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Cardiac Disease in Childhood Cancer Survivors
Risk Prediction, Prevention, and Surveillance:
JACC CardioOncology State-of-the-Art Review

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ABSTRACT
Cardiac diseases in the growing population of childhood cancer survivors are of major concern. Cardiotoxicity as a consequence of anthracyclines and chest radiotherapy continues to be relevant in the modern treatment era. Mitoxantrone has emerged as an important treatment-related risk factor and evidence on traditional cardiovascular risk factors in childhood cancer survivors is accumulating. International surveillance guidelines have been developed with the aim to detect and manage cardiac diseases early and prevent symptomatic disease. There is growing interest in risk prediction models to individualize prevention and surveillance. This State-of-the-Art Review summarizes literature from a systematic PubMed search focused on cardiac diseases after treatment for childhood cancer. Here, we discuss the prevalence, risk factors, prevention, risk prediction, and surveillance of cardiac diseases in survivors of childhood cancer. (J Am Coll Cardiol CardioOnc 2020:2:363–78) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

The survival of children with cancer has considerably increased over the last decades with 5-year survival rates currently exceeding 80% (1). However, the long-term health effects in the growing population of childhood cancer survivors (CCS) are of major concern (2). Cardiac disease, as a consequence of treatment with anthracyclines, mitoxantrone, and/or chest-directed radiotherapy (chest RT), can manifest as myocardial dysfunction and heart failure but also as valvular

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CARDIAC DISEASES AND TREATMENT-RELATED RISK FACTORS IN CHILDHOOD CANCER SURVIVORS

HEART FAILURE. Multiple studies have shown that left ventricular (LV) systolic function deteriorates as a result of cardiotoxic treatment (7-15). Anthracyclines are clearly associated with cardiomyocyte damage. Although the exact mechanism of anthracycline-induced cardiotoxicity has not been fully elucidated, early studies indicate that cardiotoxicity through reduction-oxidation reaction cycling and the generation of reactive oxygen species may be the cause. More recently, topoisomerase 2β has been proposed to be a mediator of doxorubicin-induced cardiac injury (16).

Systolic dysfunction can eventually progress to heart failure. Heart failure is one of the most frequent cardiac late effects in CCS (17,18), and contributes to significant morbidity and non-cancer-related mortality later in life (19,20). A large cohort from the Childhood Cancer Survivor Study investigated the occurrence of heart failure, defined by the Common Terminology for Criteria Adverse Events as grade 3 to 5. Based on questionnaires concerning long-term CCS, the reported cumulative incidence is 4.8% by 45 years of age (17). These results confirmed earlier reports that anthracyclines and chest RT are strongly associated with heart failure (21). Recently, it has been shown that even low-to-moderate chest RT doses increase the risk of heart failure substantially (22,23). In the Dutch LATER (Late Effects After Childhood Cancer) cohort, Feijen et al. (10) reported a cumulative heart failure incidence of 10.6% 40 years after childhood cancer diagnosis in CCS who received cardiotoxic cancer treatment. Higher exposure to mitoxantrone and cyclophosphamide were suggested as novel treatment-related risk factors (10). Although mitoxantrone has traditionally been classified as an anthracycline, it has been suggested that mitoxantrone results in cardiotoxicity through mechanisms different from anthracyclines (24,25). Mitoxantrone has a nonlinear dose-response relationship with heart failure risk (10,26-28), and compared to doxorubicin, mitoxantrone is 10 times more cardiotoxic. In addition, a younger age at diagnosis and presence of traditional cardiovascular risk factors may play a role in the development of heart failure (29). The influence of sex on the development of myocardial dysfunction is still incompletely conclusive (8,9,11,12,30).

CORONARY ARTERY DISEASE. The risk of coronary artery disease (CAD) is substantially increased in CCS. In the Childhood Cancer Survivor Study, the cumulative incidence of CAD by age 45 years was 5.3% in survivors with and without exposure to cardiotoxic cancer treatments (17). This risk is dependent on chest RT dose with no established safe dose; this risk is also higher in males. The cumulative incidence of symptomatic CAD at age 50 years...
increases to 20% in males exposed to >35 Gy of radiation (18,31). The St. Jude Lifetime cohort study detected CAD based on either history, electrocardiogram (ECG), or echocardiography in 3.8% of asymptomatic CCS 22.6 years after cardiotoxic therapy (30). However, evidence from (non)invasive coronary angiography is scarce. A study evaluating computed tomography in asymptomatic Hodgkin lymphoma CCS 55 years old or younger (n = 31) exposed to chest RT showed coronary artery lesions to be very proximal, placing large portions of the myocardium at risk (32).

VALVULAR HEART DISEASE. Several studies have investigated valvular abnormalities in CCS (11,17,30,33-35) with a reported prevalence of up to 31% (30,33,35). Chest RT has been identified as an
important risk factor that increases at higher doses (35). Other risk factors are treatment with anthracyclines, hypertension, congenital heart disease, and younger age at diagnosis, although these have not been uniformly shown in all studies (11,30,33). Mild tricuspid regurgitation was most prevalent in 2 studies describing valvular disease, but this is also very common in the general population (30,33,36). In lymphoma CCS who were exposed to chest RT, valvular heart disease, defined as mild or higher for left sided valves and moderate or higher for right sided valves, was most frequently detected in the aortic and mitral valves (35). Valvular abnormalities after chest RT are most likely caused by direct irradiation injury to the valve cusps or leaflets, causing thickening, fibrosis, and calcification (30,37). These processes progress with age and increase in prevalence over time (30,35). Hence, CCS without echocardiographic abnormalities after a short follow-up period are still at risk of severe valvular heart disease.

**PERICARDIAL DISEASE.** Besides paraneoplastic and infectious causes, pericardial disease can arise from chest RT. Late constrictive pericarditis, in particular, can lead to disabling symptoms and a poor prognosis (38). However, data on pericardial disease in CCS are limited. The Childhood Cancer Survivor Study showed a 10-fold higher risk of pericardial disease in
all CCS versus siblings (30-year cumulative incidence, 3.0%) and a dose-response relation with chest RT (11). A single-center study in CCS older than 5 years after diagnosis (n = 1,362; 47% no cardiotoxic therapy), reported symptomatic pericarditis in only 2 CCS (18). Although the diagnosis of constrictive pericarditis is difficult by echocardiography, thickening of the pericardium as well as hemodynamic consequences (e.g., “septal bounce,” abnormal respiratory variations in Doppler findings) can be suggestive. Upon high clinical suspicion, cardiac computed tomography, magnetic resonance imaging (MRI), and/or invasive hemodynamic evaluation may be needed to confirm the diagnosis (39).

ARRHYTHMIAS. The prevalence of symptomatic cardiac arrhythmias in long-term CCS is reportedly low (11,17,18,40). In 10,724 CCS, the cumulative incidence of grade 3 to 5 arrhythmia by 45 years of age was 1.3% (17). A subsequent study (n = 23,462) showed that chest RT ≥35 Gy, anthracycline dose ≥250 mg/m2, dyslipidemia, and hypertension are risk factors for symptomatic arrhythmia (11). Myocardial fibrosis caused by chest RT may contribute to the occurrence of arrhythmias. Other frequently used cancer agents for pediatric cancers such as cisplatin, cyclophosphamide, and tyrosine kinase inhibitors may also be associated with supraventricular and ventricular arrhythmias (41,42). Prolonged QTc interval, which has arrhythmogenic potential, has been shown in CCS who received anthracyclines with and without chest RT (43,44). Also, rhythm disturbances such as premature ectopic beats and atrioventricular blocks have been reported in CCS (45–47). The literature on ECG abnormalities in large cohorts of long-term CCS is sparse (46,47). Data on the use of ambulatory ECG monitoring to define the prevalence of brady- and tachyarrhythmias induced by cardiotoxic cancer treatments are needed, but must be carefully weighed against the potential patient burden and clinical significance.

PREVENTION OF CARDIAC DISEASE IN CHILDHOOD CANCER SURVIVORS

PREVENTIVE MEASURES FOR CANCER TREATMENT-INDUCED CARDIOTOXICITY. As the risk of cardiac disease is high in chest RT and anthracycline-treated survivors, and as omitting or diminishing the use of cardiotoxic treatments is not always possible, prevention is critical (48). Advanced radiotherapy techniques to minimize exposure to the heart have been developed; the impact of those improvements is reflected by the decrease in CAD in more recent treatment eras (11).

Extensive research has been devoted to the identification of possible cardioprotective interventions during anthracycline treatment that do not have negative effects on antitumor efficacy or other noncardiac adverse effects. Below we discuss 3 preventive measures that have been studied during anthracycline treatment. We focus primarily on randomized controlled trials (RCTs) as they provide the highest level of evidence to answer this type of question. Because of developmental changes and the differences in the body composition of children, data from adults cannot be reliably extrapolated to children (49).

Dexrazoxane. Dexrazoxane is one of the most widely investigated cardioprotective pharmacologic interventions. It has been shown in adult cancer patients to prevent clinical and subclinical cardiac damage (4). The few published pediatric RCTs have included participants diagnosed with leukemia, lymphoma, and sarcoma (50–52). These studies suggest that there are no significant differences in clinical heart failure between dexrazoxane and control patients (4,53), although dexrazoxane might have a protective effect on asymptomatic cardiotoxicity (53,54). All studies included relatively short-term follow-up, and the impact on outcomes after longer follow-up is yet unknown.

Currently, dexrazoxane is not routinely used in clinical practice for all children treated with anthracyclines. This might be explained by a concern over interference with antitumor efficacy and the occurrence of secondary malignancies (55). However, high-quality evidence to support an increased risk of secondary malignancy is lacking. A Cochrane systematic review identified no significant differences between treatment groups (4), which is in line with more recently published randomized trials (50,53).

A recently published nonrandomized study in pediatric patients with acute myeloid leukemia (n = 1,014) added important knowledge about the efficacy and adverse effects of continuous use of dexrazoxane versus no dexrazoxane. Results showed that after a median follow-up period of 3.5 years, cardiac function was preserved with dexrazoxane without negative influence on antitumor efficacy or noncardiac toxicities. Importantly, the influence of possible differences in cumulative anthracycline dose per treatment group could not be evaluated in this study (56).

At the moment clear guidance on the use of dexrazoxane is missing. Since it will take many years to add relevant knowledge by new RCTs, additional observational studies are needed. The International Late Effects of Childhood Cancer Guideline...
Harmonization Group (IGHG) is currently preparing recommendations based on the existing evidence.

**Liposomal anthracyclines.** Another option is to limit drug exposure in healthy tissues such as the heart and increase drug activity in malignant cells by altering the tissue distribution as with liposomal anthracyclines (57). Liposomal anthracyclines have shown promising results in adults with breast cancer (5). In a meta-analysis of 2 studies, liposomal-encapsulated doxorubicin significantly reduced both clinical and subclinical heart failure when compared to the same dose of conventional doxorubicin, without negative effects on antitumor efficacy and without cardiac adverse effects. In 1 of the studies, patients received a higher cumulative anthracycline dose in the liposomal group. However, again, follow-up was relatively short and we do not know how longer-term follow-up will influence these results (5). One study compared liposomal-encapsulated doxorubicin to the same dose of conventional epirubicin. No significant difference in cardiotoxicity was shown, but that might have been the result of inadequate power or a limited follow-up period (5). To our knowledge, no pediatric RCTs have been performed, so the benefits and harms of liposomal anthracyclines in children remain unclear. High-quality research in children is needed before definitive conclusions can be made.

**Infusion duration.** The use of longer anthracycline infusion durations may play a role in primary prevention of cardiotoxicity. A Cochrane systematic review compared different anthracycline infusion durations in children and adults with cancer (6). An anthracycline infusion duration of 6 h or longer seemed to reduce the risk of both clinical heart failure and subclinical cardiotoxicity. A clinical practice guideline for children treated with anthracyclines has suggested that although it was not possible to formulate a recommendation regarding a precise and optimal prolonged infusion duration, the use of an anthracycline infusion duration of at least 1 h was strongly recommended (58). Because data in children are limited, different anthracycline infusion durations should be evaluated further in children.

**Cardiovascular Risk Factors and Healthy Lifestyle.** For both primary and secondary prevention of cardiovascular disease in CCS, management of cardiovascular risk factors and counseling on healthy lifestyle are essential, although most evidence is still derived from the general population.

**Metabolic syndrome.** Hypertension, obesity, dyslipidemia, and diabetes, together clustered as metabolic syndrome, are well-known risk factors for cardiovascular disease (59). Some CCS are at increased risk of developing metabolic syndrome because of previous cancer treatment. Metabolic syndrome has been established in 9% of French childhood leukemia survivors and in 32% of the St. Jude Lifetime cohort at median attained ages of 21 to 32 years (60,61). Survivors treated with cranial radiotherapy are at risk of developing metabolic syndrome, especially obesity (62). Furthermore, abdominal radiation and nephrotoxic treatment may result in the development of cardiovascular risk factors (63,64). Hypertension is the most prevalent cardiovascular risk factor in CCS, approaching 40% in survivors aged 50 years or older, versus 26% in siblings (17). The Childhood Cancer Survivor Study (n = 10,724) investigated cardiovascular risk factors with longitudinal questionnaires and showed that hypertension had the strongest association with all cardiac events and mortality compared to diabetes, dyslipidemia and obesity (17). In the St. Jude Lifetime study, hypertension was also the only cardiovascular risk factor associated with an abnormal left ventricular ejection fraction (LVEF) (7). Management of cardiovascular risk factors is essential in all CCS and particularly in those at risk for cardiac disease. No studies have assessed whether more aggressive approaches and treatment goals than in the general population are beneficial in CCS with a high lifetime risk of cardiovascular disease. Lifestyle interventions may prevent the occurrence of cardiovascular risk factors and cardiac disease and may complement pharmacologic risk factor modification.

**Healthy lifestyle.** A healthy lifestyle, including cessation and abstinence from smoking, a sufficient level of physical activity, a healthy diet, and less than moderate alcohol use may benefit cardiovascular health. It may prevent the onset and/or reduce the severity of cardiovascular disease directly or indirectly by lowering the risk of metabolic syndrome (59). Although the association between lifestyle factors and cardiovascular disease has been well established in youth and aging adults (59), there are few studies that have examined the association between lifestyle and either cardiovascular disease or cardiovascular risk factors in CCS. In the Childhood Cancer Survivor Study, smoking was not associated with cardiac events, most likely because of short exposure time and follow-up (17). In the St. Jude Lifetime cohort study, CCS who did not meet most of the lifestyle recommendations from the World Cancer Research Fund/American Institute for Cancer Research were more likely to have metabolic syndrome than CCS who did meet these recommendations (61). In recent studies in the St. Jude Lifetime
cohort, CCS were shown to have substantially less exercise capacity than community controls on maximal cardiopulmonary fitness testing in recent studies. Exercise capacity was associated with all-cause mortality, cardiac function (global longitudinal strain [GLS], but not LVEF), chronotropic incompetence, and worse pulmonary and muscle function (65). Furthermore, CCS with lower exercise capacity had more emotional distress and worse attainment of social roles and health-related quality of life (66). Although causal relations have not been established, based on the above results in the general population and CCS, it is widely assumed that healthy lifestyle interventions will contribute to less cardiac morbidity and mortality. However, the effectiveness of lifestyle interventions on cardiovascular risk factors or cardiovascular disease has not been established in CCS.

Several studies have been performed to support CCS to adapt to a healthy lifestyle, of which most have focused on increasing physical activity. In a meta-analysis of 9 studies, aerobic exercise was positively related to cardiopulmonary fitness in CCS (67). A systematic review by Raber et al. (68) identified 12 studies on physical activity interventions in CCS. Of these, 5 studies found that exercise training improved strength, functional mobility, and flexibility and/or anthropometric fitness (68). Another systematic review on lifestyle interventions in adolescent and young adult cancer survivors targeting 1 or more health behaviors identified 12 studies, of which 6 were successful in changing health behavior (69). Three of these were focused on influencing multiple behaviors, including an individually tailored counseling program on smoking and alcohol consumption. One-half of the reviewed studies delivered lifestyle interventions remotely, using phone calls or online contact. Personalized e-health interventions seem a relatively cost-effective and feasible way to improve lifestyle in CCS, but more studies are needed to examine its efficacy and effectiveness.

RISK PREDICTION MODELS

Knowledge of the risk of cardiac adverse events before or early after cardiotoxic cancer treatments can be very useful to guide the care for CCS. Multivariable risk prediction models have the potential to accurately estimate risk in individual survivors and should ideally be linked to a proven effective action to prevent or reduce the severity of cardiotoxicity (70,71).

Development of prediction models broadly includes a development and validation phase (70). In the development phase, relevant predictors are selected based on subject knowledge and/or regression (72). Subsequently, model discrimination and calibration are assessed. Discrimination is the ability of the model to discriminate between patients who develop the event and those who do not and is typically quantified by the C-statistic or area under the receiver operating characteristic curve (72,73). Calibration refers to how well the predicted risks match the actual risks and can be assessed with a calibration plot (71). In the validation phase, discrimination and calibration are assessed in a distinct cohort, a critical step before the prediction model can be applied to patients (70,71). In CCS, risk prediction models have been developed for heart failure, ischemic heart disease, and cardiovascular mortality. An overview of validated prediction models in CCS is provided in Supplemental Table 2.

HEART FAILURE PREDICTION MODELS. Practical models to predict heart failure onset before the age of 40 years in CCS at 5 years after cancer diagnosis have been developed by Chow et al. (29). Here, prediction models in 13,060 CCS (285 patients with heart failure) from the Childhood Cancer Survivor Study were derived and subsequently validated in 3,421 CCS (93 with heart failure) from the Dutch Emma Children’s Hospital, the National Wilms Tumor Study, and the St. Jude Lifetime Cohort Study. Using a backward selection procedure, being female, younger age at cancer diagnosis, anthracycline dose, and chest RT dose were selected as predictors and assigned integer risk scores for clinical applicability. The final prediction model showed reasonable discrimination between CCS who developed heart failure and those who did not (C statistic: 0.76 and 0.68 to 0.82 in the development and validation cohorts, respectively). The discriminatory abilities of the model were further shown by a cumulative incidence of heart failure at age 40 years of 0.5% in the low-risk group, whereas this was 11.7% in the high-risk group. Importantly, 45.2% of the CCS were at low risk according to the model and thus unlikely to develop heart failure.

ISCHEMIC HEART DISEASE PREDICTION MODELS. A similar approach was used by the same investigators to develop and externally validate a prediction model for ischemic heart disease before the age of 50 years (31). Being male and having a higher chest RT dose were selected as predictors. The Cox regression model achieved modest discrimination between CCS who developed ischemic heart disease and those who did not (C statistic of 0.70 in the development cohort and 0.66 in the validation cohort). Cumulative incidences of ischemic heart disease at the age of 50 years ranged from 2.3%
(95% confidence interval [CI]: 1.5% to 3.1%) in the low-risk group to 19.9% (95% CI: 15.0% to 24.7%) in the high-risk group, whereas this was only 1.2% (95% CI: 0.4% to 2.0%) in siblings. Although a clear segregation was observed between the low- and high-risk groups, the C statistics were modest. For both the heart failure and ischemic heart disease prediction models, calibration was not assessed.

TRADITIONAL CARDIOVASCULAR RISK FACTORS IN THE PREDICTION FOR HEART FAILURE AND ISCHEMIC HEART DISEASE. Modifiable cardiovascular risk factors in CCS are known to increase the risk for cardiovascular events and their prevalence is strongly related to age (17). Thus, early, at 5 years after diagnosis, cardiovascular risk factors have been shown to provide little incremental information to prediction models for heart failure and ischemic heart disease (29,31).

