalTrials.gov), potentially preventing cancer patients from receiving life-saving therapies. While exclusion of patients at increased risk of potentially fatal arrhythmias may be warranted, clinical guidance is available to manage drug-induced arrhythmia risk (3, 6, 7), including specific recommendations for cancer patients (5, 8). One common strategy to reduce the risk of drug-induced arrhythmias is discontinuation of concomitant medications that prolong QTc (7, 8). For non-antiarrhythmics, alternative therapies often exist, even within the same medication class, that do not prolong QTc (9). Therefore, therapeutic substitution to reduce the number of QT-prolonging drugs may be a viable strategy to prevent exclusion of patients from clinical trials, particularly since past investigations have found that concomitant administration of multiple QT-prolonging drugs produced incremental increases in QTc prolongation (10, 11).

The potential for the administration of QT-prolonging drugs to affect clinical trial eligibility is supported by numerous investigations that have demonstrated high rates of prescriptions for QT-prolonging drugs in cancer patients (12–15). Moreover, various cancer therapies, including many tyrosine kinase inhibitors (TKIs), result in clinically relevant QTc prolongation (16–19). However, the impact of QT-prolonging drugs on trial eligibility has not been directly studied. Accordingly, the purpose of this research was to assess the potential for drug-induced QTc prolongation to affect clinical trial eligibility within a cohort of adult patients with advanced cancer. Our specific objectives included the following: (1) to survey study protocols for ongoing or recently completed cancer clinical trials, in order to document their exclusionary QTc thresholds; (2) to determine associations between demographic factors and administration of QT-prolonging drugs with QTc values obtained from electronic health records (EHRs); and (3) to assess the impact of demographic factors and administration of QT-prolonging drugs on clinical trial eligibility based on the exclusionary QTc thresholds used by cancer clinical trials and recommended by professional organizations.

Materials and methods

Patient enrollment and eligibility

Our study population consisted of adult patients with advanced solid cancers who were treated at the Indiana University Health Precision Genomics Clinic in Indianapolis, Indiana, US and enrolled in the Indiana University Total Cancer Care Protocol (part of the larger Oncology Research Information Exchange Network-wide Total Cancer Care initiative). Patients enrolled in the Total Cancer Care Protocol were selected for inclusion in this study if their EHR

Abbreviations: ACC, American College of Cardiology; AHA, American Heart Association; ECG, electrocardiogram; HER, electronic health record; ICD, implantable cardioverter-defibrillator; QTc, heart rate-corrected QT interval; TdP, torsades de pointes; TKI, tyrosine kinase inhibitor; US, United States.

included, after their date of diagnosis of cancer, at least one Bazett's-corrected QT value and administration of at least one medication. Bazett's correction was used throughout our analyses since, relative to other correction methods, it has the strongest data associating QTc threshold values with arrhythmia risk (3). The EHR data were obtained *via* query of the Indiana Health Information Exchange, a state-wide EHR repository with data from 38 health systems. Using these criteria, we identified 275 eligible patients. We excluded four patients since their only QTc measurements were those taken within 1 day of death or cardiac resuscitation (2 patients) or after they had been implanted with implantable cardioverter-defibrillators (ICDs) with functioning ventricular pacemakers (2 patients). As a result, our final cohort included 271 patients who were enrolled at clinic visits between February 2015 and February 2018 (**Supplementary Figure 1** in **Data Sheet 2**). The research protocols for this study and the parent Total Cancer Care Protocol were approved by the Indiana University Institutional Review Board, and all patients provided written informed consent.

Survey of corrected QT eligibility requirements in clinical trials

Using the ClinicalTrials website[1] (20), which is maintained by the US National Library of Medicine, we conducted a survey of clinical trial eligibility requirements related to exclusionary QTc thresholds. We searched for oncology trials involving any pharmacotherapeutic intervention, as well as specifically for trials including the following TKIs that are known to prolong the QT interval: bosutinib, cabozantinib, ceritinib. cobimetinib, crizotinib, dabrafenib, dasatinib, encorafenib, entrectinib, gilteritinib, lapatinib, lenvatinib, necitumumab, nilotinib, osimertinib, pazopanib, sorafenib, sunitinib, vandetanib, or vemurafenib. We limited our search to trials available within the US that were enrolling patients between January 1, 2010 and December 31, 2020 to match our study population. We further limited our search to protocols that contained the keyword "QT." We then manually reviewed each protocol to identify QTc values that served as exclusionary thresholds.

Study data collection and classification

Electronic health record (EHR) data were obtained from the Indiana Health Information Exchange through April 20, 2020 and included demographic data (age, date of first cancer diagnosis, date of death, sex, and race), all inpatient and outpatient prescriptions, QTc measurements, and ventricular arrhythmia-related diagnoses and interventions (list of queried International Classification of Diseases and Current Procedural Terminology codes provided in **Supplementary Table 1** in **Data Sheet 2**). All prescriptions, QTc measurements, diagnoses, and interventions had associated dates. In addition, prescription data included the dispensing location (i.e., whether administered in a medical setting, including outpatient clinics, or whether dispensed from an outpatient pharmacy). Within our analyses, we classified medications as "QT-prolonging" if they were categorized by the FDA-supported CredibleMeds® database[2] as having a "known" or "possible" risk of TdP (9). All other medications were classified for our purposes as "non-QT-prolonging." Medications classified by CredibleMeds® as having a "conditional risk of TdP," meaning that they do not independently prolong QT but can trigger clinical conditions that lead to QT prolongation (e.g., thiazide diuretic-induced hypokalemia), were not considered as "QT-prolonging" in our analyses; this decision was made since evidence of the associated QT-prolonging conditions was not routinely collected in the EHR, which did not allow us to verify whether the conditions were met for these drugs to prolong QT.

QTc measurements were collected for each patient since their respective date of first cancer diagnosis. We then reviewed the dates of QTc measurements relative to interventions or diagnoses that may be associated with alterations to QTc. QTc measurements that occurred (1) within 24 h of cardiac arrest or death from any cause or (2) any time after placement of cardioverter-defibrillator or pacemaker devices were excluded. From the remaining values for each individual, we calculated the maximum, minimum, median, and mean QTc measurements, and the difference between maximum and minimum QTc measurements, termed the delta QTc. We determined whether each individual's QTc measurements exceeded QTc thresholds from our survey of ClinicalTrials.gov or those established as potentially proarrhythmic by scientific statements from the American Heart Association (AHA) and the American College of Cardiology (ACC): 450 ms for men and 460 ms for women (the 95th percentile of normal QTc variation); 470 ms for men and 480 ms for women (the 99th percentile); and 500 ms in both sexes (3, 21).