In a more recent study, diabetes, hypertension, and dyslipidemia were used in the prediction of heart failure and ischemic heart disease in CCS who were 20, 25, 30, or 35 years of age at time of prediction, with relative risks comparable to moderate doses of anthracyclines (74). Cardiovascular risk factors were present in approximately 10% of the CCS at the age of 35 years and were strong predictors of heart failure and ischemic heart disease. Although the discrimination of the prediction models improved with the addition of cardiovascular risk factors, the C statistics were modest for both events ranging from 0.69 to 0.79 in the derivation cohort with successful replication in the other one-half of the cohort. Both the heart failure and the ischemic heart disease predictions models showed good calibration. A small, very-high-risk group was identified with cumulative incidences of heart failure or ischemic heart disease of
~10% at age 50 years; survivors in this very-high-risk group may benefit from more frequent surveillance and/or early interventions to modify their risk. However, low-risk survivors who may be excluded from further surveillance could not be identified with these models as cumulative incidences of heart failure (~1.5% to 2.5%) and ischemic heart disease (~1% to 1.5%) were still significantly higher compared to siblings at the age of 50 years.

**CARDIOVASCULAR MORTALITY PREDICTION MODELS.** A population-based study from the Surveillance, Epidemiology, and End Results Program in 28,811 CCS was used to develop and validate a clinical risk score for cardiovascular mortality ≥5 years after diagnosis (75). Being male, of non-white race, age at diagnosis, lymphoma history, and at any radiation dose were selected as predictors in the Cox regression model. This simple model showed modest discrimination (C statistic: 0.72 to 0.75) and good separation between low-risk and high-risk survivors (cumulative incidence at 30 years after cancer diagnosis of 0.7% and 6.0%, respectively).

**GENETIC RISK PREDICTION MODELS.** There is large interindividual variation in the susceptibility for cardiotoxicity after anthracycline treatment (76). Genetic predisposition may explain why some children will develop cardiotoxicity at lower anthracycline doses whereas others who are treated with high doses will not and thus enable risk stratification of children before anthracycline treatment. Several genetic variants implicated in DNA damage, oxidative stress, iron metabolism, sarcomere dysfunction, and anthracycline metabolism and transport have been described and replicated in anthracycline cardiomyopathy (Figure 2, Supplemental Table 3) (77,78). For a comprehensive overview of genetic variants implicated in anthracycline cardiomyopathy we refer the reader to an upcoming State-of-the-Art Review in JACC: CardioOncology and other systematic reviews (76,77).

In the absence of single genes explaining the susceptibility for anthracycline cardiomyopathy, combining genetic and clinical risk factors in a multivariable prediction model may increase the clinical usefulness of screening for genetic variants. Visscher et al. (79,80) developed several genetic risk prediction models. Validation of the first prediction model failed in an independent cohort (79,80). An updated prediction model based on 7 genetic variants and the clinical variables age at start of treatment, anthracycline dose, sex, chest RT, and ethnicity achieved an area under the curve of 0.79 (95% CI: 0.74 to 0.85) in the derivation cohort and 0.76 (95% CI: 0.68 to 0.83) in the validation cohort, compared to 0.68 (95% CI: 0.61 to 0.75) for the model with clinical variables only (81). Although these are promising results, this genetic risk prediction model is not ready to be applied to clinical practice due to several limitations. Calibration was not performed and coefficients of the final model were not provided. In addition, a logistic regression model was used that does not take into account the time-to-event, and also does not properly address survivors who dropped out before the study was performed. Therefore, the model estimates the probability of developing anthracycline cardiomyopathy at any time during follow-up, whereas it is likely more informative for a clinician to understand the probabilities within a certain timeframe. Studies that evaluate the predictive value of genetic variants in combination with clinical variables using time-to-event analyses are needed before genetics can be used in the risk stratification for anthracycline cardiomyopathy in CCS.

**IMPROVING PREDICTION MODELS WITH ADDITIONAL PREDICTORS.** Improvements in discrimination ability of the models may be achieved with the addition of echocardiographic parameters, ECG, blood biomarkers, and/or genetic variants (7,47). Updating risk estimates in a particular survivor with changes in echocardiographic, ECG, and/or blood biomarkers during follow-up may also improve predictions given the results in other areas of research (82). Moreover, acute or early-onset cardiotoxicity is suggested as a predictor for late-onset cardiotoxicity (83).

**CLINICAL APPLICATIONS AND CLINICAL IMPACT ANALYSES OF PREDICTION MODELS.** When a potentially high-risk patient is identified by a risk prediction model, preventive measures such as the use of dexrazoxane or liposomal anthracyclines may be considered. Prediction models using covariates that are known before cancer treatment, such as genetic variants or treatment protocols, may be useful for this purpose.

As a future application of prediction models, the predicted risk for cardiotoxicity can be weighed against the survival benefit associated with a particular treatment to guide therapy decisions. Risk estimates from a prediction model can also be used to individualize surveillance for asymptomatic cardiac dysfunction in CCS. Closer follow-up can be recommended in high-risk patients while at the same time the surveillance burden can be decreased in patients at low risk for cardiotoxicity.
Although the above-mentioned prediction models may be used to inform survivors and clinicians on individual risks for cardiotoxic events, there is a lack of evidence-based clinical actions that can be taken based on the risk estimates from current models. This emphasizes the need for clinical impact analyses to investigate changes in clinical management linked to the results from a prediction model. A trial with a cluster randomization design evaluating usual survivorship care compared to care based on results from a prediction model will provide the strongest evidence but may be impractical to perform in CCS because of the long follow-up needed (84).

Another approach to assess clinical impact is decision modeling (84,85). Decision curves can evaluate the net benefit of a prediction model across a range of disease probability thresholds for intervention (86). In the context of prediction model-guided surveillance, this can be seen as the benefit of early detection of asymptomatic cardiac dysfunction among those who will develop heart failure (true positives) weighted against the potential harm of an unnecessary diagnostic workup and/or treatment in those who will not develop heart failure (false positives).

Through decision modeling using simulations it has been shown that routine echocardiographic surveillance for asymptomatic cardiomyopathy every 10 years may be more cost-effective, especially in those treated with an anthracycline dose <250 mg/m² (85). Decision modeling provides weaker evidence on the clinical impact compared to an RCT, but it requires no follow-up and is less expensive to perform. Such analyses could be performed to assess clinical impact and cost-effectiveness before conducting an RCT.

DETECTION METHODS AND GUIDELINES

There are different methods and techniques available to detect anthracycline treatment induced cardiomyopathy. Much of the research in detection of cardiac diseases is focused on improving early detection of myocardial dysfunction. We will describe diagnostic methods that have been studied over the past decade in CCS.

CONVENTIONAL ECHOCARDIOGRAPHY. Echocardiographic measurement of the fractional shortening (FS) and biplane LVEF are widely used techniques to quantify cardiac dysfunction in survivors of childhood cancer. FS is discouraged in patients secondary to potential regional wall motion abnormalities (87). Moreover, LVEF and FS decreases may reflect later stages of cardiotoxicity. To overcome these limitations, developments in advanced imaging techniques are of great importance. Application of 3-dimensional echocardiography has improved inter-observer and intra-observer variability, which is desirable for longitudinal follow-up (88). Armstrong et al. (89) showed that the sensitivity and false-negative rate of 3-dimensional echocardiography for detection of LVEF <50% measured by cardiac MRI as the gold standard was improved compared to 2-dimensional echocardiography (89).

STRAIN IMAGING AND DIASTOLIC FUNCTION. One of the markers that may detect myocardial dysfunction at an early stage is GLS. In adult cancer patients, strain imaging has potential to predict subsequent LVEF deterioration (90,91). A relative GLS decrease of >15% from baseline is suggested as potentially abnormal, whereas a relative decrease of <8% seems not clinically relevant (92). Evidence on strain imaging in CCS is accumulating. Mavinkurve-Groothuis et al. (93) showed a significant difference in GLS between asymptomatic CCS (n = 111) approximately 15 years after anthracycline treatment and healthy controls. A large study of the St. Jude Lifetime cohort of 1,807 CCS with a median follow-up of 23 years determined an abnormal GLS in 28% of the cohort who were exposed to anthracyclines and/or chest RT and had normal LVEFs. Both cumulative anthracycline dose >300 mg/m² and any cardiac RT dose were associated with an increased risk for abnormal GLS (7). It is currently unknown whether an abnormal GLS is associated with development of an LVEF <50% or clinical heart failure in CCS.

Diastolic dysfunction after cardiotoxic cancer treatment has also been described in CCS (8,94). In the St. Jude Lifetime cohort, diastolic dysfunction grades 1 to 3 (based on peak mitral flow velocity, mitral septal and lateral early diastolic velocity, and left atrial volume) was detected in 11% of all CCS who were exposed to cardiotoxic treatment and in 8.7% with normal LVEF (7). One must be aware of the difficulties in the classification of diastolic dysfunction and there is a question of whether grading diastolic dysfunction according to the 2016 recommendations (95) has added value in CCS. Whether diastolic dysfunction is associated with asymptomatic systolic dysfunction and predictive of heart failure development warrants further investigation.

CARDIAC MRI. Cardiac MRI is a well-suited imaging technique because geometric assumptions are not needed and the high resolution images enables accurate function assessment with high reproducibility (96). A study in 114 adult survivors showed a significant difference in mean LVEF measured by MRI (55.9%) and 2-dimensional echocardiography (61.0%). Cardiomyopathy (LVEF <50% measured with MRI)
was identified in 12 CCS (11%) previously undiagnosed by 2-dimensional echocardiography (89). The added value of this modality could lie in the abilities of tissue characterization (i.e., edema and fibrosis), right ventricle systolic function assessment, precise volumetric and strain assessment of other cardiac chambers aside from the LV. Thus, cardiac MRI enables evaluation of structural and functional changes induced by cancer treatment. Yet, studies investigating the role of cardiac MRI in CCS are scarce (97–100).

**BLOOD BIOMARKERS AND ELECTROCARDIOGRAPHY.** The limited diagnostic value of the blood biomarkers N-terminal pro-B-type natriuretic peptide and (high-sensitive) cardiac troponins in the detection of myocardial dysfunction by echocardiography more than 1 year after cancer diagnosis was shown in a recent systematic review (101). Conflicting results on the predictive value of natriuretic peptides and troponins measured during cancer treatment for subsequent anthracycline cardiomyopathy exist in CCS (102,103). In adult cancer patients, the predictive value of elevated high-sensitive cardiac troponins during cancer treatment for early-onset cardiotoxicity may be more suggestive at specific time-points (91,104).

ECG parameters may also aid in the prediction of myocardial dysfunction. A recent study in anthracycline-treated CCS showed that the QTc interval after chemotherapy was associated with subsequent LV dysfunction (105).

**GUIDELINES FOR SURVEILLANCE AND TREATMENT OF CARDIAC DISEASE IN CHILDHOOD CANCER SURVIVORS.** The IGHG aims to develop guidelines for surveillance of survivors of childhood cancer and young adult survivors by a global interdisciplinary collaboration (106). Within the guideline development process, recommendations are formulated based on existent national follow-up guidelines and evidence summaries (107–110). Recommendations cover the clinical questions: 1) Who needs surveillance?; 2) Which surveillance modality should be used?; 3) At what frequency and for how long should surveillance occur?; and 4) What should be done when abnormalities are found?

**Cardiomyopathy surveillance guideline.** The IGHG cardiomyopathy surveillance guideline was published in 2015 (111) and efforts are underway to update this guideline. It serves to define risk groups for the development of cardiomyopathy based on cardiotoxic exposure. CCS treated with anthracycline doses ≥250 mg/m², chest RT dose ≥35 Gy, or a combination of anthracyclines ≥100 mg/m² and chest RT dose ≥15 Gy are regarded as high risk. Anthracycline doses of 100 to 250 mg/m² or chest RT doses 15 to 35 Gy are regarded as moderate risk, and anthracycline doses <100 mg/m² as low risk. Echocardiographic surveillance is strongly recommended every 5 years or more frequently in high-risk CCS. It is reasonable to also surveil every 5 years in moderate- and low-risk CCS. Surveillance should start no later than 2 years after the completion of cardiotoxic therapy. The IGHG furthermore strongly recommends routine screening for and management of cardiovascular risk factors and counseling on smoking cessation and regular exercise.

Participation rates of high-risk CCS to guideline-based echocardiographic surveillance were shown to be less than one-third. In one RCT, telephone counselling more than doubled the participation rate in the subsequent year after correction for recommended surveillance frequency (112).

Until now, the IGHG did not formulate treatment recommendations for cardiomyopathy in CCS. When abnormalities are detected, this guideline recommends referral to a cardiologist. Clinical practice guidelines applied by (pediatric) cardiologists after referral are summarized in the section below on guidelines for management of cardiomyopathy in CCS. **Coronary artery disease surveillance guideline.** The IGHG is currently finalizing a guideline for asymptomatic CAD surveillance in childhood, adolescent, and young adult cancer survivors (113). Preliminary studies suggest that there is insufficient evidence to recommend a particular surveillance modality in asymptomatic CCS treated with chest RT. Emphasis is placed on awareness of premature CAD risk in survivors treated with chest RT. Risk assessment and surveillance and management of modifiable cardiovascular risk factors is needed. Knowing that there is already a difference in the incidence of CAD between CCS and siblings in their late 20s, clinicians should be aware of the potential atypical presentation of CAD in younger patients (17,107).

**Other cardiac disease surveillance guidelines.** As the modality of choice for the evaluation of valvular disease is echocardiography, assessment of valve function and structure are usually incorporated in the surveillance of CCS who are at risk with chest RT doses >15 Gy (111). Furthermore, assessment of pericardial structural abnormalities is possible as well. When abnormalities are detected, a cardiologist should be consulted as specified in some national guidelines (107,109). To detect arrhythmia in an early phase, some national groups suggest performing an electrocardiogram at the initiation of long-term follow-up (107,109).
Guidelines for management of cardiomyopathy in CCS. The IGHG cardiomyopathy guidelines refers to (pediatric) cardiology guidelines for further investigation and management of cardiac abnormalities (114–116). However, an exact threshold for abnormal systolic function is not defined. In the general adult population, a LVEF <40% is a robust indicator that medical therapy reduces mortality, regardless of heart failure symptoms. Treatment decisions for patients with a LVEF 40% to 49% should be a “shared decision” balancing prognosis, heart failure symptoms, and the individual’s treatment tolerance (115,116). In practice, these thresholds are often extrapolated to CCS in the absence of survivor-specific evidence.

There is a lack of evidence to support treatment recommendations in CCS. A Cochrane systematic review identified only 1 RCT that evaluated the initiation of angiotensin-converting enzyme-inhibitors for CCS with asymptomatic cardiac dysfunction (117). This study only showed improvement in LV wall stress by echocardiography. Possible reasons for failure to show an effect on clinical endpoints are the relatively short follow-up time (median, 2.8 years) and liberal inclusion criteria (118).

The European Society of Cardiology published a position paper for the diagnosis and management of cancer patients and survivors in adult cardiology (119). The paper recommends prompt initiation of an angiotensin-converting enzyme inhibitor and β-blocker in those with cardiac dysfunction during cancer therapy based on the high risk of developing heart failure. However, these recommendations were not based on RCT data. In long-term follow-up, general heart failure guidelines should be followed (115,116).

FUTURE PERSPECTIVES

Looking forward, there is a critical need for prospective and interventional studies to address most open research questions (Table 1). The current lack of

### Table 1 Future Directions in Cardio-Oncology Research in Childhood Cancer Survivors

| Future Research Directions                                                                 | Study Design(s) to Answer Research Question                                                                 |
|-------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| Cardiac diseases                                                                          |                                                                                                             |
| Detailed risk and risk factor analysis of cardiac diseases after childhood cancer          | Cohort studies and case control studies                                                                     |
| Prevention of anthracycline cardiotoxicity                                                |                                                                                                             |
| Safety and effectiveness of dexrazoxane                                                   | RCTs and observational studies in high-risk survivors and risk prediction model-guided studies |
| Effectiveness of liposomal anthracyclines                                                  |                                                                                                             |
| Effectiveness of longer infusion duration                                                  |                                                                                                             |
| Effectiveness of pharmacologic heart failure treatments                                    | A RCT on low-dose carvedilol in high-risk CCS is ongoing (123)                                             |
| Management of cardiovascular risk factors                                                 |                                                                                                             |
| Effectiveness of risk factor modifications to prevent cardiovascular events in CCS        | Prospective trials and RCTs in CCS with cardiovascular risk factors present                                |
| Effectiveness of lifestyle interventions in CCS                                           | Prospective trials and RCTs in CCS                                                                          |
| Risk prediction models                                                                     |                                                                                                             |
| Improvement with additional predictors (genetic, echocardiography, ECG, and blood biomarkers) | Cohort studies with validation in an independent cohort                                                     |
| Benefit of longitudinal measurements to update individual risk predictions                 | Landmark analysis or joint modeling within cohort studies with external validation                         |
| The incremental predictive value of machine learning algorithms compared to classical regression | Multicenter cohort studies with a large number of events.                                                 |
| Clinical impact of prediction models                                                       |                                                                                                             |
| Early detection of cardiac disease                                                        |                                                                                                             |
| Usefulness of (strain) imaging, ECG parameters, and blood biomarkers in early detection     | Cohort studies, (cluster) RCTs of different surveillance strategies                                          |
| Identification of novel blood biomarkers for cardiac disease                               | Proteomics/metabolomics in case-control studies with validation in cohort studies                          |
| Genetics                                                                                  |                                                                                                             |
| Genetic susceptibility for other diseases than anthracycline cardiomypathy                  | Cohort studies with uniform cardiotoxicity event definitions, replication in independent cohorts          |
| Identification of novel genetic variants                                                   | GWAS or WGS in large (multicenter) cohort studies                                                           |
| Clinical usefulness of genetic risk stratification                                         | Cohort studies with time to event analysis                                                                  |

CCS = childhood cancer survivors; ECG = electrocardiography; GWAS = genome-wide association studies; RCT = randomized controlled trial; WGS = whole-genome sequencing.
intervention studies in CCS may be due to the long follow-up required for clinical events. Therefore, initially, intermediate imaging or blood biomarker outcomes may be useful as a proof of concept before conducting larger trials.

The safety and effectiveness of primary prevention strategies, including dexrazoxane, and secondary prevention strategies, such as modification of cardiovascular risk factors and treatment of asymptomatic myocardial dysfunction, can ideally be studied in RCTs or large observational studies. Prevention and surveillance may be further individualized with prediction model-guided care after evaluation of their clinical impact.

Myocardial fibrosis and edema quantification with cardiac MRI are promising techniques to improve risk stratification and may facilitate earlier detection (39). The usefulness of echocardiographic strain imaging, ECG, and blood markers in the early detection of cardiotoxicity in long-term childhood cancer survivors is currently being investigated in the Dutch LATER cohort study (120). In addition, modeling complex interactions and nonlinear relationships between predictors and outcomes with machine learning algorithms may be a valuable addition to classic regression models in childhood cancer survivors when samples sizes are sufficient (121).

CONCLUSIONS

Cardiac disease after the treatment of childhood cancer is an important health problem for survivors of childhood cancer. Optimal survivorship care, including collaboration between pediatric oncologists and cardiologists, is needed to detect and treat cardiac abnormalities in an early phase. During the past decade, a large body of evidence on cardiac diseases in CCS has been collected through cohort studies that can improve current international surveillance guidelines. New insights into the impact of risk factors such as mitoxantrone should be incorporated in discussions on new treatment protocols for children with cancer and in guidelines for follow-up care. Apart from the treatment-related risk, lifestyle interventions may be important to modify cardiovascular risk factors and prevent cardiovascular events in aging survivors. Prediction models that have been developed for heart failure, ischemic heart disease, and cardiovascular mortality await clinical impact analysis to guide individualized preventive measures, surveillance, and treatment decisions. A better understanding of genetic susceptibility for anthracycline-induced cardiomyopathy and underlying pathophysiologic mechanisms have the potential to improve both risk stratification and the development of primary and secondary prevention strategies. Translating research into the care for survivors is complex and requires a multidisciplinary approach from researchers, epidemiologists, (pediatric) oncologists, and cardiologists.