Association of QT-prolonging drugs with QTc values

For each patient, the date of maximum QTc was considered the index date. We then determined how many drugs were prescribed within 10, 30, 60, or 90 days before the index date. We categorized the patients by whether they had been prescribed QT-prolonging drugs within each time period before the index date, or only non-QT prolonging drugs (or no drugs at all).

1 www.ClinicalTrials.gov

2 www.crediblemeds.org

Our prescription data did not include the days' supply. Therefore, within our paired analysis that compared QTc values in patients while taking and not taking QT-prolonging drugs, the following assumptions were used to conservatively determine the day's supply. For prescriptions administered in a medical setting, the days' supply was assumed to be one. For prescriptions dispensed from an outpatient pharmacy, the days' supply was assumed based on the shortest days' supply for indications for which the drug is commonly prescribed (see **Supplementary Table 2** in **Data Sheet 2** for a complete list of assumed durations for all prescriptions dispensed from a pharmacy). An exception to this method was made for prescriptions dispensed from a pharmacy that were (1) dispensed for at least three consecutive regular intervals (e.g., every 30 days, every 90 days) and (2) written for medications that are commonly used as maintenance therapy for chronic medical conditions (e.g., antihypertensives). For these prescriptions, the patient was assumed to be taking the medication for the entire interval between consecutive prescriptions.

Statistical analysis

We used the Kruskal-Wallis test by ranks to investigate differences in continuous QTc-related variables (maximum, minimum, mean, median, and delta QTc) between patients grouped by discrete independent variables (e.g., patient race, whether patients were prescribed QT-prolonging drugs). When more than two discrete independent variables were compared, we performed a *post hoc* Dunn's Test with Bonferroni correction to determine which groups were different from each other. We used Spearman's rank correlations to evaluate correlations between continuous independent and dependent variables (e.g., patient age and maximum QTc). We used Chi-squared tests to investigate correlations between discrete independent and dependent variables (e.g., patient sex and whether median QTc values met various QTc thresholds). Finally, we used logistic regression to evaluate correlations with binary dependent variables, such as determining how the number of QT-prolonging drugs affected the odds of meeting exclusionary QTc thresholds. Results were considered significant at $p < 0.05$ or less than adjusted p-value thresholds after Bonferroni correction from $p = 0.05$.

Results

Survey of QTc eligibility requirements from ClinicalTrials.gov

Limiting our search of the ClinicalTrials.gov database to trials conducted in the US between 2010 and 2020, we found 158 clinical trials for oncology therapeutics that specifically mentioned QT prolongation in their protocols. Of these, $n = 93$ included the QT interval in their eligibility criteria; the remaining studies instead mentioned QT as an outcome measure. Of the 93 studies that used QT for inclusion or exclusion criteria, 37 studies excluded participants with a family or personal history of congenital long QT syndrome, and 34 studies prohibited patients from taking QT-prolonging drugs while on study. Seventy-six of the studies provided specific QTc thresholds that potential trial patients could not exceed in order to be eligible (distribution of thresholds shown in **Figure 1**). Five of these studies (6.6%) included sex-specific QTc thresholds, which consisted of 450 ms for men and 470 ms for women. These studies are represented using their least stringent QTc threshold (470 ms) in **Figure 1**. The identified exclusionary QTc thresholds were 450 ms (31.6% of studies), 470 ms (35.5%), 480 ms (28.9%), and 500 ms (3.9%), which correspond to clinically relevant QTc values established by AHA/ACC scientific statements.

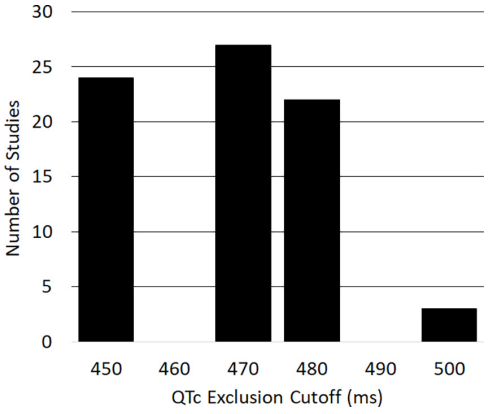

FIGURE 1
Histogram of corrected QT (QTc) measurements used as exclusion criteria in United States clinical oncology trials from 2010 to 2020 ($n = 76$).

Summary of patient demographic and clinical data

Our cohort consisted of 271 adults with advanced cancer who had at least one medication prescription and QTc measurement since their respective date of first cancer diagnosis. As displayed in **Table 1**, our cohort was 58 (49, 64) [median (1st quartile, 3rd quartile)] years old, was evenly split by sex (50.9% female), and was mostly white (88.9%). The most common cancer types at first diagnosis were pancreatic (12.9%), breast (9.6%), and colorectal (9.2%). Ventricular arrhythmia-related diagnoses occurred in 9 patients (3.3%) and included ventricular tachycardia and cardiac arrest, which occurred in 6 and 3 patients, respectively. The rate of ventricular arrhythmias was higher in our cohort than those estimated in the general