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KEY WORDS cardiotoxicity, cardiovascular risk factors, childhood cancer survivors, prevention, risk prediction

APPENDIX For supplemental tables and references, please see the online version of this paper.
Remarkable progress in cancer treatments has resulted in improved long-term survival. However, cardiovascular complications related to these therapies may result in treatment interruptions and worse oncologic and cardiovascular outcomes. Early diagnosis and management of cancer therapy cardiotoxicity are therefore critical for safe and effective cancer treatment and long-term survival. In this population, established clinical biomarkers such as cardiac troponin (cTn) and natriuretic peptides (B-type natriuretic peptide [BNP], and N-terminal pro-B-type natriuretic peptide [NT-proBNP]) have shown promise, but studies have been limited by variable sensitivity, relatively small study populations with low cardiotoxicity event rates, and inconsistencies in cardiotoxicity definitions and timing of biomarker ascertainment. Additionally, these biomarkers are not specific to drug-induced toxicity. Thus, there is a need to discover novel biomarkers to accurately identify at-risk populations, diagnose toxicity early, monitor disease course, and guide therapies, enabling the implementation of precision cardio-oncology. This paper provides a brief overview of the future of biomarker discovery in cardiotoxicity, translating novel omics technologies to the clinic.

Biomarkers can be obtained from any products or parts of the body, including urine, blood, and tissues. Human-induced pluripotent stem cells (iPSCs) can be generated from patients’ somatic cells such as peripheral blood mononuclear cells and differentiated into relevant cardiovascular or immune cell types, providing a novel source of biomarkers. Figure 1 lists common sources of biomarkers and their properties. Briefly, urine and blood are abundant, noninvasive, and easy to collect. Blood cells provide an excellent source of genomic materials and immune phenotyping. However, biomarkers from those sources may lack specificity for cardiac pathology and suffer from wide variability and poor reproducibility due, in part, to the multiorgan effects of cancer treatments. Biomarkers obtained directly from cardiac tissue provide tissue-specific and physiologic information; however, those samples are often difficult to obtain due to procedural risk and cost. iPSC-derived cardiovascular cells are cardiac-specific, albeit immature. They can be cultured in a dish with a nearly limitless supply of cells and allow serial collection without requiring invasive procedures or additional clinical sampling.
They retain patient-specific genetic information and enable personalized screening. However, reprogramming and maintenance of iPSCs are costly procedures and are labor intensive, and iPSC-derived cells alone lack environmental and physiologic relevance (1).

OMICS APPROACHES FOR NOVEL BIOMARKER DISCOVERY

Omic technologies allow high-throughput generation of large amounts of data for a specific molecular type such as DNA, proteins, and metabolites (Figure 2). By using bioinformatic tools combined with detailed clinical phenotyping, researchers can determine whether particular genetic or molecular patterns are associated with increased cardiotoxicity risk. This approach may enable novel biomarker discovery, provide mechanistic and therapeutic insights, and generate hypotheses for future investigations.

GENOMICS. Genomic influence on the risk of cancer therapy-induced cardiotoxicity has been actively researched, given widely variable interindividual susceptibility. Hypothesizing shared genetic risk between dilated cardiomyopathy (DCM) and cancer therapy-induced cardiotoxicity, Garcia-Pavia et al. (2) sequenced putative DCM genes and found an increased prevalence of TTN-truncating variants in cardiotoxicity cases. In a separate study, Aminkeng et al. (3) conducted a genome-wide association study (GWAS) in 280 patients of European ancestry treated with anthracyclines (32 cases, 248 controls) and identified a protein-altering variant in RARG that was highly associated with cardiotoxicity; findings validated in other cohorts and independently supported by investigations using patient-specific and genome-edited iPSC-derived cardiomyocytes (4). Hence, genomics can be used to identify key genetic variants or develop polygenic risk scores predicting cancer therapy cardiotoxicity risk. However, for GWAS to yield meaningful discoveries, many factors need to be considered, including the overall prevalence of cardiotoxicity associated with a particular cancer drug, the number of cardiotoxicity cases and controls being studied, expected variant frequencies, and the extent to which cardiotoxicity risk is influenced by variants (5). Thus far, genomic studies in cardio-oncology have largely been limited by small numbers and relatively low cardiotoxicity event rates. This may be overcome by combining genomic data from various sources.

Another promising genetic biomarker is clonal hematopoiesis of indeterminate potential (CHIP), which has been linked to an increased risk of aging-related conditions including cardiovascular disease (6). Emerging data suggest that CHIP is more common in patients after cancer treatments such as bone marrow transplantation and is associated with higher risk of leukemia and all-cause mortality (7). Although CHIP is currently an area of active research, it may serve as an attractive biomarker to identify both cardiovascular and oncologic risk.

EPGENOMICS. Epigenomics are reversible genetic modifications that regulate gene expressions without altering the DNA sequence. Established epigenomic factors include: 1) DNA methylation and histone modifications altering chromatin accessibility and structure; and 2) expression of noncoding RNA, such as microRNA (miRNA) and long noncoding RNA (lncRNA), directly interacting with gene transcriptions. A patient-specific epigenomic footprint may influence the response to environmental insults or cancer therapies. Additionally, chemotherapies and radiation therapies may also alter the epigenome, further impacting gene expression and subsequent phenotypes.

Recent developments in epigenetic technologies to sequence accessible chromatin regions or quantitatively interrogate methylation sites across the genome have advanced the understanding of epigenetic regulations. With advances in technologies, investigations using miniscule amounts of genomic samples (<1 µg) can now provide comprehensive epigenomic information. Epigenomic profiling of the heart, however, has been hampered by difficulties in obtaining myocardial tissues from patients. Meder et al. (8) performed epigenome-wide mapping of DNA methylation in endomyocardial biopsies obtained from 41 DCM patients and 31 controls and compared the results with the methylation profiles of whole peripheral blood samples from the same patients. The authors observed distinct epigenetic patterns associated with DCM, identifying 27 epigenetic loci significantly enriched in the DCM cohorts, and also identified a minor subset of DCM-specific methylation sites conserved in cardiac and blood tissues. Further studies are needed to examine whether epigenetic signatures of the heart or other biosamples can serve as a useful biomarker of cardiotoxicity.

TRANSCRIPTOMICS. Next-generation sequencing (NGS) has enabled rapid and affordable sequencing of RNA, making it possible to quantitatively assess thousands of gene transcripts. Transcriptomic profiling of drug-treated iPSC-derived cardiac cells revealed distinct expression patterns and
mechanistic insights for \((9,10)\) tyrosine kinase inhibitor-\((11)\), and trastuzumab-related cardiotoxicity \((12)\). Together, these studies have also demonstrated interindividual variation correlating with cellular toxicity, suggesting underlying genetic contributions in modulating cellular response to various treatments. These findings exemplify potential utility of transcriptomic biomarkers to improve accurate diagnosis of cardiotoxicity. Additionally, the recent development of single-cell RNA sequencing (scRNA-seq) technologies allows cell-specific transcriptomic evaluation, enabling the discovery of new, relevant cell populations such as inflammatory cells and important genes and pathways that mediate cardiotoxicity \((13)\).

Circulating miRNAs are attractive biomarkers as they are readily detectable in serum and are stable against degradation with a long half-life. More than 1,900 human miRNAs have been annotated in the miRBase database (miRBase: Microrna, University of Manchester, Manchester, United Kingdom) and a number of miRNAs have already been shown to be associated with myocardial injury and cardiovascular death. Oatmen et al. \((14)\) compared serum miRNA profiles in anthracycline-treated pediatric patients with age-matched controls. Using a customized microarray of 84 miRNAs associated with cardiovascular diseases, the authors observed significantly altered miRNA expression with anthracyclines and identified 8 miRNAs that correlated with

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**FIGURE 1**

Common Sources of Biomarkers and Their Properties

| Common Sources of Biomarkers | Urine | Serum | Blood Cells | Cardiac Tissue | iPSC-derived Cells |
|-----------------------------|-------|-------|-------------|---------------|-------------------|
| Abundance                   | ++++  | ++++  | ++++        | +             | +++               |
| Cost                        | $     | $$    | $$          | $$$$          | $$$$             |
| Invasiveness                | None  | Minimal | Minimal    | High          | Minimal          |
| Serial Collection           | Yes   | Yes   | Yes         | No            | Yes              |
| Variability                 | High  | Moderate | Moderate   | Low           | Moderate         |
| Physiologic Relevance       | Low   | Low/Moderate | Low      | High          | Low/Moderate     |

DNA

| Access To                   | DNA | +     | ++++    | +++   | +++ |
|-----------------------------|-----|-------|---------|-------|-----|
| CV Epigenetic Markers       | -/? | ---   | ?       | ++++  | ++  |
| Cardiac Lineage Cells       | --- | ---   | ---     | ++++  | +++ |
| Immune Cells                | +   | ---   | ++++    | +++   | ++ (\*) |
| CV Specific Exosomes        | -/? | ++    | ---     | +++   | +++ |

Common sources of biomarkers include urine, blood (serum and blood cells), cardiac tissues, and iPSC-derived cardiac lineage cells (e.g., cardiomyocytes, endothelial cells, vascular smooth muscle cells, fibroblasts) and iPSC-derived immune cells such as T cells and lymphocytes (*). Their comparative strengths and weaknesses to identify clinically useful biomarkers are summarized. CV = cardiovascular; iPSC = induced pluripotent stem cells.
Although these findings suggest the potential utility of miRNAs as cardiotoxicity biomarkers, few markers have been validated, due in part to differences in isolation and measurement techniques. Future endeavors in optimized measurement and unbiased sequencing of miRNA may accelerate the discovery of novel miRNA cardiotoxicity biomarkers.

**PROTEOMICS.** Although transcriptomic information provides insights into the proteome, significant discordance exists, due in part to complex protein regulation in cells. First, protein synthesis may be directly correlated with the abundance of RNA transcripts, but many factors contribute to protein degradation including ubiquitin-proteasome and...
lysosome-mediated proteolysis. Second, there are far more proteins in the body than there are protein-coding genes, further complicating proteomic evaluation. Third, although proteomic changes in cardiac tissues may reflect direct cardiac pathologies, the associated procedural risk has prevented its widespread use, and researchers have used plasma samples for proteomics analysis. Beer et al. (15) used mass spectrometry to comprehensively analyze plasma proteomic profiles of 3 cardiotoxicity cases versus 4 age- and cancer-matched controls without cardiomyopathy. Their results suggested that immunoglobulin E had the largest and most consistent differences in the levels between cases and controls (15). In another study in noncancer patients, researchers used aptamer-based proteomic technology to probe the plasma proteome in patients with coronary heart disease (16). The study’s 9-protein risk score outperformed the Framingham secondary event risk score in predicting cardiovascular events among patients with stable coronary heart disease. As such, proteomics-based studies may allow the discovery of novel protein biomarkers or the development of a risk score to predict those at risk for cardiotoxicity.

**METABOLOMICS.** Metabolomics is the analysis of metabolites in the body such as amino acids, lipids, and organic acids. According to the Human Metabolome Database, there are >100,000 metabolites, which include endogenous metabolites and metabolites from external sources such as food and environmental pollutants. Circulating metabolites not only reflect the end products of bodily processes but provide unique insights into the interplay between environmental exposure and development of cardiotoxicity. Although metabolomic profiling of plasma or urine samples can provide valuable insight into metabolic pathways critical to cardiotoxicity, cancer therapies typically have a broad impact in the metabolism of multiple organ systems, complicating interpretation. One way to circumvent this problem would be to use cardiac-specific tissues, such as iPSC-derived cardiovascular cells, to identify cardiac-specific metabolic perturbation by specific cancer therapies (1).

**IMMUNOLUMICS.** With the introduction of immunotherapies, there have been increasing reports of immunity-related cardiovascular complications such as myocarditis. Omics-based immune profiling of whole blood or affected tissues, immunolomics, may provide insights into new mechanisms and biomarkers. The major approaches include: 1) genetic sequencing of the complementarity-determining regions and the antigen-binding portion of the T-cell-receptor beta chain, so-called immuno-seq; 2) scRNA-seq of immune cells; and 3) a single cell-level proteomic evaluation of immune cell surface receptors using a mass spectrometry-based approach called mass cytometry by time-of-flight (CyTOF), all of which are used to characterize cellular compositions and molecular characteristics of immune cells. Johnson et al. (17) used immuno-seq to identify selective clonal T-cell populations potentially involved in immune checkpoint inhibitor myocarditis. Although immunolomics are still at an early stage, they may provide a novel class of biomarkers with which to identify immune-related complications.

**INTEGRATED MULTIO-MICS AND SYSTEMS BIOLOGY.** With advances in bioinformatics and computational biology, the aforementioned multi-level omics data can be simultaneously and longitudinally studied, which may help narrow biomarker candidates and also reveal important interaction networks. Rose et al. (18) performed integrative multi-omics profiling from patient samples collected quarterly for up to 8 years. They constructed prediction models to identify patients at risk for developing type 2 diabetes mellitus and also reported >60 clinically actionable health discoveries to implement diet and exercise changes. With accumulating integrative and longitudinal multi-omics data, comprehensive molecular signatures specific to cardiotoxicity of cancer therapy may accelerate actionable health and biomarker discoveries.

**CONCLUSIONS**

Despite significant progress, there is an important need to continue to develop the necessary infrastructure and technologies to advance biomarker science. Emerging omics technologies and bioinformatics tools coupled with access to large patient populations may provide an unparalleled opportunity to discover novel, molecularly targeted biomarkers. This would facilitate better risk stratification, prevention, and treatment of cancer therapy-associated cardiotoxicity.

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KEY WORDS cardio-oncology, epigenomics, immunolomics, metabolomics, proteomics, transcriptomics
Prospective Evaluation of Malignancy in 17,708 Patients Randomized to Ezetimibe Versus Placebo
Analysis From IMPROVE-IT

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ABSTRACT

BACKGROUND An increased risk of malignancy was reported with simvastatin/ezetimibe in 1,873 patients in the SEAS (Simvastatin and Ezetimibe in Aortic Stenosis) trial.

OBJECTIVES The purpose of this study was to clarify this unexpected finding in a larger sample size of patients stabilized after acute coronary syndrome, we conducted a prospective systematic analysis of malignancy events in IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial).

METHODS Within IMPROVE-IT, 17,708 patients post–acute coronary syndrome were randomized to either ezetimibe 10 mg or matching placebo on a background of simvastatin 40 mg who took ≥1 dose of the study drug. Suspected tumors (benign and malignant) reported by investigators or identified from a review of adverse events were adjudicated by oncologists without knowledge of drug assignment. The primary malignancy endpoint included new, relapsing, or progressive malignancies (excluding nonmelanotic skin malignancies). The secondary endpoint was death due to malignancy.

RESULTS In this trial, 1,470 patients developed the primary malignancy endpoint during a median 6 years of follow-up. The most common malignancy locations were prostate (18.9%), lung (16.8%), and bladder (8.8%) with no differences by treatment group (p > 0.05 for each location). Kaplan-Meier 7-year rates of malignancies were similar with ezetimibe and placebo (10.2% vs. 10.3%; hazard ratio: 1.03; 95% confidence interval: 0.93 to 1.14; p = 0.56), as were the rates for malignancy death (3.8% vs. 3.6%; hazard ratio: 1.04; 95% confidence interval: 0.88 to 1.23; p = 0.68).

CONCLUSIONS Among 17,708 patients receiving simvastatin 40 mg daily, those randomized to ezetimibe 10 mg daily had a similar incidence of malignancy and deaths due to malignancy compared with those receiving placebo during a median follow-up of 6 years (96,377 patient-years). (IMPROVE-IT: Examining Outcomes in Subjects With Acute Coronary Syndrome: Vytorin [Ezetimibe/Simvastatin] vs Simvastatin [P04103]; NCT00202878) (J Am Coll Cardiol CardioOnc 2020;2:385–96) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Patients with established atherosclerotic cardiovascular disease are at a higher risk of death attributed both to cardiovascular and noncardiovascular origin (1). Although malignancy is characterized by different biological pathways and pharmacological targets, it shares similar causal risk factors (e.g., age, smoking, inflammation, and diabetes) with cardiovascular disease (2). Therefore, both diseases can occur during a long-term clinical trial, resulting in competing risks for morbidity and mortality (3). A striking example came from the CANTOS (Canakinumab Antiinflammatory Thrombosis Outcome Study), in which a monoclonal antibody targeting inflammation studied primarily to reduce major adverse cardiovascular events was associated with a significant reduction of lung malignancy (4).

Meta-analyses of therapies lowering low-density lipoprotein cholesterol (LDL-C) demonstrated that prevention of cardiovascular events was proportional to the absolute reduction in LDL-C levels with no major safety concerns, specifically no increase in malignancies (5,6). However, available data from previous LDL-C-lowering clinical trials have not systematically adjudicated data for malignancy, because these were not considered disease-related events (7). An increased rate of malignancy associated with the use of the combination of ezetimibe and simvastatin was unexpectedly reported in the SEAS (Simvastatin and Ezetimibe in Aortic Stenosis) trial (8), which compared simvastatin-ezetimibe to placebo in 1,873 adults with mild-to-moderate aortic stenosis over a median follow-up of 4.4 years (8).

Ezetimibe is a nonstatin drug that inhibits the intestinal absorption of cholesterol by targeting the transmembrane protein, Nieman-Pick C1-Like 1 (9). It is recommended for further LDL-C reduction in combination with a statin (10). In IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial), the daily addition of 10 mg ezetimibe to 40 mg simvastatin in patients with acute coronary syndrome (ACS) with LDL-C levels between 50 and 125 mg/dl resulted in an incremental reduction of LDL-C to median achieved LDL-C value of 53 mg/dl versus 70 mg/dl (p < 0.001) and a significant reduction in the primary cardiovascular composite outcome compared with simvastatin alone during a median follow-up of 6 years (32.7% vs. 34.7%; hazard ratio [HR]: 0.94; 95% confidence interval [CI]: 0.89 to 0.99; p = 0.016) (11). In this present analysis, we report the results on malignancy in patients post-ACS participating in IMPROVE-IT treated with simvastatin.

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who were randomized to ezetimibe versus placebo and followed up for a median of 6 years.

METHODS

STUDY POPULATION. The design and results of IMPROVE-IT have been published previously (11,12). IMPROVE-IT was a multinational, double-blind, randomized, placebo-controlled trial that enrolled 18,144 patients from October 26, 2005 to July 8, 2010 after a period of stabilization following a hospitalization for ACS (11). Patients were randomized in a 1:1 manner to once daily treatment with either 40 mg simvastatin plus 10 ezetimibe or 40 mg simvastatin plus matching placebo. The study population included men and women age \( \geq 50 \) years who had been hospitalized within the previous 10 days for ACS, comprising myocardial infarction with or without ST-segment elevation or high-risk unstable angina. To be eligible, the LDL-C level measured within 24 h after hospital admission had to be 50 to 125 mg/dl for patients not receiving prior lipid-lowering therapy, or 50 to 100 mg/dl for patients on prior long-term prescription of lipid-lowering therapy. Major exclusion criteria most relevant to the current analysis included active malignancy or any clinically significant condition other than atherosclerotic vascular disease. Excluded were patients with malignancy diagnosed within 5 years or who were receiving treatment for malignancy, with the exception of adequately treated in situ tumors and nonmelanotic skin malignancy. Other exclusion criteria were hemodynamic instability, acute ischemic or arrhythmic events within 24 h before enrollment, creatinine clearance <30 ml/min, active liver disease, or prior use of statin therapy with a potency higher than 40 mg of simvastatin.

STUDY ENDPOINTS. The primary endpoint of IMPROVE-IT was a composite of cardiovascular death, major coronary event (nonfatal myocardial infarction, rehospitalization for unstable angina, or coronary revascularization occurring \( \geq 30 \) days after randomization), or nonfatal stroke. After the publications of the SEAS trial in 2008, the investigators of IMPROVE-IT designed and implemented a protocol to review all tumors occurring during the trial in previously and future-enrolled patients through the end of follow-up. The primary malignancy endpoint consisted of all first new, worsening, or relapsing malignancies, excluding nonmelanotic (i.e., basal and squamous) skin malignancies. The investigators were trained to report all suspected tumors (benign or malignant) using a prespecified data collection tool designed specifically for this study by the Executive Committee in consultation with the independent Oncology Clinical Endpoint Committee (CEC) (Supplemental Appendix). The completeness of data reported for tumors was closely monitored on site and supplemented by a review of all adverse events reported. All suspected tumors were adjudicated by a pair of independent oncologists unaware of assigned treatment and lipid levels. Pathology reports were used as a primary source of information when available. The tumors were initially classified as malignant or benign, and then subclassified as new versus present prior to randomization. If present prior to randomization, malignancies were additionally characterized as worsening versus relapsing versus stable. Further classifications were performed by organ system disease and malignancy extent. The malignancy extent for solid tumor was graded as: 1) local disease only; 2) spread to contiguous structures; 3) metastatic; and 4) unknown. The extent for leukemia, lymphoma, and other blood malignancies was graded as: 1) acute; 2) chronic; and 3) unknown. The relationship between the malignancy and the vital status was adjudicated by the Oncology CEC. Nonmalignancy deaths were reviewed by a central CEC consisting of neurologists (who reviewed stroke and intracranial hemorrhage) or cardiologists (all other nontumor cases). Disagreements between members were resolved by consensus.

STATISTICAL ANALYSIS. Baseline characteristics stratified by the development of post-randomization malignancy are reported as frequencies and percentages for categorical variable and compared using the chi-square test. Continuous variables were reported as medians with 25th and 75th percentiles (quartile 1 to quartile 3) for continuous variables and compared using the Wilcoxon test. All analyses were performed first on a modified intention-to-treat basis defined as patients who received at least a single dose of the study drug. If a patient developed several primary malignancy endpoints, the first event was used for the primary analysis. Malignancies are presented as Kaplan-Meier (KM) rates at 7 years stratified by the treatment group and compared using the log-rank test. The cumulative incidence plots for the primary malignancy endpoint and the secondary endpoint of death due to malignancy were presented graphically using KM product-limit method. In addition, the frequencies and percentages of malignancy by location are presented by treatment group. The Cox proportional hazard (PH) regression model was used for the analysis and data presented with HR and 95% CI comparing the ezetimibe/simvastatin arm with the placebo/simvastatin arm. We tested for effect modification by evaluating the interaction terms for high-risk subgroups, including gender, age (\( <75 \) years vs. \( <75 \) years), smoking status (current vs. past vs. ...
never), total cholesterol quartiles, body mass index categories, high-sensitive C-reactive protein, TIMI Risk Score for Secondary Prevention (13), and randomized treatment on the risk of the primary malignancy endpoint. The PH assumption was satisfied using visual inspection of Schoenfeld residual plots and performing Supremum test for PH assumption. Sensitivity analyses were performed considering different definitions of malignancy events: 1) new, worsening, and relapsing malignancies; 2) new malignancies only; or 3) worsening or relapsing malignancies only. For each malignancy endpoint, we performed additional analyses including and excluding basal cell and squamous cell malignancies.

| TABLE 1 Baseline Characteristics by Status of Primary Malignancy Endpoint |
|---------------------------------|-----------------|-----------------|-----------------|
| No Malignancy (n = 16,238)       | Malignancy (n = 1,470) | p Value |
| Age, yrs                         | 62.8 (56.5, 70.7)   | 66.8 (60.3, 74.0) | <0.001          |
| Body mass index, kg/m²           | 27.6 (24.9, 30.9)   | 27.3 (24.7, 30.7) | 0.051           |
| Weight, kg                       | 81.2 (71.0, 92.7)   | 81.2 (72.0, 92.5) | 0.37            |
| Male                             | 12,231 (75.3)       | 1,183 (80.5)     | <0.001          |
| Caucasian                        | 13,543 (83.4)       | 1,324 (90.1)     | <0.001          |
| Region of enrollment             |                  |                  |                 |
| North America                    | 6,164 (38.0)       | 613 (41.7)       | 0.005           |
| Western Europe                   | 6,428 (39.6)       | 654 (44.5)       | <0.001          |
| Eastern Europe                   | 1,330 (8.2)        | 77 (5.2)         | <0.001          |
| Asia Pacific                     | 822 (5.1)          | 53 (3.6)         | 0.014           |
| South America                    | 1,494 (9.2)        | 73 (5.0)         | <0.001          |
| Coexisting conditions            |                  |                  |                 |
| Diabetes                         | 4,412 (27.2)       | 383 (26.1)       | 0.36            |
| Hypertension                     | 9,973 (61.4)       | 872 (59.3)       | 0.11            |
| Heart failure                    | 700 (4.3)          | 66 (4.5)         | 0.75            |
| Peripheral arterial disease      | 869 (5.4)          | 115 (7.8)        | <0.001          |
| Current smoking                  | 5,290 (32.6)       | 547 (37.2)       | <0.001          |
| Previous myocardial infarction   | 3,375 (20.8)       | 327 (22.2)       | 0.19            |
| Previous percutaneous coronary intervention | 3,159 (19.5) | 306 (20.8) | 0.21 |
| Previous coronary artery bypass graft | 1,480 (9.1) | 160 (10.9) | 0.025 |
| Medications at index acute coronary syndromes |                  |                  |                 |
| Lipid-lowering therapy           | 5,730 (35.3)       | 543 (37.0)       | 0.20            |
| Statin                           | 5,564 (34.3)       | 527 (35.9)       | 0.21            |
| Aspirin                          | 6,830 (42.1)       | 632 (43.1)       | 0.48            |
| Creatinine clearance, ml/min     | 85.0 (66.1, 107.6)  | 80.7 (63.5, 98.8) | <0.001          |
| TRS2P score >3                   | 1,354 (8.5)        | 152 (10.5)       | 0.011           |
| Type of index event              |                  |                  |                 |
| MI with ST-segment elevation      | 4,634 (28.5)       | 451 (30.7)       | 0.083           |
| MI without ST-segment elevation   | 7,676 (47.3)       | 704 (47.9)       | 0.66            |
| Unstable angina                  | 3,924 (24.2)       | 315 (21.4)       | 0.018           |
| Laboratory values at index event |                  |                  |                 |
| LDL-C, mg/dl                     | 95.0 (79.0, 110.0)  | 93.8 (78.0, 109.7) | 0.23          |
| HDL-C, mg/dl                     | 40.0 (33.0, 49.0)   | 39.1 (33.0, 48.7) | 0.38          |
| Triglycerides, mg/dl             | 120.0 (85.0, 171.8) | 121.2 (85.9, 176.2) | 0.50          |
| Hs-CRP, mg/l                     | 5.1 (2.0, 17.7)    | 5.3 (2.5, 16.1)  | 0.46            |
| Hemoglobin A1c, %                | 6.1 (5.6, 7.3)     | 6.1 (5.7, 7.0)   | 0.81            |
| Medications at time of randomization |            |                  |                 |
| Aspirin                          | 15,770 (97.1)      | 1,421 (96.7)     | 0.39            |
| Beta-blocker                     | 14,171 (87.3)      | 1,277 (86.9)     | 0.69            |
| ACEI/ARB inhibitor               | 12,293 (75.7)      | 1,082 (73.7)     | 0.08            |
| Thienopyridine                   | 14,067 (86.6)      | 1,291 (87.9)     | 0.18            |

Values are median (25th, 75th percentiles) or n (%). Wilcoxon rank-sum test of differences between with and without primary malignancies for continuous variables. Chi-square test of frequencies between with and without malignancies for categorical variables. The no-malignancy group summary statistics are based on patients without malignancy diagnosis prior to death, loss to follow-up or end of the study.

ACEI = angiotensin-converting enzyme inhibitor; ACS = acute coronary syndromes; ARB = angiotensin receptor blocker; HDL-C = high-density lipoprotein cholesterol; Hs-CRP = high-sensitive C-reactive protein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; MI = myocardial infarction; TRS2P = TIMI Risk Score for Secondary Prevention (13).
of the skin, as well as limiting malignancy events to only those with available pathology reports. We also performed a competing risk analysis integrating all-cause death as a competing outcome in the model using Fine and Gray’s method. Finally, we also evaluated the HRs in both arms with increasing duration of follow-up as done in previous publications (14,15). All analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, North Carolina). A 2-sided \( p < 0.05 \) was considered as significant. The institutional review board/ethics committee at each participating center approved the protocol and amendments, and all patients gave written informed consent.

**RESULTS**

There were 436 (2.4%) of the 18,144 randomized patients who did not take any study drug, thus 17,708 patients who took at least one dose of the study drug were included in this analysis (Supplemental Figure 1). Of these, 15,895 patients had no tumor event and 231 had a benign tumor only (115 in the simvastatin/ezetimibe arm and 116 in the simvastatin/placebo arm). At the end of the trial (median [interquartile range] of follow-up: 6.0 [4.3 to 7.2] years), 1,582 patients had a malignancy of which 1,470 met the primary malignancy endpoint (726 in the placebo arm and 744 in the ezetimibe arm) and 112 had nonmelanotic skin malignancy. Among the 1,470 patients who met the primary malignancy endpoint, 1,370 patients had new malignancy, 53 had progressive malignancy, and 47 had relapsing malignancy (Supplemental Figure 1).

Patients who met the primary malignancy endpoint (\( N = 1,470 \)) compared with those who did not (\( N = 16,238 \)) were significantly older (median age: 66.8 vs. 62.8 years; \( p < 0.001 \)), had lower baseline creatinine clearance (80.7 vs. 85.0 ml/min; \( p < 0.001 \)), and were more likely to be male (80.5% vs. 75.3%; \( p < 0.001 \)), white (90.1% vs. 83.4%; \( p < 0.001 \)), a current smoker (37.2% vs. 32.6%; \( p < 0.001 \)), and to have pre-existing peripheral artery disease (7.8% vs. 5.4%; \( p < 0.001 \)) or prior coronary artery bypass graft (10.9% vs. 9.1%; \( p = 0.025 \)) (Table 1). No significant baseline
differences between patients who did versus did not experience the primary malignancy endpoint were found for lipid values or high-sensitive C-reactive protein levels, or in the prior use of statin (35.9% vs. 34.3%; *p* = 0.21).

The 7-year event rate for the primary malignancy endpoint was similar between the ezetimibe and placebo groups (7-year event rates of 10.2% vs. 10.3%; log-rank test *p* = 0.56; HR: 1.03; 95% CI: 0.93 to 1.14) (Central Illustration). There were 277 (3.8%) deaths due to malignancy in the ezetimibe versus 268 (3.6%) in the placebo arm (log-rank test *p* = 0.68; HR: 1.04; 95% CI: 0.88 to 1.23) (Figure 1).

In sensitivity analyses, the estimates were similar when including basal and squamous cell skin malignancy (905 vs. 908 cases; 12.5% vs. 12.8%; HR: 1.00; 95% CI: 0.91 to 1.10). The restriction of the primary endpoint to new malignancy did not significantly alter the results (690 vs. 674 cases; 9.6% vs. 9.7%; HR: 1.03; 95% CI: 0.92 to 1.14) (Table 2).

Simvastatin/ezetimibe (red) and placebo/ezetimibe (blue). The cumulative incidence plots were presented graphically using Kaplan-Meier product-limit method. CI = confidence interval; HR = hazard ratio.

**TABLE 2 Risks of Primary and Secondary Malignancy Endpoints by Treatment Arm**

|                       | Simvastatin Monotherapy (n = 8,855) | Simvastatin/Ezetimibe (n = 8,853) | HR (95% CI) | p Value |
|-----------------------|------------------------------------|-----------------------------------|-------------|---------|
| **Primary endpoint**  |                                    |                                   |             |         |
| New, relapsing, or progressive malignancy (excluding nonmelanotic skin malignancy) | 726 10.3 | 744 10.2 | 1.03 (0.93-1.14) | 0.56 |
| **Secondary endpoints** |                                    |                                   |             |         |
| New, relapsing, or progressive malignancy (including nonmelanotic skin malignancy) | 908 12.8 | 905 12.5 | 1.00 (0.91-1.10) | 0.99 |
| New malignancy (excluding nonmelanotic skin, relapsing, or progressive malignancies) | 674 9.7 | 690 9.6 | 1.03 (0.92-1.14) | 0.63 |
| New malignancy (including nonmelanotic skin malignancy and excluding relapsing or progressive malignancy) | 857 12.2 | 851 11.9 | 0.99 (0.90-1.09) | 0.90 |
| New, relapsing, or progressive malignancy (excluding nonmelanotic skin malignancy) | 629 8.9 | 640 8.8 | 1.02 (0.92-1.14) | 0.67 |
| Deaths due to malignancy | 268 3.6 | 277 3.8 | 1.04 (0.88-1.23) | 0.68 |

CI = confidence interval; HR = hazard ratio.
11.9% vs. 12.2%; HR: 0.99; 95% CI: 0.90 to 1.09). The results were consistent when limiting the primary endpoint definition to cases confirmed with pathology reports (640 vs. 629 cases; 8.8% vs. 8.9%; HR: 1.02; 95% CI: 0.92 to 1.14) (Supplemental Table 1). In the competing risk analysis with consideration of all-cause death, we found similar results (7-year event rate of 9.8% vs. 9.8%; HR: 1.03; 95% CI: 0.93 to 1.15; p = 0.53) (Supplemental Table 2).

In the overall population, the most common organ locations of the primary malignancy endpoint were prostate (18.9%), lung (16.8%), and bladder (8.8%). By treatment arm, the most common malignancy locations were lung (16.0% in the ezetimibe arm vs. 17.6% in the simvastatin monotherapy arm; p = 0.40), prostate in men (19.3% in the simvastatin-ezetimibe arm vs. 18.5% in the placebo arm; p = 0.72), and bladder (8.1% in the ezetimibe arm vs. 9.5% in the placebo arm; p = 0.33) (Supplemental Table 3). Breast malignancy occurred in 26.4% of female patients in the ezetimibe arm versus 18.9% patients in the placebo arm (p = 0.13) (and only 1 case in men). The rates of malignancy events did not differ by treatment group for any location (p > 0.05 for all comparisons) (Table 3). Results were similar between treatment groups for deaths due to malignancy by location.
Further analyses by gender showed no significant differences between treatment groups for both the primary malignancy endpoint (p > 0.05 for each comparison) (Supplemental Tables 5 and 6) and deaths due to malignancy (p > 0.05 for each comparison) (Supplemental Tables 7 and 8). Finally, we did not observe any differences in the extent of malignancy between treatment arms (Table 4).

In subgroup analyses, the rates of the primary malignancy endpoint were similar between treatment groups for each of the 8 pre-specified high-risk subgroups (each p for interaction > 0.05) (Figure 2). The risks for the primary malignancy endpoint with ezetimibe versus placebo were similar in men (7-year KM event rates 10.8% vs. 10.9%; HR: 1.03; 95% CI: 0.92 to 1.16; p = 0.59) and in women (8.3% vs. 8.3%; HR: 1.03; 95% CI: 0.81 to 1.30; p = 0.82; p for interaction = 0.96). The risks of death due to malignancy between treatment groups were consistent across the pre-specified high-risk subgroups (each p for interaction > 0.05) (Figure 3).

During the 7 years of follow-up, there was no divergence in the KM curves over time (Figure 1); the year-by-year HRs for the primary malignancy endpoint comparing the two treatment arms did not demonstrate a progressive trend over time (Table 5).

**DISCUSSION**

The data presented in this analysis of IMPROVE-IT are robust. A total of 17,708 patients with a median follow-up of 6 years (96,377 patient-years) after acute coronary syndromes were studied. The addition of ezetimibe to simvastatin did not affect the risk of malignancy. No differences were found in new, worsening, or relapsing malignancy, or after: 1) including or excluding nonmelanoma skin malignancies; 2) limiting the malignancy definition to new malignancies only or when including relapsing/worsening malignancies; or 3) restricting the endpoint to patients with available pathology reports. In addition, we did not observe any increased relative risk with ezetimibe versus placebo with longer follow-up duration to 7 years.

In the SEAS trial (approximately one-tenth the size of IMPROVE-IT) of patients with aortic stenosis, incident malignancy was diagnosed in 105 patients (11.1%) randomized to the ezetimibe-simvastatin combination, as compared with 70 patients (7.5%) in the placebo group (p = 0.01). Fatal malignancies diagnosed after discontinuation of the study drug or placebo
 occurred in 39 patients in the ezetimibe-simvastatin group versus 23 in the placebo group ($p = 0.05$). The excess malignancy risk in the ezetimibe-simvastatin group was not restricted to a single organ and the risk did not increase over time. Because simvastatin had been studied in more than 41,541 patients in large double-blind randomized trials without an increase in malignancy as previously reported, attention focused on whether the excess in malignancy in SEAS may have been related to ezetimibe (16).

Because ezetimibe inhibits the absorption, not only of cholesterol, but also of phytosterols and other nutrients that have possible protective roles against malignancy (17,18), a potential mechanism for increased risk of malignancy with ezetimibe has been proposed (19). To clarify whether an excess in malignancy existed in other large randomized studies with ezetimibe, in 2011 a combined analysis from the SHARP (Study of Heart and Renal Protection) trial (20) and an interim analysis from the ongoing IMPROVE-IT (analysis limited to 11,354 patients with a follow-up of 1 year) was performed in a total of 20,617 patients randomized to ezetimibe versus placebo (15). In contrast to the SEAS trial (mean follow-up: 4.1 years), the combined data from the SHARP trial (mean follow-up: 2.7 years) and the interim IMPROVE-IT data (mean follow-up: 1.0 year) did not show an increased risk of malignancy with ezetimibe (risk ratio: 0.96; 95% CI: 0.82 to 1.12; $p = 0.61$) (15). When combining data from all three datasets (SEAS, SHARP, and IMPROVE-IT), the risk ratio (RR) was 1.06 (95% CI: 0.92 to 1.22; $p = 0.46$). There was no evidence of an increased RR over time ($p$ for trend $= 0.54$), suggesting the absence of a temporal association or causal inference between ezetimibe exposure and risk of malignancy over a follow-up period of 18,604 person-years.