population for similarly aged individuals (18, 22). This may be attributable to the facts that many cancer therapies can cause ventricular arrhythmias (23) or that advanced cancer patients have an increased risk of ventricular arrhythmias relative to those with less advanced disease (24). Serum electrolyte abnormalities known to prolong the QT interval were common in our cohort, with the incidence of at least one episode of hypocalcemia, hypokalemia, and hypomagnesemia being 86.3, 83.0, and 68.3%, respectively. Of the 28 subjects with index QTc measurements > 500 ms and serum electrolyte concentrations from that same day, 18 (64.3%) had a serum electrolyte abnormality that may have contributed to their prolonged index QTc. The median heart rate was 83 (72, 96) beats per minute, and 92.6 and 85.6% of the cohort experienced at least one episode of tachycardia and bradycardia, respectively. Three patients (1.1%) had a medical history that included placement of an ICD or pacemaker. Fifty-four patients (19.9%) had recorded dates of death during the study period. The median duration of follow-up, defined as the elapsed time between the date of first cancer diagnosis and the date of any last study event (e.g., prescription, QT measurement), was 3.0 (1.2, 5.8) years. Since first cancer diagnosis, our cohort had a total of 19,306 unique prescriptions for QT-prolonging drugs with a median of 8 [6, 10] unique drugs per patient. Of the 271 patients in our cohort, 270 (99.6%) had ≥ 1 prescription for a QT-prolonging drug since first cancer diagnosis. In addition, our cohort had a total of 1,164 unique QTc measurements since first cancer diagnosis, with a median of 3 (2, 6) QTc measurements per patient. The median QTc for our cohort was 438 (423, 453) ms, and the minimum and maximum QTc values were 320 and 633 ms, respectively; the median index QTc, defined as the maximum QTc at the patient level, was 456 (437.5, 478) ms.

TABLE 1 Demographic and clinical characteristics of patients with advanced cancer included in the study.

Variable	Value in study cohort (n = 271)
Age in years at first cancer diagnosis [median (quartile 1, quartile 3)]	58 (49, 64)
Number of patients age ≥ 65 years [count (percent)]	65 (24.0%)
Duration of follow-up in years* [median (quartile 1, quartile 3)]	3.0 (1.2, 5.8)
Sex [count (percent)]	
Female	138 (50.9%)
Male	133 (49.1%)
Race [count (percent)]	
White	241 (88.9%)
Black	26 (9.6%)
Asian	4 (1.5%)
Cancer type at first diagnosis [count (percent)]	
Pancreatic	35 (12.9%)
Breast	26 (9.6%)
Colorectal	25 (9.2%)
Soft-tissue sarcoma	24 (8.9%)
Prostate	23 (8.5%)
Ovarian	13 (4.8%)
Renal	10 (3.7%)
Non-small cell lung	9 (3.3%)
Cholangiocarcinoma	8 (3.0%)
Head and neck	8 (3.0%)
Unknown primary	8 (3.0%)
Ventricular arrhythmia-related diagnoses [count (percent)]	
Ventricular tachycardia	6 (2.2%)
Cardiac arrest	3 (1.1%)
Serum electrolyte abnormalities⁺ [count (percent)]	
Hypocalcemia (< 8.5 mg/dL, ionized < 4.5 mg/dL)	234 (86.3%)
Hypokalemia (< 3.5 mEq/L)	225 (83.0%)
Hypomagnesemia (< 1.7 mg/dL)	185 (68.3%)
Heart rate values [in bpm or count(percent)]	
Heart rate [median (quartile 1, quartile 3)]	83 (72, 96)
Bradycardia (< 60 bpm)	232 (85.6%)
Tachycardia (> 100 bpm)	251 (92.6%)
Placement of implantable cardiac defibrillator or pacemaker [count (percent)]	3 (1.1%)
All-cause mortality during study [count (percent)]	54 (19.9%)
Corrected QT (QTc) values (in ms)	
Median QTc [median (quartile 1, quartile 3)]	438 (423, 453)
Minimum QTc	320
Maximum QTc	633
Index QTc⁺⁺ [Median (Quartile 1, Quartile 3)]	456 (437.5, 478)

*Duration of follow-up was defined as the time elapsed between the date of first cancer diagnosis and date of most recent prescription.
⁺The incidence of serum electrolyte abnormalities was assessed based on diagnoses and on lab values below the specified thresholds.
⁺⁺Index QTc was defined as the maximum observed QTc for each individual patient.

Association of patient demographics with prescriptions for QT-prolonging drugs and QTc values

Women were prescribed more QT-prolonging medications than men when the overall study period was considered [median: 8 QT-prolonging drugs, 1st and 3rd quartiles: (6, 10) for women versus 7 (6, 9) for men, p = 0.010]. Similarly, patients younger than age 65 were prescribed more QT-prolonging drugs during the overall study period than those over 65 [8 (6, 10) versus 7 (5, 9), p = 0.006], and age was inversely correlated with the number of QT-prolonging drugs

prescribed (Spearman's $\rho = -0.26$, $p < 0.001$). However, when the timing of prescriptions relative to the index date was considered, women and those under 65 were not more likely to be prescribed a QT-prolonging drug within 90, 60, 30, or 10 days before the index date. Demographic characteristics were not otherwise associated with the number of QT-prolonging drugs prescribed, nor were cardiac arrest, ventricular arrhythmia-related diagnoses, ICD/pacemaker implant, or patient death during the study period.

Women had significantly longer median QTc measurements than men [442 (426, 456) ms versus 435 (422, 448) ms, $p = 0.030$]. Men and women did not differ with regard to other measures of QTc (i.e., mean QTc, maximum QTc, minimum QTc, and delta QTc), and significant differences in QTc were not observed when patients were grouped by other demographic characteristics (i.e., age at first cancer diagnosis, age greater than 65, race, or cancer diagnosis). Patients who experienced cardiac arrest or who died during the study period did not differ with regard to their QTc measurements. Patients with ICDs or pacemakers ($n = 3$) had longer delta QTcs ($p = 0.010$), but did not differ with regard to other measures of QTc. Patients with ventricular arrhythmias ($n = 9$) had longer maximum QTc measurements than patients who did not [517 (495, 518) ms versus 456 (436, 476) ms, $p < 0.001$] but did not differ with regard to other QTc measures.

Association of QT-prolonging drugs with QTc values

We assessed whether prescriptions for QT-prolonging drugs were associated with QTc values. A histogram of QTc measurements stratified by whether patients were prescribed QT-prolonging drugs within 30 days of the index date is shown in **Figure 2**, with quantitative results provided in **Table 2**. Results for the other analyzed time points (90 days, 60 days, 10 days, any time before) are shown in **Supplementary Table 3** in **Data Sheet 2**. Median QTc measurements were significantly longer in patients prescribed QT-prolonging drugs [442 (425, 456) ms] within 30 days of the index date relative to those prescribed only non-QT prolonging drugs [427 (418, 440) ms] or no drugs at all [432 (423, 445) ms; $p < 0.001$]. Similar associations were also observed for maximum QTc ($p < 0.001$) and delta QTc ($p = 0.002$), and we observed similar patterns of higher QTc measurements in patients prescribed QT-prolonging medications within 10, 60, 90 days, or any time before their index date. Further, the number of QT-prolonging drugs prescribed within 30 days of the index date was correlated with median QTc (Spearman's $\rho = 0.20$, $p = 0.001$; **Table 3**) as well as maximum QTc ($p < 0.001$) and delta QTc ($p < 0.001$); these associations were also observed at the other assessed time points. Minimum QTc was not associated with prescriptions for QT-prolonging drugs

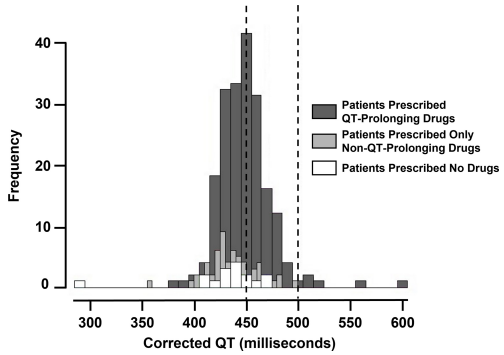

FIGURE 2
Histogram of maximum corrected QT (QTc) measurements (i.e., index QTc) in patients prescribed QT-prolonging drugs, only non-QT-prolonging drugs, and no drugs within 30 days before the index date. The vertical dashed lines at 450 and 500 milliseconds indicate minimum and maximum cancer clinical trial exclusionary QTc thresholds.

and was not correlated with the number of QT-prolonging drugs prescribed at any time point. When considering individual drugs prescribed within 30 days of the index date, patients with prescriptions for ondansetron ($p = 0.005$), promethazine ($p = 0.013$), or propofol ($p = 0.043$) had higher median QTc measurements than patients not prescribed each of these drugs (**Table 4**); these were also the three most commonly prescribed QTc prolonging drugs in our cohort.

We also performed a paired analysis in 160 patients who had QTc measurements both during and not during concomitant treatment with ≥ 1 QT-prolonging drug. For this analysis, we assigned each medication prescription a days' supply based on the type of medication and the observed prescribing patterns (see methods for additional details), and we assessed the days' supplies for temporal overlap with QTc measurements. As illustrated in **Figure 3**, median QTc values were longer in patients when concomitantly prescribed ≥ 1 QT-prolonging drug (mean of medians: 443.2 ms) than when not co-prescribed QT-prolonging drugs (mean of medians: 437.7; $p = 0.010$). A histogram displaying changes in median QTc measurements for each individual patient during concomitant treatment with QT-prolonging drugs (relative to when not treated with QT-prolonging drugs) is shown in **Supplementary Figure 2** in **Data Sheet 2**.

Association of patient demographics and prescriptions for QT-prolonging drugs with clinical trial exclusion

Based on our findings from surveying the ClinicalTrials.gov database, the number of patients in our cohort meeting common

TABLE 2 Corrected QT (QTc) measures based on whether patients were prescribed QT-prolonging drugs within 30 days of the index date.

	Patients prescribed no drugs (n = 15)	Patients prescribed only non-QT drugs (n = 57)	Patients prescribed QT-prolonging drugs (n = 199)	P-value (*post hoc* P-value for QT vs. non-QT)
Mean QTc	438 (423, 443)	429 (418, 443)	443 (429, 457)	**< 0.001 (< 0.001)**
Median QTc	432 (423, 445)	427 (418, 440)	442 (425, 456)	**< 0.001 (0.001)**
Maximum QTc	441 (426, 477)	437 (421, 469)	460 (445, 482)	**< 0.001 (< 0.001)**
Minimum QTc	424 (420, 440)	416 (405, 430)	421 (406, 442)	0.080 (0.080)
Difference between maximum and minimum QTc (Delta QTc)	0 (0, 24)	17 (0, 37)	37 (11, 64)	**< 0.001 (0.002)**

All values are in milliseconds. All data are presented as: median (1st quartile, 3rd quartile). Kruskal-Wallis test was used to compare continuous data. Bold values indicates that the p-value is significant at the <0.05 threshold.

TABLE 3 Correlation between the number of prescribed QT-prolonging drugs and corrected QT (QTc) measures at assessed time points.

	Any time before	90 days	60 days	30 days	10 days
Spearman correlation: ρ (p-value)					
Mean QTc	**0.15 (0.020)**	**0.21 (< 0.001)**	**0.22 (< 0.001)**	**0.20 (0.001)**	**0.19 (0.002)**
Median QTc	**0.14 (0.020)**	**0.19 (0.002)**	**0.21 (< 0.001)**	**0.20 (0.001)**	**0.19 (0.002)**
Maximum QTc	**0.24 (< 0.001)**	**0.31 (< 0.001)**	**0.32 (< 0.001)**	**0.28 (< 0.001)**	**0.28 (< 0.001)**
Minimum QTc	−0.01 (0.89)	0.00 (0.94)	0.02 (0.70)	0.02 (0.70)	−0.01 (0.92)
Difference between maximum and minimum QTc (Delta QTc)	**0.29 (< 0.001)**	**0.38 (< 0.001)**	**0.36 (< 0.001)**	**0.26 (< 0.001)**	**0.31 (< 0.001)**

Bold values indicates that the p-value is significant at the <0.05 threshold.

clinical trial exclusionary QTc thresholds is shown in Table 5. Overall, 27.3 and 57.9% of our cohort would be excluded from clinical trials based on their median and maximum QTc values, respectively, when applying the most stringent exclusionary QTc threshold (450 ms). In addition, 11.4% of our cohort had maximum QTc values that exceeded 500 ms, which corresponds to the least stringent exclusionary QTc threshold and is described in AHA/ACC scientific statements as being "dangerously" proarrhythmic. Women in our study were more likely to have median QTc values that exceeded the 450 ms threshold than men (33.3% of women versus 21.1% men, p = 0.030). This is notable given that < 10% of surveyed cancer trials had sex-specific exclusionary QTc thresholds and 31.6% of trials used a 450 ms threshold for all patients. If these trials used sex-specific thresholds at the 95th percentile described in the AHA/ACC scientific statements (i.e., 450 ms for men and 460 ms for women), only 15.9% of women in our cohort (rather than 33.3%) would be excluded based on median QTc. Demographic characteristics were not otherwise associated with the likelihood of meeting any assessed clinical trial exclusion or AHA/ACC thresholds.