Major strengths of our analysis are the systematic evaluation of all tumors (malignant or benign), a thorough classification of all possible malignancies with multiple sensitivity analyses, and an independent assessment by a panel of oncologists who were unaware of study treatment and lipid levels. In contrast, previous studies have usually reported malignancy as a safety outcome, without a broad review of all tumors or use of an independent blinded adjudication process as is typically performed for the primary and secondary efficacy endpoints related to the disease. Therefore, we agree with Peto et al. (14) that the malignancy findings reported from SEAS were likely due to chance. The present study reinforces the importance of testing previously unanticipated findings in a large and independent database with prospectively validated methods for malignancy events adjudication (7).
Some concern has been expressed that statin and other LDL-C-lowering drugs might be carcinogenic (21). Because simvastatin was a mandatory background therapy in both arms of IMPROVE-IT, our analysis cannot address any potential hypothesis regarding the association between simvastatin use and cancer. However, the cholesterol treatment trialist meta-analysis in 90,056 patients treated with statins and 14 trials showed no association between LDL-C reduction and an increased risk of malignancy with statin (statin: 2,810 events, 1.4% per year; controls: 2,804 events, 1.4% per year; RR: 1.00; 95% CI: 0.95 to 1.04) (5). In the updated analysis from the cholesterol treatment trialist cycle 2, including 174,149 patients and 27 trials, the reduction of LDL-C with statin (or high-intensity statin) had no effect on the incidence of newly diagnosed malignancy (RR: 1.00; 95% CI: 0.96 to 1.05) or on deaths to malignancy (RR: 1.00; 95% CI: 0.98 to 1.02) compared with control (placebo or low-intensity statin) over a median treatment duration of 4.8 years (22). Furthermore, there was no increased malignancy rate despite very low achieved LDL-C lowering with the proprotein convertase subtilisin kexin 9 (PCSK9) inhibitors in the FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects With Elevated Risk) and ODYSSEY-OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) trials (23,24).

Finally, achievement of very low LDL-C levels with ezetimibe in IMPROVE-IT (<30 mg/dl) and with evolocumab in FOURIER (<20 mg/dl) at 1 month after randomization was not associated with an increase in major safety events, including new malignancies.

We acknowledge limitations to this analysis. First, although IMPROVE-IT was a large multicenter, multinational trial that enrolled a broad population of patients after an ACS hospitalization, patients were selected for enrollment who fulfilled appropriate inclusion criteria. Frail patients at higher risk of malignancy may have been less likely to qualify and/or willing to participate in this clinical trial. Therefore, the applicability of the observations described to patients excluded from IMPROVE-IT is unknown. Second, as previously reported, the discontinuation of study medication was highest early and stabilized to 8% per year (26). However, study drug discontinuation was not related to the addition of ezetimibe and data continued to be collected and analyzed regardless of whether patients continued study treatment or not (26). Third, the average follow-up of 6 years might be not long enough to detect a carcinogenic signal, particularly for slowly growing malignancies with a long latency period. However, there is no indication that the risks by treatment arm would have changed over a longer follow-up nor that the KM curves would have started to diverge beyond the available follow-up of 6 years. Fourth, the systematic prospective collection for malignancies started in 2008, roughly 3 years after the start of the trial,

| TABLE 5 | Risks of Malignancy Events by Treatment Arm and Year of Follow-Up |
|---------|---------------------------------------------------------------|
|          | Simvastatin Monotherapy | Simvastatin/Ezetimibe |          |          |
|          | Years of Follow-Up | N | n (%) | N | n (%) | HR (95% CI) | p Value |
| Primary malignancy endpoint | | | | | | | |
| ≤1 | 8,848 | 129 | 8,847 | 133 | 1.04 (0.81-1.32) | 0.77 |
| >1 and ≤2 | 8,199 | 125 | 8,136 | 131 | 1.05 (0.82-1.34) | 0.68 |
| >2 and ≤3 | 7,798 | 117 | 7,776 | 130 | 1.11 (0.87-1.43) | 0.40 |
| >3 and ≤4 | 7,468 | 95 | 7,454 | 109 | 1.15 (0.87-1.51) | 0.33 |
| >4 and ≤5 | 6,988 | 95 | 6,971 | 96 | 1.01 (0.76-1.35) | 0.92 |
| >5 and ≤6 | 5,234 | 80 | 5,191 | 70 | 0.88 (0.64-1.22) | 0.44 |
| >6 and ≤7 | 4,168 | 57 | 4,132 | 44 | 0.78 (0.52-1.15) | 0.21 |
| Deaths due to malignancy | | | | | | | |
| ≤1 | 8,855 | 20 | 8,853 | 28 | 1.40 (0.79-2.49) | 0.25 |
| >1 and ≤2 | 8,590 | 44 | 8,557 | 36 | 0.82 (0.53-1.27) | 0.37 |
| >2 and ≤3 | 8,373 | 55 | 8,378 | 43 | 0.78 (0.52-1.16) | 0.22 |
| >3 and ≤4 | 8,144 | 42 | 8,168 | 41 | 0.97 (0.63-1.49) | 0.89 |
| >4 and ≤5 | 7,715 | 36 | 7,755 | 49 | 1.36 (0.89-2.09) | 0.16 |
| >5 and ≤6 | 5,921 | 34 | 5,891 | 44 | 1.30 (0.83-2.04) | 0.25 |
| >6 and ≤7 | 4,802 | 25 | 4,757 | 23 | 0.93 (0.53-1.64) | 0.80 |

Abbreviations as in Table 2 and 3.
and consequently part of the malignancy data was collected retrospectively. Fifth, although the analyses of outcomes stratified by subgroups were pre-specified, the power was low in these subgroups. Finally, we did not measure phytosterols or other potential protective or tumor-promoting factors in blood to evaluate the effect of ezetimibe on the potential mechanistic pathways related to malignancy.

CONCLUSIONS

We found that ezetimibe did not increase the rates of malignancy nor deaths due to malignancy in 17,708 patients with recent ACS treated with simvastatin and followed up for a median of 6 years totaling 96,377 patient-years of follow-up.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: The addition of ezetimibe to simvastatin did not affect the risk of malignancy. No differences were found in new, worsening, or relapsing malignancy. Findings remained consistent after: 1) including or excluding nonmelanoma skin malignancies; 2) limiting malignancy outcome definitions to either new malignancy or including relapsing/worsening malignancy; or 3) restricting the endpoint to patients with available pathology reports. In addition, we did not find differences between treatment groups in deaths due to malignancy, malignancy location, extent of malignancy, or among high-risk subgroups. Finally, we did not observe any increased relative risk with ezetimibe versus placebo with longer follow-up duration.

TRANSLATIONAL OUTLOOK: Our findings support the 2018 Cholesterol Guidelines recommendations to intensify lipid-lowering treatment to lower LDL-C levels in patients with ACS. The use of ezetimibe in addition to simvastatin can be safely considered for the long-term lipid-lowering management of patients with ACS. Our data highlight the importance of considering malignancy outcomes in the design of long-term cardiovascular trials because the incidence of cancer endpoints approaches the incidence of traditional cardiovascular endpoints over time.
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KEY WORDS acute coronary syndromes, cancer, ezetimibe, lipid-lowering therapy, lipids, malignancy, statin

APPENDIX For supplemental Methods and tables, please see the online version of this paper.
Low-density lipoprotein-cholesterol (LDL-C)-lowering strategies are the cornerstone of coronary heart disease prevention and treatment. Therapies to reduce LDL-C such as statins or ezetimibe have significantly improved cardiovascular outcomes; this benefit has been ascribed to pleiotropic effects beyond lowering LDL-C. However, such pleiotropic mechanisms have raised safety concerns about long-term use. A potential carcinogenic effect has been of particular concern as earlier studies suggested an association between baseline low LDL-C levels and incident cancer risk (1). Thus far, a wealth of outcome data on statin therapies has established no significant cancer risk of statins (2). The safety of other cholesterol-lowering therapies in regard to their cancer risk has not been well established. Moreover, the threshold for reporting safety analyses and the level of acceptable evidence, including exploratory analyses, are different than that of efficacy analyses.

In 2008, the SEAS (Simvastatin and Ezetimibe in Aortic Stenosis) investigators reported the initial results of their randomized trial investigating the effects of cholesterol-lowering therapies on cardiovascular outcomes among patients with aortic stenosis (AS) (3). Given the association between hyperlipidemia and AS, the study sought to examine potential beneficial effects of intensified cholesterol lowering with combined simvastatin/ezetimibe therapies in AS. Ezetimibe works differently from statins in that it inhibits the biliary and intestinal cholesterol absorption by binding to Niemann-Pick C1 Like 1. The study involved 1,873 patients with mild-to-moderate asymptomatic AS and the patients received either simvastatin/ezetimibe (40/10 mg) or placebo daily. Although the study did not find significant benefits from the intensive lipid-lowering therapy, it reported an incidental finding of increased cancer occurrence (105 vs. 70; p = 0.01) and cancer-associated death (39 vs. 23; hazard ratio [HR]: 1.67; p = 0.05) with simvastatin/ezetimibe compared to placebo. The cancer incidence did not cluster in particular organ sites. As prior studies have shown no significant link between statins and cancer risk, the results raised a concern that adding ezetimibe to statin therapy might increase the risk of cancer. This safety signal needed further investigation.

Subsequently, several studies have investigated the effects of combined ezetimibe and simvastatin therapy. First, the SHARP (Study of Heart and Renal Protection) trial investigated whether combination of simvastatin plus ezetimibe as compared to placebo would decrease major atherosclerotic events in 9,270 patients with chronic kidney disease (4). During a median follow-up of 4.9 years, the combination cholesterol-lowering therapy yielded a 17% risk reduction in major atherosclerotic events without increasing the risk of cancer (438 vs. 439; p = 0.89). Similarly, other retrospective analyses and meta-analyses (2) did not find a significant association between ezetimibe and increased risk of cancer. The U.S. Food and Drug Administration thus released an updated statement regarding the safety of the ezetimibe/simvastatin therapy and stated that it is unlikely that the drug would increase the risk of cancer or cancer-related death, although the existing data on
ezetimibe are “insufficient to definitely rule out a cancer risk” (5).

In this issue of JACC: CardioOncology, Giugliano et al. (6) report their systematic analysis of cancer incidences in IMPROVE-IT (Improved Reduction of Outcomes: Vyetorin Efficacy International Trial), a largest trial to date to investigate the cardiovascular effects of combined ezetimibe/simvastatin therapy (6). Here, 17,708 patients with recent acute coronary syndrome were randomized 1:1 to receive either simvastatin/ezetimibe (40/10 mg) or simvastatin (40 mg) plus matching placebo. To address a carcinogenic risk of the combined therapy, the IMPROVE-IT trial implemented: 1) an independent Oncology Clinical Endpoint Committee was formed to guide their study design and data adjudication; 2) training for investigators to report all suspected tumor cases, benign or malignant, during the trial period; and finally 3) independent adjudication of suspected tumors by oncologists unaware of treatment assignments or lipid levels. At the end of the trial with median follow-up of 6.0 years, 1,470 (8.3%) met the primary malignancy endpoint and no significant difference in cancer incidence was observed between the 2 treatment groups (744 in the ezetimibe arm and 726 in the placebo arm; HR: 1.03; p = 0.56). The rates of cancer-associated death were also similar (277 vs. 268; HR: 1.04; p = 0.68).

The IMPROVE-IT trial has several strengths over the SEAS trial in addressing the cancer risk of ezetimibe. First, the number of participants in IMPROVE-IT is approximately 10-fold more than in SEAS, improving the power to detect significant differences and lowering the chance to observe random events. Second, the trial was specifically designed to prospectively collect and analyze relevant tumor data, which were then independently adjudicated by oncologists. Third, the trial population is ethnically more diverse than SEAS (white race being 84% vs. 99%). Overall, the findings from this study (6) strengthen the accumulating data that the combined simvastatin/ezetimibe therapy does not increase cancer risk and suggest that the results from the SEAS trial may have been due to random imbalance of the cancer events. Similar observations were made previously in regard to the safety of statins. One trial reported an increased risk of breast cancer with pravastatin, an outcome which was not replicated in >20 other trials with the drug (7). As such, some imbalances may exist particularly when studies are not sufficiently powered and/or not specifically designed to see differences in the events of interest.

Although reassuring, it is worth highlighting several points. First, the event rates of each cancer type in IMPROVE-IT were low with lung cancer being the highest at 1.3% in the ezetimibe arm and 1.4% in the placebo arm. Therefore, despite the trial being the largest, it may not have been adequately powered to detect differences or interactions related to cancer subtype. Additionally, when evaluating breast cancer rates among female subjects who met the primary malignancy endpoints, there was a nonstatistically significant trend towards higher rates of breast cancer in the ezetimibe arm versus the placebo arm (26.4% vs. 18.9%; p = 0.13). The low representation of females in the trial (~25%) leaves a degree of uncertainty whether the observation is truly just an imbalance of the events. Finally, the 2 studies differ in that IMPROVE-IT specifically recruited patients with acute coronary syndrome whereas SEAS recruited patients with mild-to-moderate AS without a history of coronary artery disease. Despite this, it is reassuring that 21 additional months of registry follow-up of the original SEAS cohort demonstrated no increased risk of cancer or related mortality with combined treatment with ezetimibe and simvastatin as compared to placebo (6).

In summary, the IMPROVE-IT study appears to provide confirmatory reassurance that there is no link between ezetimibe and increased cancer risk. Although smaller concerns regarding unknown risk of cancer subtypes may exist, the available data provide no indication to dissuade clinicians from using ezetimibe clinically. Given that lipid-lowering therapy is generally a chronic medication for patients, continued surveillance of drug safety and cancer outcomes in this population is warranted. As new cardiovascular drugs are being introduced to clinics every day, the field of cardio-oncology faces additional challenges to address their oncologic risk beyond the traditional cardiovascular risk of cancer therapies. This is further challenged by difficulties to interpret and apply the results of exploratory safety analyses as they are inherently more prone for errors as seen in the case of SEAS, albeit critically important. Hence, multidisciplinary collaborations between cardiologists, oncologists, and population scientists will be essential to delineate potential cancer risks, optimize interventions to mitigate risk, and identify potential interactions between heart disease and malignancy to maximize survival and quality of life for patients and guidance for shared decisions.

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KEY WORDS cancer, ezetimibe, hyperlipidemia, lipid-lowering therapy, malignancy, statins
Prediction of Lifetime and 10-Year Risk of Cancer in Individual Patients With Established Cardiovascular Disease

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ABSTRACT

BACKGROUND Cardiovascular disease (CVD) and cancer share many common risk factors; patients with CVD also may be at risk of developing cancer.

OBJECTIVES The aim of this study was to derive and externally validate prediction models for the estimation of lifetime and 10-year risk for total, colorectal, and lung cancer in patients with established CVD.

METHODS Data from patients with established CVD from the UCC-SMART cohort (N = 7,280) were used for model development, and from the CANTOS trial (N = 9,322) for model validation. Predictors were selected based on previously published cancer risk scores, clinical availability, and presence in the derivation dataset. Fine and Gray competing risk-adjusted lifetime models were developed for the outcomes total, colorectal, and lung cancer.

RESULTS Selected predictors were age, sex, smoking, weight, height, alcohol use, antiplatelet use, diabetes, and C-reactive protein. External calibration for the 4-year risk of lung, colorectal, and total cancer was reasonable in our models, as was discrimination with C-statistics of 0.74, 0.64, and 0.63, respectively. Median predicted lifetime and 10-year risks in CANTOS were 26% (range 1% to 52%) and 13% (range 1% to 31%) for total cancer; 4% (range 0% to 13%) and 2% (range 0% to 6%) for colorectal cancer; and 5% (range 0% to 37%) and 2% (range 0% to 24%) for lung cancer.

CONCLUSIONS Lifetime and 10-year risk of total, colorectal, and lung cancer can be estimated reasonably well in patients with established CVD with readily available clinical predictors. With additional study, these tools could be used in clinical practice to further aid in the emphasis of healthy lifestyle changes and to guide thresholds for targeted diagnostics and screening. (J Am Coll Cardiol Cardio Onc 2020;2:400–10) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Treatment for cardiovascular disease (CVD) has improved substantially over the past decades, with more patients surviving CVD and living long enough to develop other diseases such as cancer. Besides an increased risk of new cardiovascular events, patients with established CVD have a higher risk of cancer compared with the general population (standardized incidence ratio of 1.19; 95% confidence interval [CI]: 1.10 to 1.29 adjusted for age, sex, and calendar year) (1), most likely due to shared risk factors including obesity, smoking, and low-grade inflammation (2,3). Furthermore, even though CVD is still the leading cause of mortality worldwide among adults, in some higher- and middle-income countries, cancer has become the predominant cause of death, partly due to improved prevention and treatment of CVD (4).

Given one’s absolute individual cancer risk varies, several risk prediction models have been developed to estimate the absolute risk for incident cancer of a specific type, notably lung cancer and breast cancer (5–9). However, no prediction models are available for patients with established CVD specifically. Furthermore, from a patient’s perspective, risk of any cancer might be a more relevant metric, and no risk prediction models estimate total cancer risk. Furthermore, classic risk prediction models estimate prognosis in terms of absolute 5- or 10-year risk of cancer, and may not identify those patients who have a relatively low 5- or 10-year absolute risk, but a high cumulative lifetime risk (10). Finally, traditional 10-year risk prediction scores often do not consider the competing risk of noncancer mortality, and are prone to several types of bias (11). Especially in a population of patients with established CVD, the competing risk of noncancer mortality including cardiovascular death should be taken into account to prevent overestimation of cancer risk.

Estimating individualized probabilities could help in patients’ and clinicians’ understanding of cancer risk. As several modifiable risk factors are related to cancer (2) as well as to CVD, discussing these cancer risks with patients could potentially aid in emphasizing healthy lifestyle changes, such as smoking cessation or weight loss. The aim of the current study was to develop and externally validate prediction models to estimate the 10-year and lifetime risk for total, colorectal, and lung cancer in patients with established CVD.

METHODS

STUDY POPULATIONS. Model development was conducted in the UCC-SMART (Utrecht Cardiovascular Cohort–Second Manifestations of ARTerial disease) study, an ongoing prospective cohort study, including 18- to 79-year-old patients referred to the University Medical Center Utrecht with clinically manifest vascular disease or atherosclerotic risk factors. The cohort was initiated in 1996 and is still recruiting patients annually. For the current study 7,280 patients age 45 to 80 years with clinically manifest vascular disease and who gave permission for data requests to other medical authorities were included.

External model validation was performed in the CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study; NCT01327846), a double-blind, placebo-controlled, randomized clinical trial, that included 10,061 participants with a myocardial infarction at least 1 month before study entry and elevated C-reactive protein (CRP) concentration (≥2 mg/l). Eligible patients were randomized to receive either placebo or canakinumab at a dose of 50 mg, 150 mg, or 300 mg (12). For the current study 9,322 patients were included, after exclusion of patients younger than 45 years or older than 80 years. Detailed descriptions of the UCC-SMART cohort and the CANTOS trial have been published elsewhere (12–14). The studies were approved by institutional review boards and all participants provided written informed consent. An overview of eligibility criteria is provided in Supplemental Table 1.

Ingelheim, Amphera, AstraZeneca, Takeda, and Roche; and own stock in Amphera. Dr. Asselbergs is supported by UCL Hospitals NIHR Biomedical Research Centre. All other authors have reported that they have no relationships relevant to the contents of this manuscript.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the JACC: CardioOncology author instructions page.

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OUTCOMES. During follow-up, participants enrolled in the UCC-SMART cohort received biannual questionnaires, gathering information on occurrence of recurrent CVD, bleeding events, incident diabetes mellitus, and end-stage renal disease. Additional information was collected from the hospital or general practitioner’s records. An endpoint committee of 3 physicians adjudicated all clinical events independently and conflicting classifications were resolved through consensus. For data on cancer incidence, the UCC-SMART database was linked to the Dutch National Cancer Registry (INKL), a national registry receiving notifications of all new cancer diagnoses in the Netherlands through the Nationwide Network and Registry of Histopathology and Cytopathology, and hospital discharge diagnoses.

Participants in the CANTOS trial were followed for incident CVD as well as cancer diagnoses. Even though the primary endpoint of the trial was CVD incidence, patients’ records were investigated for cancers reported during follow-up, as prespecified in the trial safety monitoring plan. Incident cancer reports were adjudicated by an endpoint committee of oncologists, blinded to treatment allocation (15). An overview of cancer diagnoses during follow-up for both study populations is provided in Supplemental Tables 2 and 3. For the current study, total cancer was defined as any invasive neoplasm, excluding nonmelanoma skin cancer. As lung and colorectal cancer are the most common (not sex specific) cancers worldwide (16), these were chosen as separate outcomes. For the endpoint of total cancer, only first diagnoses of cancer were counted. For lung and colorectal cancer, the first diagnosis of that particular cancer type was included, possibly being the second or third primary diagnosis of cancer for a certain patient during follow-up.