Results from our analyses associating prescriptions for QT-prolonging drugs with the odds of meeting clinical trial exclusionary QTc thresholds are shown in Table 6. Prescriptions for QT-prolonging drugs within 30 days of the index date were associated with increases in the percentage of patients having a maximum QTc that exceeded the > 450 ms threshold (all sexes pooled) identified *via* ClinicalTrials.gov (p < 0.001) and the > 450/460 ms thresholds in men and women, respectively, from AHA/ACC scientific statements (p = 0.001). Additionally, each QT-prolonging medication prescribed within 30 days increased the odds that a patient's maximum QTc would exceed the > 450 ms (odds ratio = 1.40, p < 0.001), 450/460 ms (odds ratio = 1.30, p < 0.001), 470 ms (odds ratio = 1.28, p = 0.002), 470/480 ms (odds ratio = 1.20, p = 0.010), and 480 ms thresholds (odds ratio = 1.21, p = 0.030; Table 7). These associations were similar at the other assessed time points, and each QT-prolonging medication significantly increased the odds that a patient's maximum QTc exceeded the 500 ms threshold when prescribed at 90 days (odds ratio = 1.24, p = 0.030), 60 days (odds ratio = 1.18, p = 0.046), or at any time before the index date (odds ratio = 1.13, p = 0.030). When individual drugs were considered, prescriptions for ondansetron within 30 days were associated with increased odds of a patient's maximum QTc exceeding all assessed exclusionary thresholds (Table 4). Prescriptions for promethazine within 30 days were associated with increased odds of a patient's maximum QTc exceeding the 450 ms, 450/460 ms, and > 470 ms thresholds, and, similarly, propofol prescriptions were associated with increased odds of exceeding the 450 ms and 450/460 ms thresholds. The associations of increased odds of exceeding exclusionary QTc thresholds with prescriptions for ondansetron, promethazine,

TABLE 4 Most commonly prescribed QT-prolonging drugs, ranked by number of patients prescribed the drug at 30 days before the maximum corrected QT (QTc) index date, with associated median QTc values and number of patients that exceeded QTc thresholds.

	All patients				Patients meeting maximum QTc > 450/450 ms threshold		Patients meeting maximum QTc > 470/480 ms threshold		Patients meeting maximum QTc > 500 ms threshold	
Drug name	**Number of Patients (%)**	**Median QTc for patients on drug**	**Median QTc for patients not on drug**	***P*-value**	**Patients on drug**	***P*-value**	**Patients on drug**	***P*-value**	**Patients on drug**	***P*-value**
Ondansetron	159 (58.7%)	443 ± 31	433 ± 29	**0.005**	97 (61.0%)	< **0.001**	58 (36.5%)	< **0.001**	26 (9.6%)	**0.003**
Promethazine	101 (37.3%)	446 ± 32	435 ± 28	**0.013**	61 (60.4%)	**0.024**	33 (32.7%)	0.16	13 (4.8%)	0.56
Propofol	34 (12.5%)	449 ± 40	437 ± 27	**0.043**	25 (73.5%)	**0.006**	14 (41.2%)	0.067	5 (1.8%)	0.56
Palonosetron	29 (10.7%)	446 ± 20	438 ± 31	0.39	14 (48.3%)	0.85	5 (17.2%)	0.27	3 (1.1%)	1.00
Azithromycin	20 (7.4%)	446 ± 41	438 ± 29	0.41	15 (75.0%)	**0.036**	10 (50%)	**0.034**	4 (1.5%)	0.26
Tramadol	17 (6.3%)	438 ± 35	438 ± 30	0.94	9 (52.9%)	1.00	4 (23.5%)	0.79	2 (0.7%)	1.00
Ciprofloxacin	17 (6.3%)	440 ± 29	438 ± 30	0.54	10 (58.8%)	0.62	6 (35.3%)	0.58	3 (1.1%)	0.42
Levofloxacin	12 (4.4%)	443 ± 14	438 ± 31	0.41	10 (83.3%)	**0.035**	7 (58.3%)	**0.022**	5 (1.8%)	**0.006**
Escitalopram	10 (3.7%)	438 ± 23	438 ± 31	0.78	4 (40%)	0.53	3 (30%)	1.00	0 (0%)	1.00
Mirtazapine	8 (3%)	442 ± 15	438 ± 31	0.90	4 (50%)	1.00	2 (25%)	1.00	1 (0.4%)	1.00

All QTc values are in milliseconds. Data are presented as median ± interquartile range and percentages. Kruskal-Wallis test was used to compare continuous data, and Fisher's exact test was used to determine if the proportion of patients who exceeded a given QTc exclusionary threshold was higher among patients prescribed the drug compared to patients not prescribed the drug. AHA/ACC scientific statements identify 450 ms (men)/460 ms (women) and 470 ms (men)/480 ms (women) as the 90th and 99th percentiles of the normal QTc intervals, respectively. QTc > 500 ms for all patients was identified as a relevant QTc threshold by both the AHA/ACC and from our survey of ClinicalTrials.gov. Due to space issues, only the QTc thresholds identified from the AHA/ACC are shown on this table. Associations between QT-prolonging drugs and the proportions of patients exceeding QTc thresholds from our survey of ClinicalTrials.gov is shown in **Data Sheet 1**. Bold values indicates that the *p*-value is significant at the <0.05 threshold.