DATA PREPARATION AND PREDICTOR SELECTION. Missing data (per variable ≤1.1% for UCC-SMART and ≤0.2% for CANTOS) were singly imputed by weighted probability matching using multivariable regression for the baseline and outcome data. Complete case analysis yielded similar model coefficients. Continuous variables were truncated at the 1st and 99th percentile to limit the effect of outliers on the model coefficients (i.e., leverage) (17). To prevent overfitting, predictors were preselected based on presence in previously published risk prediction models of multiple cancer types. Antiplatelet use (aspirin, P2Y12-ADP receptor antagonist, or other, such as dipyridamole) was added as a predictor, due to its inclusion in multiple previously published prediction models for colorectal cancer and due to the common use of antiplatelet therapy in patients with CVD. Furthermore, it was required that the variables were readily clinically available, as well as present in the derivation dataset. This led to the following predictors: age, sex, smoking status, weight, height, alcohol use, use of antiplatelet medication, and diabetes mellitus (Supplemental Table 4 details an overview of predictor selection). In addition, CRP was added as a predictor after a literature search for predictors of cancer was performed (3,15,18,19). Definitions of the predictors in the UCC-SMART cohort and CANTOS trial are provided in Supplemental Table 5.

DEVELOPMENT OF A PREDICTION MODEL FOR TOTAL CANCER, COLORECTAL CANCER, AND LUNG CANCER. Methods have been described in detail previously (10,11). Three separate complementary Fine and Gray competing risk-adjusted subdistribution hazard functions (20,21) were developed in the UCC-SMART cohort for 10-year and lifetime risk predictions of: 1) total cancer; 2) colorectal cancer; and 3) lung cancer, with consideration for the competing risks of: 1) noncancer death; 2) non–colorectal cancer death; and 3) non-lung cancer death, respectively. As the endpoints colorectal and lung cancer included potential second or third primary diagnoses of cancer for a particular patient, the competing risks for these outcomes did not include other cancer types. The models were developed with left truncation: age rather than follow-up time was used as the underlying time scale. This way, patients contributed person-years between age at study entry and age at study exit, resulting in overlapping observations that allow for lifetime predictions across the range of baseline ages. Because a limited number of patients and events in certain age groups led to instability of predictions, the age range at baseline was restricted to 45 to 80 years.

The proportional hazards assumption was assessed visually by plotting scaled Schoenfeld residuals against time, and interactions with age (underlying time scale) were added to the model when a violation was observed. Log and quadratic associations between continuous predictors and the outcome variable were assessed by comparing model fit based on Akaike’s Information Criterion (AIC) (17), and transformations were applied when appropriate to improve robustness of the model. AICs of models with and without addition of CRP as a predictor were compared to assess differences in model fit. Coefficients of the predictors were adjusted to account for optimism using a shrinkage factor acquired by bootstrapping with 1,000 bootstrap samples.
INDIVIDUAL CANCER RISK PREDICTIONS. Individual 10-year and lifetime risk of total, colorectal, and lung cancer, as well as life expectancy without cancer were estimated using the respective models. These predictions were derived from an individual lifetable with 1-year time intervals (22). First, starting at the baseline age for each patient, the risk of the event of interest (a) and the risk of the competing event (b) was calculated for each following life-year. Next, for each subsequent age year the probability of being healthy and alive at the start of that time interval (age year) \( e_{t+1} \) was calculated by multiplying the survival probability \( e_t \) by the event-free survival probability during that year \( 1 - a_t - b_t \). These steps were repeated from the age at baseline of an individual patient to the maximum age of 90 years, and together these predictions form an individual lifetable (10,23). The cancer-free life expectancy was determined as the age at which the median estimated cancer-free survival curve is 50%. For 10-year and lifetime risk of cancer, the cumulative cause-specific risks were truncated at 10 years after the age at baseline, and at the age of 90 years, respectively.

INTERNAL AND EXTERNAL VALIDATION OF VALIDATION OF THE MODELS. Internal validation of the total cancer, colorectal cancer, and lung cancer models was performed at 10 years of follow-up in the UCC-SMART data. External validation of the total, colorectal, and lung cancer models was evaluated in outcome data from the CANTOS trial at 4 years of follow-up (approximation of the median follow-up time in the CANTOS trial) by implementing the 4-year baseline hazard from the derivation dataset (UCC-SMART). To adjust for treatment effects of canakinumab, hazard ratios of treatment effects of canakinumab on cancer outcomes and their competing mortality were determined and added to the respective models. Discrimination was assessed using Harrell’s c-statistic for survival data, and goodness of fit was assessed by calibration plots of the predicted versus observed risks. For the calibration plot, patients were divided into equal groups of increasing predicted risk. Based on the number of events, patients were divided into 10 equal groups for the total cancer model, and patients were divided into 6 equal groups for the colorectal and lung cancer models. Observed risks were estimated in these groups by using a cumulative incidence function, accounting for competing risks. Recalibration was performed based on the expected to observed ratio. Predicted risks in the CANTOS trial were estimated after recalibration. The Brier score was calculated for 4-year predictions in CANTOS, with confidence intervals based on the percentile method with 1,000 bootstrap samples with replacement.

For comparison, simple models for total, colorectal, and lung cancer with sex and smoking status as the only predictors and with age as the underlying time scale were developed in the UCC-SMART study and externally validated in the CANTOS study population by the same methodology.

All analyses were performed in R statistical software, version 3.5.1, for model development, and 3.6.0. for external validation analyses (packages Hmisc, rms, cmprsk, car). To facilitate the use of this model in clinical practice, an online calculator will be developed.

RESULTS

Baseline characteristics of the UCC-SMART and CANTOS study populations are shown in Table 1. During a median follow-up time of 8.1 years (interquartile range 4.5 to 12.1 years), a total number of 1,143 first cancers were diagnosed in patients enrolled in the UCC-SMART cohort. Lung cancer occurred in 258 patients and colorectal cancer in 180 patients. Incidence rates for total cancer and noncancer mortality as a competing event were 1.97 (95% CI: 1.85 to 2.08) and 1.91 (95% CI: 1.80 to 2.02) per 100 person-years, respectively. Median follow-up time of the CANTOS trial was 3.8 years (interquartile range: 3.2 to 4.5 years), during which a total number of 509 incident cancers were diagnosed, 123 lung cancers, and 72 colorectal cancers. Incidence rates of total cancer and noncancer mortality were 1.48 (95% CI: 1.35 to 1.61) and 2.21 (95% CI: 2.05 to 2.37), respectively. An overview of incidence rates is shown in Supplemental Table 6.

DEVELOPMENT OF LIFETIME RISK PREDICTION MODELS FOR COLORECTAL, LUNG, AND TOTAL CANCER IN UCC-SMART. Results of model development are shown in Supplemental Tables 7 to 10. Transformations of continuous predictors, and interactions with age for continuous as well as categorical predictors are shown in Supplemental Table 7. Age-specific baseline survival is shown in Supplemental Table 8. Subdistribution hazard ratios and shrinkage factors are shown in Supplemental Table 9, and model formulas of the total cancer, colorectal cancer, and lung cancer models are provided in Supplemental Table 10. The AIC was lower for total cancer, colorectal cancer, and lung models with CRP compared with the same model without CRP.

INTERNAL AND EXTERNAL VALIDATION OF TOTAL, COLORECTAL, AND LUNG CANCER MODELS. Internal validation showed good agreement between the predicted and observed 10-year risk for total,
TABLE 1  Baseline Characteristics of UCC-SMART and CANTOS Study Populations

|                         | UCC-SMART  | CANTOS      |
|-------------------------|------------|-------------|
|                         | (N = 7,280)| (N = 9,322) |
| Male                    | 5,470 (75)| 6,869 (74) |
| Age, yrs                | 62 ± 9    | 62 ± 8     |
| Former smoking          | 3,582 (49)| 4,437 (48) |
| Current smoking         | 2,146 (29)| 2,197 (24) |
| Alcohol consumption >0  | 3,850 (53)| 1,654 (18) |
| Alcohol consumption >10 | 2,173 (30)| 1,124 (12) |
| Medical history         |           |             |
| Cerebrovascular disease | 2,128 (29)| 712 (8)    |
| Coronary heart disease  | 4,530 (62)| 9,322 (100)|
| Peripheral vascular disease | 1,300 (18)| 844 (9)  |
| Diabetes mellitus       | 1,321 (18)| 3,829 (41) |
| Physical examination and laboratory measurements | | |
| Body mass index, kg/m²  | 27 ± 4    | 31 ± 6     |
| Systolic blood pressure, mm Hg | 140 ± 20 | 130 ± 16 |
| Diastolic blood pressure, mm Hg | 81 ± 11 | 78 ± 9  |
| LDL cholesterol, mmol/l | 2.7 (2.1-3.5)| 2.1 (1.7-2.8)|
| C-reactive protein, mg/l | 2.0 (0.9-4.4)| 4.2 (2.8-7.1)|
| Creatinine, μmol/l      | 91 ± 23   | 86 ± 29    |
| Medication              |           |             |
| Lipid-lowering medication | 5,038 (69)| 8,711 (93) |
| Blood pressure-lowering medication | 5,549 (76)| 7,591 (81)|
| Antiplatelet therapy    | 5,652 (78)| 8,488 (91) |
| Anticoagulants          | 816 (11)  | 718 (8)    |

Values are n (%), mean ± SD, or median (25th and 75th percentile).
CANTOS = Canakinumab Anti-Inflammatory Thrombosis Outcomes Study; LDL = low density lipoprotein; UCC-SMART = Utrecht Cardiovascular Cohort-Second Manifestations of ARterial disease.

Compared with a simple model with sex and smoking status as the only predictors and with age as the underlying time scale, the full model had a better fit according to the likelihood ratio tests for total and lung cancer (p = 0.005 and p < 0.001, respectively). For the colorectal cancer model, the full model did not improve model fit (p = 0.174). Although the C-statistics of the simple models in CANTOS were similar or even slightly higher; 0.65; 95% CI: 0.62 to 0.67 for total cancer, 0.65; 95% CI: 0.62 to 0.66 for colorectal cancer, and 0.74; 95% CI: 0.70 to 0.79 for lung cancer, and although calibration was similar for colorectal and lung cancers, calibration was worse for colorectal cancer and for the competing risks (Supplemental Figure 5). As calibration is a more clinically relevant performance measure for risk prediction accuracy than the C-statistic (24), the full model for total cancer was considered superior. As all predictors are needed for estimations of total cancer risk, the advantage of a simple model with a limited number of predictors was no longer relevant, and full models were used for risk predictions of total, colorectal, and lung cancer.

PREDICTED 10-YEAR AND LIFETIME RISK OF CANCER.
Median predicted absolute 10-year risks were 13% (range 1% to 31%) for total cancer, 2% (range 0% to 6%) for colorectal cancer, and 2% (range 0% to 24%) for lung cancer in the CANTOS study population. In the UCC-SMART study population, predicted 10-year risks were 16% (range 2% to 33%) for total cancer, 2% (range 0% to 5%) for colorectal cancer, and 2% (range 0% to 20%) for lung cancer. Median predicted absolute lifetime risks were 26% (range 1% to 52%) for total cancer, 4% (range 0% to 13%) for colorectal cancer, and 5% (range 0% to 37%) for lung cancer in the CANTOS study population. In the UCC-SMART study population, median predicted absolute lifetime risks were 35% (range 2% to 59%) for total cancer, 5% (range 0% to 11%) for colorectal cancer, and 7% (range 0% to 32%) for lung cancer. Median predicted 10-year and lifetime risks per age group with a 5-year interval for the UCC-SMART and CANTOS study populations are provided in Supplemental Table 11. The distribution of lifetime risks for total, colorectal and lung cancer for UCC-SMART and CANTOS study populations is shown in Figures 2A to 2C.

As an example, for a 50-year-old man with average values of UCC-SMART for all other predictors, his predicted lifetime risk of total cancer is 48% if he is a current smoker, 45% if he is a former smoker, and 35% if he has never smoked. The predicted lifetime risks of colorectal cancer for this 50-year-old male are 6% (current smoker), 7% (former smoker), and 6% (never smoker). This 50-year-old male has a predicted lifetime risk of lung cancer of 18% if he is a smoker,
Calibration plots are shown of the predicted versus observed 4-year risk of total (A), colorectal (B), and lung cancer (C) in the CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) study population, before and after recalibration. The study population is divided into quantiles based on the predicted risk, and ordered according to increasing predicted risk. The diagonal dotted line represents perfect calibration.
The present study demonstrates that the lifetime and 10-year risk of total, colorectal, and lung cancer can be estimated reasonably well in individual patients with established CVD (Central Illustration). Although discrimination was moderate with C-statistics of 0.63 to 0.74, calibration of the total, colorectal, and lung cancer models was reasonable. Given the wide distribution of predicted lifetime risks for total cancer and lung cancer (Figures 2A to 2C), these models can enable the identification of patients at the highest risk for cancer. Innovative and notable aspects of our work include the applicability to patients with established CVD specifically; the relative ease of use with readily clinically available predictors; the prediction of the combined endpoint total cancer; the external validation; and the estimation of lifetime risks with adjustment for competing risks.

Several risk prediction models with clinical predictors have previously been published for specific types of cancer, including lung (5,6), colorectal (6,25–28), and breast (6–9) cancers. None of these models were developed for patients with established CVD specifically, even though these patients are at higher risk for total and lung cancer compared with the general population, with standardized incidence ratios of 1.19 (95% CI: 1.10 to 1.29) for total cancer and 1.56 (95% CI: 1.31 to 1.83) for lung cancer (1), due to similar risk factors for CVD and cancer (2). Furthermore, the endpoint total cancer has a different distribution of cancer types in patients with established CVD (1), and patients with established CVD are at higher risk for the competing risks (i.e., dying from CVD) compared with the general population (29), emphasizing the need for a prediction model in patients with established CVD specifically. It has even been hypothesized that CVD itself influences cancer development, for example through cardiac excreted factors in heart failure (30,31), potentially leading to a higher baseline risk independent of traditional risk factors. Even though cancer is a very heterogeneous disease and prognoses are divergent for the various cancer types, from a patient’s perspective, risk of any cancer is relevant, with respect to the potential mortality and morbidity associated with the malignancy, frequent hospital visits, demanding treatments (32), and psychological distress (33,34). Furthermore, in patients with CVD, specific cancer types are more common, including cancers of the respiratory tract (1), leading to restricted variation in cancer types.

Our cancer prediction models performed reasonably well, and calibration plots before and after

10% if he is a former smoker, and 4% if he is a never smoker. In order to facilitate risk predictions in clinical practice, the prediction model is available in the Supplemental Appendix.
recalibration were similar. Only lung cancer risk was slightly underestimated in the CANTOS population before recalibration, probably due to variations in smoking habits, or genetic factors causing a higher baseline risk. The higher discriminative power of the lung cancer model (C-statistic 0.74) compared with the total and colorectal cancer models (C-statistics 0.63 and 0.64, respectively), is possibly due to the strong relation between the predictor smoking status and lung cancer. For the prediction of lung and colorectal cancer, a simple model with just age, sex, and smoking status could be sufficient; however, for total cancer and the competing risks, the full model was necessary to achieve the most accurate predictions. For lung cancer, even though the calibration plot showed a 4-year risk of ±3% in the highest risk group, the model allowed for a widespread lifetime risk distribution, assigning lifetime risks up to 37% to a small proportion of patients. As young patients generally have a low 10-year risk of cancer, despite...
high-risk factor levels, lifetime risk predictions might provide more accurate estimations of their “true” risk. The lifetime risk of cancer estimated by the total cancer model ranged from 1% to 52%, enabling identification of patients at the highest risk. Median predicted risks for total cancer were higher in the UCC-SMART study population, corresponding with a higher observed incidence rate for total cancer (1.97 vs. 1.48 per 100 person-years), most likely due to more current smokers in UCC-SMART compared with CANTOS (29% vs. 24%). The distribution of colorectal cancer risk predictions is slightly limited, possibly partly due to absence of family history of colorectal cancer as a predictor in the model, and this model might be less appropriate for selecting patients at very high risk for colorectal cancer.

C-reactive protein was included in the risk prediction models based on previous observational research showing a relation between CRP and incident (lung) cancer (3,18,19), and based on results from the CANTOS trial demonstrating that lowering inflammation with an IL-1β inhibitor lowered the incidence of lung cancer and lung cancer mortality (15). Implementing CRP as a marker of low-grade inflammation in risk scores for determining cancer risk could lead to more accurate predictions. In current models for total, colorectal, and lung cancer, CRP improved model fit based on the AIC. Previous research has shown that CRP improved discrimination in a prediction model for lung cancer in the general population, but only for diagnoses within the first 2 years after measuring CRP (18). In the current models for total and lung cancer, an interaction with age resulted in a higher coefficient of CRP with increasing age, potentially representing a higher predictive value of CRP closer to cancer diagnosis.

There are multiple potential applications of this work, which each require further study. Personalized risk assessment is considered informative and motivational by patients (35), and effective risk communication can lead to changes in behavior (36). Although observed effects of personalized risk communication on healthy behavior changes have been small and evidence is inconsistent (37), effects are dependent on risk information (36). Lifetime risk predictions for cancer, especially in patients at a younger age, could potentially aid in discussions on the importance of healthy lifestyle habits, including smoking cessation. Future prospective studies are needed to evaluate lifestyle improvements and clinical outcomes in patients at high risk for cancer identified by these current models. Moreover, we hypothesize that these models could be used to further inform screening. Results from a recent lung cancer screening trial (NELSON [Nederlands-Leuvens Longkanker Screenings Onderzoek]) showed that screening for lung cancer could reduce lung cancer mortality in men (cumulative rate ratio for death from lung cancer at 10 years of 0.76; 95% CI: 0.61 to 0.94) (38). The NELSON trial included 50- to 74-year-old current or former smokers who had smoked more than 15 cigarettes a day for more than 25 years or more than 10 cigarettes a day for more than 30 years, and showed a 10-year risk for lung cancer of approximately 6% in the screening group (incidence rate of 5.58 cases per 1,000 person-years) (38). Similarly, it could be hypothesized that patients with stable CVD with a high 10-year predicted risk of lung cancer may benefit from screening computed tomography imaging of the chest. A predicted 10-year lung cancer risk of 6% (close to the 90th percentile in CANTOS) that corresponds to the observed risk in the NELSON study, could potentially be used as one threshold. In addition, application of the predicted lung cancer risk could be used to inform thresholds for targeted diagnostics in patients with early symptoms and high predicted 10-year risks, potentially leading to earlier detection and treatment of cancer.