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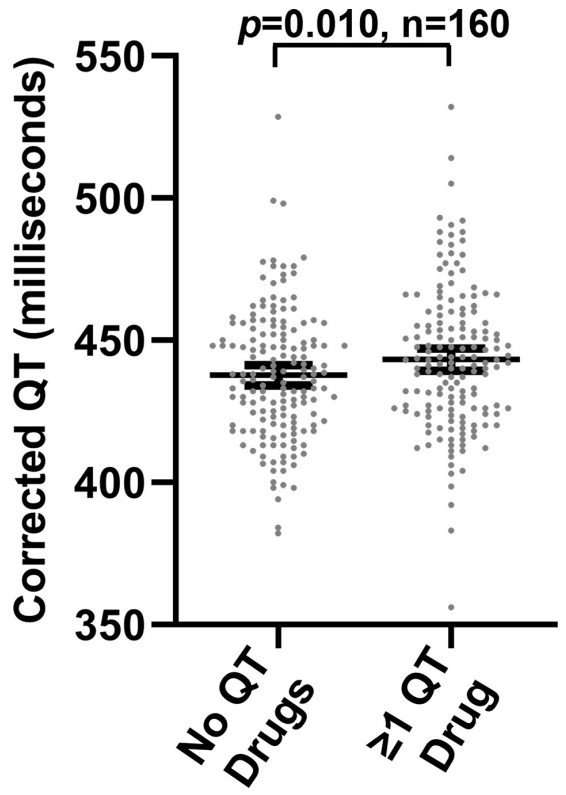

FIGURE 3
Median corrected QT measurements in 160 patients in the absence and presence of concomitant prescriptions for one or more QT-prolonging drugs.

TABLE 5 Clinical trial corrected QT (QTc) exclusion criteria and numbers of patients in this study who would potentially be excluded from clinical trials.

Exclusion criterion	Number of patients meeting exclusion criterion
AHA/ACC criteria	
Median QTc > 450 (men)/460 (women) ms	50 (18.5%)
Median QTc > 470 (men)/480 (women) ms	17 (6.3%)
Median QTc > 500 ms (all patients)	4 (1.5%)
Maximum QTc > 450 (men)/460 (women) ms	140 (51.7%)
Maximum QTc > 470 (men)/480 (women) ms	75 (27.7%)
Maximum QTc > 500 ms (all patients)	31 (11.4%)
Exclusion criteria described in ClinicalTrials.gov	
Median QTc > 450 ms (all patients)	74 (27.3%)
Median QTc > 470 ms (all patients)	23 (8.5%)
Median QTc > 480 ms (all patients)	8 (3.0%)
Median QTc > 500 ms (all patients)	4 (1.5%)
Maximum QTc > 450 ms (all patients)	157 (57.9%)
Maximum QTc > 470 ms (all patients)	88 (32.5%)
Maximum QTc > 480 ms (all patients)	60 (22.1%)
Maximum QTc > 500 ms (all patients)	31 (11.4%)

AHA/ACC scientific statements identify 450 ms (men)/460 ms (women) and 470 ms (men)/480 ms (women) as the 90th and 99th percentiles of the normal QTc intervals, respectively. QTc > 500 ms for all patients was identified as a relevant QTc threshold by both the AHA/ACC and from our survey of ClinicalTrials.gov.

and propofol were also observed at the other assessed time points. Data for all assessed drugs and time points are provided in **Data Sheet 1**.

Discussion

In this investigation, we demonstrate the potential for the administration of QT-prolonging drugs to impact clinical trial eligibility in a cohort of adults with advanced cancer. Our findings indicate that advanced cancer patients are commonly prescribed QT-prolonging drugs, as evidenced by 99.6% of our cohort having ≥ 1 prescription for a QT-prolonging drug since first cancer diagnosis. We also found that prescriptions for QT-prolonging drugs were robustly associated with prolonged QTc intervals across the many time points assessed in our analyses and in our paired analysis that compared QTc intervals in the same patients when co-prescribed and not co-prescribed QT-prolonging drugs. When considering exclusionary QTc thresholds from ongoing and recently completed clinical trials for cancer therapeutics, we found that (1) over half of our cohort (57.9%) had maximum QTc values that would exclude them from trials with the most stringent QTc thresholds (> 450 ms) and (2) the number of QT-prolonging drugs prescribed increased the odds of meeting exclusionary QTc thresholds by 9–40%. In addition, our analyses identify (1) specific demographic characteristics, including female sex, that were associated with increased prescriptions for QT-prolonging drugs and with greater odds of meeting exclusionary QTc thresholds and (2) specific drugs, including ondansetron, promethazine, and propofol, that were associated with > 10 ms increases in QTc and with increased risk of meeting exclusionary QTc thresholds.

Although we are not aware of previous investigations that have directly assessed the potential for the administration of QT-prolonging drugs to affect cancer trial eligibility, results from past studies do support our findings that QT-prolonging drugs can affect trial eligibility. Past studies have consistently found that prescriptions for QT-prolonging drugs are common in cancer patients and that numerous cancer therapeutics prolong QTc. With regard to the prevalence of prescriptions for QT-prolonging drugs, retrospective studies have found that 17.1% (15), 28.4% (14), and 92.6% (12) of cancer patients were prescribed ≥ 1 QT-prolonging drug as determined by CredibleMeds® . The variability in these results likely stems from the type of cancer populations that were studied and the duration of follow-up. The prevalence of prescriptions for ≥ 1 QT-prolonging drug in our cohort (99.6%) is higher than those found in past investigations, and this is likely due to the fact

TABLE 6 Probability of meeting clinical trial corrected QT (QTc) exclusion criteria based on whether patients were prescribed QT-prolonging drugs within 30 days of the index date.

	Patients prescribed no drugs (n = 15)	Patients prescribed only non-QT drugs (n = 57)	Patients prescribed QT-prolonging drugs (n = 199)	P-value (*post hoc* P-value for QT vs. non-QT*)
AHA/ACC criteria				
Patients with maximum QTc > 450/460 milliseconds (ms)	4 (26.7%)	19 (33.3%)	116 (58.3%)	< **0.001 (0.003)**
Patients with maximum QTc > 470/480 ms	0 (0%)	12 (21.1%)	63 (31.7%)	**0.007** (0.42)
Patients with maximum QTc > 500 ms	0 (0%)	3 (5.3%)	28 (14.1%)	0.076 (0.31)
Exclusion criteria from ClinicalTrials.gov				
Patients with maximum QTc > 450 ms	5 (33.3%)	20 (35.1%)	132 (66.3%)	< **0.001** (< **0.001**)
Patients with maximum QTc > 470 ms	1 (6.7%)	13 (22.8%)	74 (37.2%)	**0.010** (0.17)
Patients with maximum QTc > 480 ms	0 (0%)	8 (14.0%)	52 (26.1%)	**0.010** (0.23)
Patients with maximum QTc > 500 ms	0 (0%)	3 (5.3%)	28 (14.1%)	0.076 (0.10)

**Post hoc* p-values were Bonferroni-corrected (multiplied by 3) to account for multiple comparisons. Fisher's exact test was used to compare percentages among groups. AHA/ACC scientific statements identify 450 ms (men)/460 ms (women) and 470 ms (men)/480 ms (women) as the 90th and 99th percentiles of the normal QTc intervals, respectively. QTc > 500 ms for all patients was identified as a relevant QTc threshold by both the AHA/ACC and from our survey of ClinicalTrials.gov. Bold values indicates that the p-value is significant at the <0.05 threshold.

TABLE 7 Correlation between the number of QT-prolonging drugs prescribed and the odds of meeting clinical trial corrected QT (QTc) exclusion criteria at multiple assessed time points.

Logistic regression: Odds ratio, 95% CI (p-value)

AHA/ACC criteria

	Any time before	90 days	60 days	30 days	10 days
Maximum QTc > 450/460 milliseconds (ms)	1.1, 1.1–1.2 (**0.001**)	1.3, 1.2–1.4 (< **0.001**)	1.4, 1.2–1.5 (< **0.001**)	1.3, 1.2–1.5 (< **0.001**)	1.4, 1.2–1.6 (< **0.001**)
Maximum QTc > 470/480 ms	1.1, 1.0–1.2 (**0.040**)	1.2, 1.1–1.4 (**0.003**)	1.3, 1.1–1.4 (**0.002**)	1.2, 1.1–1.4 (**0.010**)	1.3, 1.1–1.6 (**0.003**)
Maximum QTc > 500 ms	1.1, 1.0–1.3 (**0.030**)	1.2, 1.0–1.4 (**0.046**)	1.2, 1.1–1.5 (**0.030**)	1.2, 1.0–1.5 (0.060)	1.2, 1.0–1.5 (0.14)

Exclusion criteria from ClinicalTrials.gov

	Any time before	90 days	60 days	30 days	10 days
Maximum QTc > 450 ms	1.1, 1.1–1.2 (**0.001**)	1.4, 1.2–1.5 (< **0.001**)	1.4, 1.3–1.6 (< **0.001**)	1.4, 1.2–1.6 (< **0.001**)	1.4, 1.2–1.7 (< **0.001**)
Maximum QTc > 470 ms	1.1, 1.1–1.2 (**0.002**)	1.3, 1.2–1.5 (< **0.001**)	1.4, 1.2–1.5 (< **0.001**)	1.3, 1.1–1.5 (**0.002**)	1.3, 1.1–1.6 (**0.002**)
Maximum QTc > 480 ms	1.1, 1.1–1.2 (**0.007**)	1.2, 1.1–1.4 (**0.004**)	1.3, 1.1–1.4 (**0.004**)	1.2, 1.1–1.4 (**0.030**)	1.3, 1.1–1.5 (**0.020**)
Maximum QTc > 500 ms	1.1, 1.0–1.3 (**0.030**)	1.2, 1.0–1.4 (**0.046**)	1.2, 1.1–1.5 (**0.030**)	1.2, 1.0–1.5 (0.060)	1.2, 1.0–1.5 (0.14)

AHA/ACC scientific statements identify 450 ms (men)/460 ms (women) and 470 ms (men)/480 ms (women) as the 90th and 99th percentiles of the normal QTc intervals, respectively. QTc > 500 ms for all patients was identified as a relevant QTc threshold by both the AHA/ACC and from our survey of ClinicalTrials.gov. Bold values indicates that the p-value is significant at the <0.05 threshold.

that we studied patients since their date of first cancer diagnosis (median duration of follow-up: 3.0 years), which was longer than study periods from past investigations that ranged from 1 week to 1 year (12, 14, 15).

The most commonly prescribed QT-prolonging drugs in our study were also similar to those from past studies and included antiemetics, antimicrobials, antidepressants, and analgesics (12–15). Past investigations have also demonstrated that cancer therapeutics, including capecitabine, arsenic trioxide, combination epirubicin/cyclophosphamide, vorinostat, and numerous TKIs, are associated with prolonged QTc in greater than 10% of patients, based on the Common Terminology Criteria for Adverse Events thresholds (QTc > 450 ms or increase in QTc > 60 ms from baseline) (16–19). Abu Rmilah, et al. found that 28.8% of patients with mixed cancers treated with TKIs had QTc prolongation, with life-threatening QTc prolongation, including the development of ventricular arrhythmias, occurring in 5.4% of patients (16). Our study expands on these findings by demonstrating that ondansetron, promethazine, and propofol, which are commonly prescribed to cancer patients, were each associated with QTc prolongation of > 10 ms. While the number of patients treated with each

drug wasn't large enough to allow an adequately powered statistical analysis, the median QTc values were > 450 ms in patients receiving a prescription in the preceding 30 days for a number of other medications in our analyses; these included the TKIs lenvatinib, crizotinib, and sunitinib, the anti-androgen degarelix, and the supportive therapies hydroxychloroquine, flecainide, clarithromycin, nortriptyline, nicardipine, tolterodine, methadone, and dextromethorphan.

Finally, a study by Kim, et al. compared QTc intervals between patients with cancer and healthy stem cell donors, and demonstrated that cancer patients had prolonged QTc values (mean Bazett's-corrected QT was 427 ms in cancer patients and 413 ms in healthy donors) (13). While our investigation only included cancer patients, the median Bazett's-corrected QT value of 438 ms in our cohort numerically supports the association found by Kim, et al. In addition, our finding suggests that patients with advanced cancer may have further prolonged QTc values relative to the Kim, et al. cancer cohort, which consisted of general cancer patients, though caution is warranted when comparing QTc values among patient populations from different health systems.

Our study also expands upon past investigations to discover novel insights with important implications for clinical oncology. Based on information listed on ClinicalTrials.gov, we found that the most common QTc thresholds used for cancer clinical trial exclusion were 450, 470, and 480 ms (**Figure 1**). It is noteworthy, and likely not coincidental, that these thresholds correspond to the 95th and 99th percentile values for QTc that are identified by AHA/ACC scientific statements as portending arrhythmia risk (3, 21). Our findings also demonstrate that demographic variables, including female sex, increased the odds of meeting exclusionary QTc thresholds. Although it is well-established that women have longer baseline QTc intervals than men (3, 21, 25), we found that < 10% of cancer trials considered patient sex when setting exclusionary QTc thresholds, which would result in significantly more women being excluded from the majority of trials. Standardized incorporation of sex into exclusionary QTc thresholds, as is done in the AHA/ACC scientific statements, is likely to prevent undue exclusion of women from cancer trials while still minimizing arrhythmia risk. In addition, the number of QTc prolonging medications was also associated with increased odds of meeting exclusionary QTc thresholds. Though demographics are immutable, clinicians can appreciate the increased risk of QTc prolongation in at-risk demographic subgroups; conversely, concomitant prescriptions for QT-prolonging drugs can be clinically managed to reduce the risk of arrhythmia and clinical trial exclusion. Guidance for the management of QTc prolongation in cancer patients recommends therapeutic substitution of QT-prolonging drugs as a major clinical strategy to mitigate QTc prolongation (5, 8). While therapeutic substitution might not always be possible for cancer therapeutics without sacrificing efficacy, our findings support the feasibility of therapeutic substitution for supportive therapies, since non-QT-prolonging alternatives exist for the majority QT-prolonging drugs commonly prescribed in our cohort. Substitution to non-QT-prolonging drugs may involve administering a different drug class (e.g., ondansetron must be substituted to a drug from a different class, like aprepitant, since all serotonin receptor 5-HT_3 antagonist antiemetics prolong QT), but, in many cases, non-QT-prolonging alternatives exist within the same drug class (e.g., opioid analgesics, selective serotonin reuptake inhibitors). Thus, our findings suggest that therapeutic substitution to non-QT-prolonging alternative drugs may be a viable strategy to enhance clinical trial eligibility for advanced cancer patients.

We acknowledge the following limitations of our investigation. First, all QTc values in our study were collected from ECGs that were obtained during normal clinical care. Since ECGs are more likely to be obtained when cardiac abnormalities are suspected, our data collection methods may have enriched for QTc values that are prolonged relative to those from otherwise healthy adults with cancer undergoing ECG screening before enrollment into cancer clinical trials. Second, our extracted medication data did not include sufficient information to determine the days' supply for each prescription or whether medications were prescribed on an "as needed" (so-called "PRN") basis. To account for these limitations, (1) we performed our analyses associating prescriptions for QT-prolonging drugs with QTc values using multiple time points (e.g., 10, 30, and 60, and 90 days) and (2) within our paired analysis that assessed QTc values in patients while co-prescribed and not co-prescribed QT-prolonging drugs, we used conservative methods to estimate the days' supply for each prescription. Given our findings that prescriptions for QT-prolonging drugs were consistently associated with prolonged QTc values across our analyzed time points and in our paired analysis, we do not believe that limitations related to our medication data meaningfully impacted our results. Additionally, there are a host of clinical factors that are known to prolong the QT interval (26). While we attempted to account for the effect of serum electrolyte abnormalities and extreme heart rates on the QTc values observed in our cohort, these analyses were limited by the fact that electrolyte and heart rate data were not regularly captured simultaneously with QTc measurements in the EHR. Given that electrolyte abnormalities and extreme heart rates were common in our cohort, these factors likely influenced our observed QTc values; however, since we excluded "conditional" QT-prolonging drugs from our analyses (which affect QTc *via* alteration of these clinical factors), we believe our analyses demonstrate the effect of QT-prolonging drugs on cancer trial eligibility independent of these clinical factors.

This investigation demonstrates the potential for the administration of QT-prolonging drugs to limit trial eligibility, based on exclusionary QTc thresholds from current or recently completed cancer clinical trials. In addition, our work identifies specific demographic characteristics and medications that are associated with reduced trial eligibility. Importantly, our findings suggest that therapeutic substitution

to non-QT-prolonging alternative drugs may be a potentially viable clinical strategy to enhance trial eligibility. However, prospective studies are needed to validate our findings and to determine the clinical validity of therapeutic substitution.

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 Indiana University Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ER and TS analyzed the data and performed the statistical analyses. ER, TS, RL, and TCS wrote the manuscript. All authors contributed to the conception, design of the study, manuscript revision, read, and approved the submitted version.

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Conflict of interest

MRa reports financial connections to ArQule, Inc., Boston Biomedical, Inc., Eli Lilly and Company, Immunomedics, Inc., LifeOmic, Inc., Macrogenics, Inc., and Tyme Technologies, Inc., and is currently an employee of Caris Life Sciences, Inc.

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.

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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.2022.894623/full#supplementary-material

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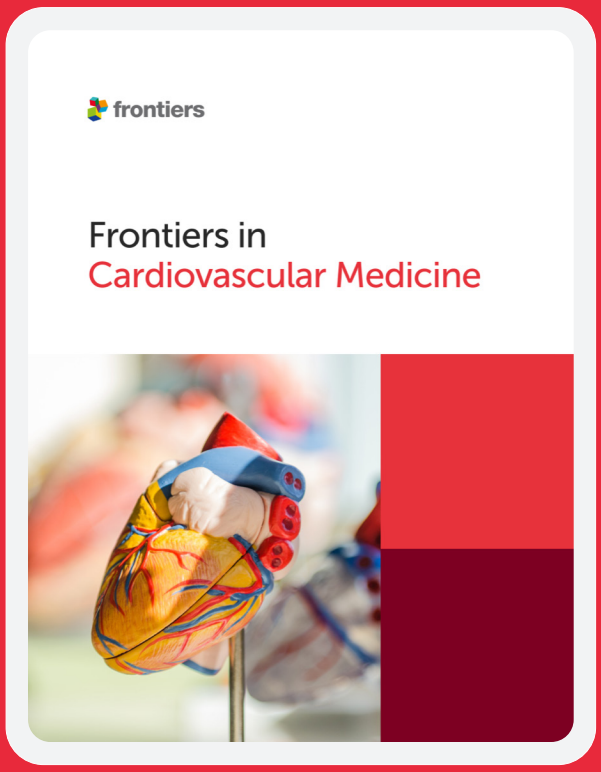

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