**STUDY STRENGTHS AND LIMITATIONS.** Strengths of the present study include the large study populations for both the derivation and external validation of the cancer risk prediction models. Another important strength is the competing risk-adjusted analyses, preventing overestimation of the event of interest, especially in a population of patients with established CVD. Furthermore, by using age as the underlying time scale in the models, predictions are not limited by follow-up time in the derivation cohort and lifetime predictions are enabled. Last, the prediction model is available in the Supplemental Appendix. Limitations, however, should be considered. These include the smaller number of lung cancer and colorectal cancer in the development and validation study populations. Furthermore, external validation in the CANTOS trial could be performed only up to 4 years due to limited length of follow-up, although internal validation of 10-year predictions in UCC-SMART showed good calibration. Previous studies have shown that lifetime predictions based on the current methodologies provide adequate estimates for up to at least 17 years (10), and the advantage of CANTOS is the large number of patients with CVD and detailed information on incident cancer. C-statistics for the total cancer, colorectal cancer, and lung cancer models were moderate (0.62 to 0.74), comparable to previous cancer risk predictions models (5,7,25) and recurrent CVD risk prediction models in patients with...
established vascular disease (24,39,40). However, evaluation of discrimination with the C-statistic is not optimal in assessing performance of risk prediction models. Calibration is a more clinically relevant performance measure for risk prediction accuracy (24), and calibration of the total, colorectal, and lung cancer predictions models in the CANTOS trial population were all reasonable. Although patients were included in stable phase after a qualifying cardiovascular event, patients potentially changed lifestyle habits, such as smoking, during follow-up, and the single baseline measurement might not reflect such time varying covariates. Last, several potentially important predictors, including level of education, socioeconomic status, race, and family history of cancer were unavailable in the derivation cohort and could not be included in the prediction models, possibly limiting model performance.

**CONCLUSIONS**

Lifetime and 10-year risk of total cancer, colorectal cancer, and lung cancer can be estimated reasonably well with easy clinically available predictors in patients with established CVD. The wide distribution of predicted lifetime risks for total and lung cancer enables identification of patients at the highest risk for cancer. With additional study, the lifetime total and lung cancer models could be used in clinical practice to further promote healthy lifestyle changes, and application of these models, particularly theo-year lung cancer risk model, could potentially lower thresholds for targeted diagnostics and screening.

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**PERSPECTIVES**

**COMPETENCY IN PATIENT CARE:** Patients with established CVD are at higher risk for cancer compared with the general population, due to similar risk factors, such as smoking and obesity. The currently developed and externally validated prediction models estimate the 10-year and the lifetime risks for total, colorectal, and lung cancer in patients with established CVD.

**TRANSLATIONAL OUTLOOK:** Careful consideration of cancer risks in CVD clinical practice could lead to improved patient care by increasing patients’ efforts to improve lifestyle habits, such as smoking cessation, and potentially by lowering thresholds for targeted diagnostics and screening. Prospective studies are needed to evaluate lifestyle improvements and clinical outcomes in patients at high risk for cancer identified by these models.

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APPENDIX For supplemental tables and figures, please see the online version of this paper.

KEY WORDS colorectal cancer, lung cancer, risk prediction
EDITORIAL COMMENT

Praise to Robust Prediction Modeling in Large Datasets*

Ewout W. Steyerberg, PhD, Edouard F. Bonneville, MSc

It is known that patients with cardiovascular disease have a higher risk of cancer compared to the general population; this is likely attributable to the presence of several common risk factors such as smoking. Adequate estimation of absolute individual cancer risk requires the combination of such risk factors in clinical prediction models. In this issue of *JACC: CardioOncology*, a team of international researchers should be applauded for presenting a promising risk calculator for cancer in individual patients with cardiovascular disease (1).

As risk prediction is receiving increasing attention in the current Big Data era, a fundamental question is: Are the predictions valid? Validity of a prediction model may be limited by poor reproducibility (internal validity) and poor transportability (external validity) (2). Prediction models are often developed in relatively small datasets with data hungry methods, that is, methods that only work in very large datasets (3). We see this unfortunate practice in the many prediction models that appear for coronavirus disease 2019 diagnosis and prognosis (4). This current study stands out positively with sensible modeling approaches in carefully collected datasets and large sample sizes with complete follow-up. Impressive numbers are noted in the development dataset (UCC-SMART [Utrecht Cardiovascular Cohort Second Manifestation of Arterial Diseases]: 1,143 incident cancers among 7,280 patients with median follow-up of 8.1 years) and the validation dataset (CANTOS [Canakinumab Anti-inflammatory Thrombosis Outcomes Study]: 509 incident cancers among 9,322 patients with median follow-up of 3.8 years). For specific cancers, numbers are smaller (CANTOS: 123 lung cancers, 72 colorectal cancers). As such, detailed modeling can be difficult, even in overall large datasets. Indeed, some shrinkage of model coefficients was needed, especially for the colorectal cancer prediction model (1). Internal validity of the calculator may be improved with larger numbers of patients, particularly for the lung cancer and colorectal cancer prediction models. Longer follow-up is also important to corroborate the current 10-year and lifetime risk estimates, which are to some extent extrapolations.

An additional threat to validity is the limited transportability due to various sources of heterogeneity between populations. The current models were developed within a broader range of clinically manifest cardiovascular disease, as compared to the validation setting (UCC-SMART: coronary artery disease, cerebrovascular disease, or peripheral artery disease; CANTOS: myocardial infarction and elevated C-reactive protein) (1). The geography also differed (the Netherlands vs. the United States). Nevertheless, adequate performance was found in this rather strong test of validity. Given this, we might be tempted to claim that the predictions from the calculator apply to all patients in the Western world. However, such a strong claim would require further validation. To further support generalizability, additional clinical settings need to be studied, as some form of local updating is often required for prediction models (5). Preferably, extensions are sought to non-Western hospitals, with large numbers of patients, high-quality data, and long-term follow-up. Such extensions may reveal heterogeneity in performance,
motivating locally adapted versions of the calculator, that is, model updating (6).

Many methodological issues in the presented study are dealt with in an exemplary way. The study may serve well for teaching purposes to those interested in predictive analytics. The high-quality data are analyzed with regression techniques that take into account competing risks. Absolute risks of cancer are thus adequately estimated (with cumulative incidence functions) in a context where many die of cardiovascular disease and other causes. Missing data are minimal and statistically imputed. Predictors were pre-selected based on previous literature with easy clinical applicability. Age was used as the timescale, which is natural and allows for 10-year and lifetime prediction horizons. A simple model only considered sex and smoking as additional predictors, whereas a full model added height and weight, C-reactive protein, diabetes mellitus, alcohol use, and antiplatelet use. This robust approach in selecting predictors is in contrast to modern machine-learning approaches, which may explore a wider range of potential predictors with highly flexible functions. Interestingly, with external validation, the more complex model (“full model”) versus the simple model did not demonstrate improved discrimination (1). Two lessons might be drawn. First, the key set of predictors in prediction of cancer may be age, sex, and smoking status. This is in line with other prediction studies where a simple model may generalize better than a more complex model (7). Second, complex models based on machine learning would not be expected to provide improved performance or better prognostication in the current study, consistent with other large-scale external validation studies (8,9).

From a clinical perspective, a key concern should be whether the predictions from the risk calculator are well calibrated (5). For example, the 70-year-old male in the example calculation sheet has a predicted 1.1% risk of lung cancer, while the risk may in fact be 0.5%, or 2%. This type of miscalibration has been common in earlier evaluations of lung cancer prediction models (10). Indeed, the investigators state that “calibration is a more clinically relevant concept than prediction accuracy than the C-statistic.” We should be specific on what “clinically relevant” refers to in this risk prediction context. Predictions may serve to inform patients about their individual risk. The C-statistic is a commonly used measure to indicate discrimination, or how well we can separate low-risk patients from high-risk patients. As the investigators state, this may be less of a concern to individual patients. Beyond informing patients, clinical relevance may refer to decision support, such as selecting patients for lung cancer screening. Prioritizing calibration over discrimination may be an oversimplification because both calibration and discrimination properties of the calculator are relevant to quantify its clinical usefulness. Better discrimination is needed for better decision support than possible with the current models, where the lung cancer prediction model was best with a C-statistic of only 0.74 (1). An increasingly popular summary measure for clinical usefulness is “net benefit.” Net benefit is a classic measure, proposed in 1884 to quantify the quality of predictions (11). It was recently rediscovered and presented through a “decision curve” (12,13), which is unfortunately missing from the current report. Future work should consider the clinical decision-making perspective more fully, with net benefit in a decision curve as a step towards a more comprehensive cost-effectiveness analysis.

In sum, the presented risk calculator is very promising given its high-quality data sources, large numbers, and sensible methodology. Some caution is needed with regard to clinical application, and we await further model validation and the potential need for local updating. Further research is also needed to identify more robust predictors beyond age, sex, and smoking status, which may provide a solid knowledge base when counseling individual patients on their cancer risks and emphasizing healthy lifestyle changes.

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**KEY WORDS** big data, prediction, regression analysis, validation
Diuretic Dose and NYHA Functional Class Are Independent Predictors of Mortality in Patients With Transthyretin Cardiac Amyloidosis

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ABSTRACT

BACKGROUND With increasing diagnoses and available treatment options for transthyretin amyloidosis cardiomyopathy (ATTR-CM), risk stratification of ATTR-CM patients is imperative.

OBJECTIVES We hypothesized that diuretic dose and New York Heart Association (NYHA) functional class are independent predictors of mortality in ATTR-CM and would be incrementally additive to existent risk scores.

METHODS Consecutive ATTR-CM patients referred to a single center were identified. Adjusted Cox proportional hazards models determined the association between diuretic dose (furosemide equivalent in mg/kg) at time of diagnosis and the primary outcome of all-cause mortality. The incremental value of adding diuretic dose and NYHA functional class to existing ATTR-CM risk scores was assessed for discrimination and calibration.

RESULTS 309 patients were identified, with mean age 73.2 ± 9.8 years, 84.1% male, and 66% wild type. Daily mean diuretic dose was 0.6 ± 1.0 mg/kg and significantly associated with all-cause mortality (unadjusted hazard ratio: 2.12 per 1-mg/kg increase, [95% confidence interval: 1.71 to 2.61] and fully adjusted hazard ratio: 1.43 [95% confidence interval: 1.06 to 1.93]). Testing previously published ATTR risk scores, adding diuretic dose as categories (0 mg/kg, >0 to 0.5 mg/kg, >0.5 to 1 mg/kg, and >1 to 2 mg/kg) improved the area under the curve of the Mayo risk score from 0.693 to 0.767 and the UK risk score from 0.711 to 0.787 while preserving calibration. Adding NYHA functional class further improved the area under the curve to 0.798 and 0.816, respectively.

CONCLUSIONS Diuretic dose and NYHA functional class are independent predictors of mortality in ATTR-CM patients and provide incremental value to existing ATTR-CM risk scores. (J Am Coll Cardiol CardioOnc 2020;2:414–24) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Transthyretin amyloid cardiomyopathy (ATTR-CM) is being increasingly diagnosed, secondary to growing clinical recognition, the emergence of noninvasive methods to confirm ATTR-CM such as nuclear scintigraphy, and the availability of treatment with transthyretin tetramer stabilizers (1). The ability to accurately risk stratify these patients is essential for guiding clinical care and treatment options.

There are 2 commonly used risk models for the ATTR-CM population. The Mayo risk model includes the cardiac biomarkers N-terminal pro-B-type natriuretic peptide (NT-proBNP) and troponin-T, stratifying wild-type ATTR-CM (wtATTR) patients into 3 stages (2). The UK risk model includes both wtATTR and hereditary ATTR-CM (hATTR) patients, and uses NT-proBNP and estimated glomerular filtration rate (eGFR) (3). However, neither model incorporates well-established, easily obtained predictors of outcomes in heart failure (HF), including diuretic dose (4–7) and New York Heart Association (NYHA) functional class. Further, the Seattle Heart Failure Model (SHFM) (7) is another widely used risk score that has been validated in other HF cohorts but has not been tested in ATTR-CM.

We hypothesized that diuretic dose and NYHA functional class would be robust predictors of mortality in ATTR-CM. We thus sought to define the associations of diuretic dose and NYHA functional class with all-cause mortality in ATTR-CM and assess whether diuretic dose and NYHA functional class are additive to the existing Mayo and UK ATTR-CM risk models. We applied the SHFM to this cohort to both test and compare its ability to predict risk in ATTR-CM patients.

**METHODS**

Consecutive ATTR-CM patients referred to a single, quaternary care center (Columbia University, New York, New York) between February 2002 and November 2018 were enrolled in a registry. All patients over 18 years of age with either wtATTR or hereditary hATTR were included. Approval for the study was obtained from the Columbia University Irving Medical Center Institutional Review Board. Demographics, clinical characteristics, and laboratory data including diuretic dose and NYHA functional class assessment were obtained at the baseline clinical visit. Patients were followed over time. Outcomes, including death and cardiac transplantation, were adjudicated manually from chart review. The current study is a retrospective cohort analysis of these previously collected data. The date of data lock was August 1, 2019.

Daily loop diuretic dose was converted to furosemide equivalence normalized by body weight, with standard conversion factors of bumetanide 1 mg oral = torsemide 20 mg oral = furosemide 40 mg oral and divided by weight (in kilogram). Diuretic dose was treated as a continuous variable and also categorized into furosemide equivalent dosages of 0, >0 to 0.5, >0.5 to 1, and >1 mg/kg for comparison in risk models and for ease of interpretation. For diuretic dosing in the risk models, and to facilitate comparisons with the Mayo and UK models, 0 points were assigned for 0 mg/kg, 1 point for >0 to 0.5 mg/kg, 2 points for >0.5 to 1 mg/kg, and 3 points for >1 mg/kg. NYHA functional class was obtained using the standard convention and assigned 1 point per NYHA functional class, respectively, ranging from 1 to 4 points.

Current ATTR-CM risk stratification models are from the Mayo Clinic (2) and the UK data (3). The Mayo classification identified elevated NT-proBNP >3,000 pg/ml and troponin-T >0.05 ng/ml as risk factors. The UK classification used an elevated NT-proBNP >3,000 pg/ml and decreased eGFR <45 ml/min/1.73 m². As our patients had a combination of BNP and NT-proBNP at baseline, we estimated a BNP cutoff >600 pg/ml to correspond to NT-proBNP >3,000 pg/ml (on the basis of a commonly used 5- to 6-fold estimated conversion in clinical settings) (8). Similarly, there was a combination of troponin-T and troponin-I assays at baseline. We chose a troponin-I cutoff of >0.1 ng/ml to correspond to troponin-T >0.05 ng/ml; this was based on the Boston University analysis on light chain amyloid patients for equivalence between troponin-I and troponin-T (9). All patients had eGFR estimates at baseline. For the Mayo risk score, patients were assigned 1 point for either elevated NT-proBNP or BNP and 1 point for elevated troponin-T or troponin-I, for a range of 0 to 2 points. For the UK risk score, patients were assigned 1 point for either elevated NT-proBNP or BNP and 1 point for eGFR <45 ml/min/1.73 m², for a range of 0 to 2 points.

We additionally evaluated the SHFM model to provide a separate comparator with a well-established risk tool used in other HF cohorts. SHFM includes age, sex, ejection fraction, systolic blood pressure, weight, NYHA functional class, etiology, medications (diuretic dose, allopurinol, statins,

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**ABBREVIATIONS AND ACRONYMS**

ATTR = transthyretin amyloidosis

ATTR-CM = transthyretin amyloid cardiomyopathy

AUC = area under the curve

CI = confidence interval

eGFR = estimated glomerular filtration rate

hATTR = hereditary transthyretin amyloidosis

HF = heart failure

HR = hazard ratio

NRI = net reclassification index

NT-proBNP = N-terminal pro-B-type natriuretic peptide

NYHA = New York Heart Association

SHFM = Seattle Heart Failure Model

wtATTR = wild-type transthyretin amyloidosis
Diuretics and NYHA Functional Class in ATTR-CM

TABLE 1 Baseline Characteristics by Diuretic Dose

| Diuretic Dose | Total (N = 309) | 0 mg/kg (n = 92) | >0 to 0.5 mg/kg (n = 95) | >0.5 to 1 mg/kg (n = 70) | >1 mg/kg (n = 52) |
|---------------|----------------|------------------|--------------------------|--------------------------|------------------|
| Age, yrs*     | 73.2 ± 9.8     | 70.1 ± 12.2      | 75.1 ± 7.1               | 74.2 ± 9.2               | 74.1 ± 8.7       |
| Male *        | 84.1           | 77.2             | 92.6                     | 81.4                     | 84.6             |
| Race*         |                |                  |                          |                          |                  |
| White         | 72.5           | 79.3             | 80.0                     | 60.0                     | 63.5             |
| Black         | 23.6           | 18.5             | 16.8                     | 31.4                     | 34.6             |
| Other         | 3.9            | 2.2              | 3.2                      | 8.6                      | 1.9              |
| ATTR type*    |                |                  |                          |                          |                  |
| Wild-type     | 66.0           | 56.5             | 84.2                     | 61.4                     | 55.8             |
| Hereditary    | 34.0           | 43.5             | 15.8                     | 38.6                     | 44.2             |
| Height, cm*   | 172.9 ± 8.9    | 171.6 ± 9.1      | 176.0 ± 8.1              | 171.5 ± 9.2              | 171.5 ± 8.2      |
| Weight, kg*   | 78.8 ± 13.7    | 76.1 ± 14.3      | 84.4 ± 12.2              | 78.4 ± 14.7              | 73.8 ± 9.8       |
| BMI, kg/m²*   | 26.5 ± 4.7     | 25.7 ± 3.9       | 27.4 ± 4.8               | 27.0 ± 6.0               | 25.3 ± 3.6       |
| SBP, mm Hg*   | 115.7 ± 16.3   | 122.4 ± 16.2     | 118.3 ± 15.9             | 109.5 ± 13.9             | 108.0 ± 14.4     |
| DBP, mm Hg*   | 70.4 ± 9.7     | 73.7 ± 9.6       | 72.0 ± 10.0              | 68.0 ± 8.0               | 65.1 ± 8.4       |
| Heart rate, beats/min | 75.2 ± 13.3 | 74.6 ± 12.2 | 74.3 ± 13.7 | 75.9 ± 14.3 | 77.4 ± 13.2 |
| NYHA functional class* |              |                  |                          |                          |                  |
| I             | 9.4            | 27.2             | 4.2                      | 0.0                      | 0.0              |
| II            | 45.3           | 53.3             | 56.8                     | 34.3                     | 25.0             |
| III           | 41.7           | 17.4             | 37.9                     | 64.3                     | 61.5             |
| IV            | 3.6            | 2.2              | 1.1                      | 1.4                      | 13.5             |
| Prevalent atrial fibrillation/flutter‡ | 17.2         | 10.7             | 12.1                     | 20.9                     | 32.7             |
| Creatinine, mg/dl‡ | 1.3 ± 0.5    | 1.1 ± 0.4        | 1.3 ± 0.4                | 1.6 ± 0.7                | 1.5 ± 0.6        |
| eGFR, ml/min/1.73 m²‡ | 60.1 ± 22.6   | 73.3 ± 25.5      | 58.6 ± 17.5              | 51.2 ± 20.4              | 52.2 ± 18.4      |
| BNP or NT-proBNP elevated†† | 40.1          | 19.5             | 38.4                     | 56.5                     | 55.9             |
| Troponin-I or troponin-T elevated†† | 37.8          | 15.6             | 35.6                     | 55.6                     | 57.1             |
| LVEF, %*      | 45.1 ± 15.1    | 51.8 ± 12.8      | 44.3 ± 15.1              | 39.4 ± 15.7              | 42.2 ± 14.4      |
| Lasix dose, mg/kg* | 0.6 ± 1.0     | 0.0 ± 0.0        | 0.3 ± 0.1                | 0.7 ± 0.2                | 1.7 ± 0.8        |
| SHFM score*   | 1.0 ± 0.8      | 0.6 ± 0.7        | 0.8 ± 0.6                | 1.2 ± 0.7                | 1.6 ± 0.8        |
| HF GDMT       |                |                  |                          |                          |                  |
| ACE inhibitor/ARB | 31.7          | 21.7             | 38.9                     | 34.3                     | 32.7             |
| Beta-blocker* | 49.8           | 38.0             | 57.9                     | 51.4                     | 53.8             |
| MRA*          | 23.3           | 11.2             | 22.3                     | 32.9                     | 31.4             |

Values are mean ± SD or %.*p < 0.05 across diuretic categories. †Defined as NT-proBNP >3,000 pg/ml or BNP >600 pg/ml. ‡Defined as troponin-T >0.05 ng/ml or troponin-I >0.1 ng/ml.

ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; ATTR = transthyretin amyloidosis; BMI = body mass index; BNP = B-type natriuretic peptide; DBP = diastolic blood pressure; eGFR = glomerular filtration rate; GDMT = guideline directed medical therapy; HF = heart failure; LVEF = left ventricular ejection fraction; MRA = mineralocorticoid receptor antagonist; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; SBP = systolic blood pressure; SHFM = Seattle Heart Failure Model.

renin-angiotensin receptor blockers, beta-blockers, potassium sparing diuretics, biventricular pacing or defibrillators, and laboratory values (sodium, total cholesterol, hemoglobin, lymphocyte percentage, and uric acid) (7). The SHFM model does not include BNP or troponin.

**Statistical Analysis.** Baseline characteristics were compared between the different diuretic groups, as well as Mayo Clinic and UK risk scores 0 to 2. For continuous variables, distributions were assessed for normality, and means were compared using 1-way analysis of variance. All variables were normally distributed. Categorical variables were compared using the chi-square test.

The relationship between each interval increase in diuretic dose category or NYHA functional class was not completely equal. For this reason, we tested weighted models estimated from beta-coefficients for the variables. There was only a modest, nonsignificant gain in discrimination with the more complex weighted scoring system, so we opted to implement the diuretic dose categories and NYHA functional class as a nonweighted increase for simplicity.

Cox proportional hazards models were generated for diuretic dose as a continuous predictor with all-cause mortality as the primary outcome. Patients were censored at time of cardiac transplantation or time of last follow-up. Multivariable models were
TABLE 2  Daily Diuretic Dose as Predictor of All-Cause Mortality and Mortality or Heart Transplantation

| Model       | Hazard Ratio (95% CI) per 1 mg/kg increase (Continuous Scale) | p Value |
|-------------|-----------------------------------------------------------|---------|
| All-cause mortality |                                            |         |
| Unadjusted   | 2.12 (1.71-2.61)                                          | <0.001  |
| Adjusted 1   | 1.80 (1.38-2.34)                                          | <0.001  |
| Adjusted 2   | 1.49 (1.11-2.01)                                          | 0.009   |
| Adjusted 3   | 1.43 (1.06-1.93)                                          | 0.020   |
| Mortality or heart transplantation |                               |         |
| Unadjusted   | 2.09 (1.72-2.54)                                          | <0.001  |
| Adjusted 1   | 1.73 (1.37-2.19)                                          | <0.001  |
| Adjusted 2   | 1.49 (1.15-1.93)                                          | 0.003   |
| Adjusted 3   | 1.35 (1.03-1.77)                                          | 0.031   |

*Adjusted 1 – adjusted for age, sex, SBP, hereditary vs. wild type, LVEF; Adjusted 2 – adjusted for age, sex, SBP, hereditary vs. wild type, LVEF, and also adjusted for eGFR, BNP or NT-proBNP elevation, and troponin I or T elevation; Adjusted 3 – adjusted for age, sex, SBP, hereditary vs. wild type, LVEF, eGFR, BNP or NT-proBNP elevation, and troponin I or T elevation, and also adjusted for NYHA functional class.

CI = confidence interval; other abbreviations as in Table 1.

initially adjusted for age, sex, systolic blood pressure, wtATTR versus hATTR, and left ventricular ejection fraction (LVEF). We additionally tested the model after adjusting for renal function, troponin-I or -T elevation, and for either NT-proBNP or BNP elevation; this included variables in the Mayo and UK ATTR-CM risk models. Third, we adjusted for NYHA functional class in the diuretic model. Analyses were repeated with the composite of death or heart transplantation as a secondary outcome. Data are expressed in the tables as hazard ratio (HR) with 95% confidence intervals. Kaplan-Meier curves are used to display survival probabilities for individual risk markers. The final combined scores for Mayo + diuretic dose + NYHA functional class and UK + diuretic dose + NYHA functional class were subdivided into tertiles and plotted to estimate median survival. Comparisons between groups were made using log-rank statistic.

We tested the discrimination of the various risk models using time-dependent receiver-operating characteristic (ROC) curves with estimation of the time-dependent area under the curve (AUC) at the 2-year time point (10). This time point was chosen due to reported median survival of 2.5 years in hATTR V122I mutation subtype (1). Additionally, given the high early mortality and the contemporary enrollment of patients, the models are less stable over time; hence, the point estimates at 2 years for the models are more accurate than with longer follow-up. To test the robustness of our models, we ran sensitivity analyses calculating Harrell’s c-statistic, which is the weighted average of the time-dependent AUC across all available survival times. The likelihood ratio test was used to compare nested models to determine whether the addition of diuretic dose and/or NYHA functional class improved the model fit to either the Mayo or UK scores. The continuous net reclassification index (NRI), integrated discrimination improvement, and median improvement (11) were used to determine incremental benefit of adding diuretic dose and NYHA functional class to either the Mayo or the UK risk scores. Calibration was tested with a modified Hosmer-Lemeshow statistic for goodness of fit incorporating survival data (12). Internal validation of the predictive accuracy of each model was performed using 1,000 bootstrap samples to estimate optimism-corrected AUCs and 95% confidence interval (CI).

Statistics were performed using a combination of STATA SE 15 (StataCorp LLC, College Station, Texas) and R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). A 2-sided p value <0.05 was considered significant.

RESULTS

A total of 309 ATTR-CM patients were included in this study. The mean follow-up time was 1.92 ± 1.82 years for the total cohort. During this time, 33.3% died and 38.8% died or had cardiac transplantation. Cardiac transplantation occurred in 6.8% of the total cohort. The median survival time was 4.0 years, and the estimated 1-year survival was 87.4% (95% CI: 83.3% to 91.8%) and 5-year survival was 36.7% (28.3% to 47.5%).

For the total cohort, the mean age was 73.2 ± 9.8 years, 84.1% were male, and 72.5% were White; 66.0% had wtATTR and 34.0% had hATTR. For those with hATTR, the most common mutation was V122I (61.0%). The majority of patients were NYHA functional class I to III. Mean eGFR was 60.1 ± 22.6 ml/min/1.73 m², 40.1% had elevated BNP or NT-proBNP, and 37.8% had elevated troponin I or T (Table 1). We had no missing data for diuretic dose or NYHA functional class. For the other variables relevant to the Mayo and UK models, we had BNP data on 95.1% of patients (n = 294), troponin data on 95.8% of patients (n = 296), and eGFR on 95.1% of patients (n = 294). The percentage of missing data for these variables was <5%.

Baseline characteristics stratified by diuretic dose categories are shown in Table 1. Patients on higher doses of diuretic agents were more likely to have hATTR, lower weight, lower systolic blood pressure, lower eGFR, elevated BNP or NT-proBNP, elevated
troponin-I or -T, lower LVEF, and higher NYHA functional class. Characteristics by Mayo stage and UK stage are shown in Supplemental Table 1. Patients with higher Mayo stage were older, had lower weight, lower systolic blood pressure, higher NYHA functional class, lower eGFR, and lower LVEF. Higher UK stage was associated with older age, lower BMI, higher NYHA functional class, and lower LVEF.

Diuretic dose was a strong predictor of all-cause mortality in unadjusted models. It remained significant after consideration of baseline demographics, and after adjusting for BNP, troponin, eGFR, and NYHA functional class (fully adjusted HR: 1.43 per 1 mg/kg increase [95% CI: 1.06 to 1.93]; p = 0.020) (Table 2). Similarly, diuretic dose predicted the secondary outcome of all-cause mortality or heart transplantation (adjusted HR: 1.35 [95% CI: 1.03 to 1.77]; p = 0.031). NYHA functional class was predictive of both all-cause mortality (adjusted HR: 1.85 [95% CI: 1.22 to 2.80]; p = 0.004) and the composite of mortality or transplantation (adjusted HR: 2.02 [95% CI: 1.39 to 2.93]; p < 0.001). Models with the Mayo and UK scores individually are also shown in Supplemental Table 2.

Kaplan-Meier curves were plotted for the Mayo (log-rank p < 0.001) and UK scores (p < 0.001), as well as for diuretic dose (p < 0.001), SHFM (p < 0.001), and NYHA functional class (p < 0.001) for freedom.

(A) Kaplan-Meier curves are shown for diuretic dose categories. (B) New York Heart Association functional class; (C) Mayo risk score categories; (D) UK risk score categories; and (E) Seattle Heart Failure Model (SHFM) score rounded to the nearest integer. For each model, comparison between groups was statistically significant with log-rank p < 0.001.
from all-cause mortality (Figure 1). Additionally, Kaplan-Meier curves were generated for freedom from death or heart transplantation (Supplemental Figure 1).

Diuretic dose alone yielded an AUC of 0.713 (95% CI: 0.673 to 0.779) for all-cause mortality (Table 3). The Mayo model had a baseline AUC of 0.693 (95% CI: 0.609 to 0.777). Adding diuretic dose to the Mayo model improved the AUC to 0.767 (95% CI: 0.692 to 0.843), whereas adding NYHA functional class further increased this to 0.798 (95% CI: 0.729 to 0.868) for Mayo + diuretic dose + NYHA functional class. The UK model had a baseline AUC of 0.711 (95% CI: 0.630 to 0.792) (Table 3). Adding diuretic dose improved the AUC to 0.787 (95% CI: 0.717 to 0.856), and adding NYHA functional class further improved the model to 0.816 (95% CI: 0.749 to 0.883) for UK + diuretic dose + NYHA functional class (Figure 2).

For the final model of Mayo + diuretic dose + NYHA functional class, patients were divided into 3 risk groups (score 1 to 3, 4 to 6, and 7 to 9). The estimated mean survival was 6.5 years for the low-risk group, 4.0 years for the intermediate risk group, and 2.2 years for the high-risk group (log-rank p < 0.001) (Figure 3A). Similarly, for the UK + diuretic dose + NYHA functional class model, survival was 6.5 years, 3.8 years, and 1.9 years for the low-, intermediate-, and high-risk groups, respectively (log-rank p < 0.001) (Figure 3B).

We also tested the ability of the risk scores to predict a combined outcome of mortality or cardiac transplantation (Table 3). The Mayo model had an AUC of 0.685 (95% CI: 0.605 to 0.764), which improved to 0.780 (95% CI: 0.713 to 0.847) when adding diuretic dose and NYHA functional class. The UK model had an AUC of 0.688 (95% CI: 0.608 to 0.767), which improved to 0.791 (95% CI: 0.721 to 0.861) with diuretic dose and NYHA functional class.

We tested SHFM in this cohort because it is widely used in other HF populations. The continuous SHFM score provided an AUC of 0.820 (95% CI: 0.751 to 0.889) for mortality and 0.802 (95% CI: 0.732 to 0.871) for mortality or transplantation. SHFM predicted and observed 1-year survival were similar (88.0% vs. 87.4%), although the 5-year predicted survival was higher than observed (53.7% vs. 36.7%).

To confirm the robustness of our results, we tested Harrell’s c-statistic for the different models to incorporate all available follow-up times (Supplemental Table 3), which confirmed that adding diuretic dose and NYHA functional class to the Mayo or UK risk models substantially increased the c-statistic. Likelihood ratio tests between nested models showed that adding diuretic dose and NYHA functional class to each of the Mayo or UK models improved the models significantly in a stepwise manner (Table 4). Additionally, to further assess the incremental value of adding diuretic dose and NYHA functional class to either the Mayo or UK scores, we assessed integrated discrimination improvement, NRI, and median improvement (Table 5). For all-cause mortality, the addition of diuretic dose and NYHA functional class to both the Mayo and UK scores provided added value compared with either score alone. The results were similar for the combined outcome of mortality or transplantation. The values of the event and nonevent NRI are provided in Supplemental Table 4. Survival-based Hosmer-Lemeshow goodness-of-fit comparisons were nonsignificant across all tested models, suggesting that there were no statistically significant differences between predicted versus observed event rates (Supplemental Table 5). Lastly, we performed internal validation by bootstrapping to calculate the
optimism-adjusted AUCs at 2 years (Table 6). The adjusted AUCs were similar to the AUCs calculated in our sample, suggesting the models were well-validated internally.

**DISCUSSION**

ATTR-CM has increasingly been diagnosed in recent years, due to improved accuracy of noninvasive imaging modalities, enhanced awareness, and development of effective disease-specific treatment options. The spectrum of patients diagnosed ranges from preclinical hATTR mutation carriers to HF with preserved ejection fraction patients presenting with clinical HF, and to those with end-stage disease. It is critical to accurately risk stratify ATTR-CM patients to identify those that need closer clinical follow-up and are more likely to benefit from current and future treatments.

Diuretic dose has been shown to be a strong predictor of mortality in other HF cohorts (4–7,13,14). Putative mechanisms for worse outcomes with loop diuretic agents include activation of the renin-angiotensin-aldosterone and sympathetic nervous systems (15) leading to increases in ventricular filling pressures, decreased glomerular filtration rate due to changes in renal blood flow (16), and exacerbation of arrhythmias (17). For ATTR-CM patients with decreased reserve, increased filling pressures, worsening renal function, and arrhythmias could exacerbate an already precarious condition. It has also been proposed that a higher diuretic dose may serve as an indicator of worse disease severity rather than as a mediator of outcomes (4). ATTR-CM results in a predominantly restrictive HF phenotype, resulting in declines in stroke volume and cardiac output with high right atrial pressures. Hence, a decline in renal perfusion pressure leads to progressive cardio-renal syndrome and is likely a contributor to the higher diuretic requirements over time. The UK model has shown that eGFR is a strong predictor of outcomes in ATTR-CM. Although diuretic dose and eGFR may be interrelated, our analyses show that diuretic dose remains an independent predictor of mortality, even after adjusting for eGFR.

The Mayo Clinic (2) and the UK data (3) risk models are most frequently used in clinical practice. There has, however, been interest in other potential predictors of risk in ATTR-CM. For example, despite the effectiveness of nuclear imaging in the diagnosis of ATTR-CM (18,19), its ability to risk stratify those with confirmed ATTR-CM has yielded discordant findings as to whether or not increased radiotracer uptake is associated with mortality (18,20–22). There has also been interest in the use of echocardiographic-derived parameters including global longitudinal strain, early mitral inflow, deceleration time, myocardial performance index, and stroke volume index as predictors of adverse outcomes; however, there is not, as of yet, any formal staging system that incorporates these parameters for ATTR-CM (18). Cardiac magnetic resonance imaging parameters including native T1, extracellular volume, and the presence and pattern of late gadolinium enhancement have also shown promise as prognostic markers in cardiac amyloidosis, but it requires advanced imaging, and much of the data have been with light chain, rather than ATTR, amyloidosis, with extracellular volume perhaps the most robust predictor in ATTR-CM.
In a comprehensive study that included noninvasive parameters including demographics, laboratory testing, electrocardiography, echocardiography, nuclear scintigraphy, and cardiac magnetic resonance imaging, univariable analysis found that NT-proBNP, troponin-T, mitral annular plane systolic excursion and left ventricular hypertrophy index were predictors of mortality; however, on multivariable analysis, only troponin-T predicted survival (22). A more recent analysis included a combination of light chain and ATTR amyloidosis patients, and evaluated parameters that included demographics, right heart catheterization, echocardiography, and biomarkers. In ATTR-CM, the strongest predictors of all-cause mortality were QRS duration, high-sensitivity troponin-T, and NT-proBNP (24). As far as we know, there have not been attempts to assess diuretic dose or NYHA functional class as predictors of adverse outcomes in ATTR-CM, despite the extensive data for diuretic dose and NYHA functional class as risk factors in other HF populations.

In this study, we validate the previously published Mayo and UK ATTR-CM models in a relatively large ATTR-CM cohort. We confirm that they each have moderate discriminatory ability in their current forms. A recent study comparing the 2 risk models in 175 ATTR-CM patients found that the UK model had better discrimination than the Mayo model (25). Our analysis found similar results, with the UK model performing slightly better than the Mayo model, although the difference was not significant.

We sought to increase the accuracy of risk stratification while keeping the overall model parsimonious with easily obtainable data from the clinical setting. Adding either diuretic dose or NYHA functional class individually resulted in improved discrimination over the Mayo or UK models. Adding both of these variables further improved discrimination. In routine clinical care, diuretic dose and NYHA functional class...
are readily available data points. Adding these easily obtainable parameters resulted in a substantial incremental gain in AUC, increasing the AUC for the Mayo risk model from 0.693 to 0.798 and the UK risk model from 0.711 to 0.813 for all-cause mortality (Central Illustration). The gain in AUC of ~0.10 for both the Mayo and UK models when adding diuretic dose and NYHA functional class supports the use of these variables for clinical decision-making.

Although not the focus of the current study, we did test the SHFM in this cohort, as it is widely used in other HF populations. Despite the model not being developed for ATTR amyloid patients or HF with preserved ejection fraction, it also showed robust discriminatory ability in ATTR-CM with an AUC of 0.820, probably due to inclusion of diuretic dose and NYHA functional class in the SHFM. However, the SHFM is complex and includes many variables, which may not be readily available in a routine clinic visit. Further, the benefit of HF medications including neurohormonal blockade, which is included in the SHFM, has not been proven in the ATTR-CM population. In fact, there is some concern that standard HF guideline directed medical therapies for reduced LVEF, such as beta-blockers, may be detrimental in this population. We find that the much simpler Mayo or UK models, after the addition of diuretic dose and NYHA functional class, was similar in discriminatory ability to the full SHFM despite containing fewer variables and being more readily accessible.

ATTR-CM is an emerging disease with increasing recognition over the last decade. As more patients are diagnosed, accurate risk stratification will become more important. Currently, the Mayo and UK risk models provide patients with an estimate of their prognosis. The addition of diuretic dose and NYHA functional class to these models offers incremental insight into disease severity. Given the recently observed difference in benefit of treating ATTR-CM for NYHA functional class I to II versus class III with the ATTR-ACT (Tafamidis in the Transthyretin Amyloidosis Cardiomyopathy Clinical Trial) (26), better understanding of disease progression is needed to guide therapeutic decision-making. There may be a “point of no return” due to progressive ATTR amyloid

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**TABLE 5** Continuous IDI and NRI

|                     | IDI† (95% CI) | p Value | NRI‡ (95% CI) | p Value | Median Improvement† (95% CI) | p Value |
|---------------------|--------------|---------|--------------|---------|-----------------------------|---------|
| 2-yr all-cause mortality |              |         |              |         |                             |         |
| Mayo vs. Mayo + diuretic dose | 0.07 (-0.00 to 0.13) | 0.073 | 0.68 (-0.08 to 0.96) | 0.12 | 0.08 (0.00 to 0.20) | 0.047 |
| Mayo + diuretic dose vs. Mayo + diuretic + NYHA functional class | 0.05 (0.01 to 0.07) | <0.001 | 0.86 (0.36 to 1.12) | <0.001 | 0.06 (0.01 to 0.09) | <0.001 |
| Mayo vs. Mayo + diuretic dose + NYHA functional class | 0.10 (0.04 to 0.19) | <0.001 | 0.70 (0.38 to 1.02) | <0.001 | 0.13 (0.05 to 0.25) | <0.001 |
| UK vs. UK + diuretic dose | 0.07 (-0.01 to 0.14) | 0.027 | 0.46 (-0.10 to 0.98) | 0.126 | 0.12 (-0.05 to 0.26) | 0.146 |
| UK + diuretic dose vs. UK + diuretic + NYHA functional class | 0.05 (0.02 to 0.09) | 0.013 | 0.88 (0.34 to 1.12) | 0.007 | 0.07 (0.02 to 0.11) | 0.007 |
| UK vs. UK + diuretic dose + NYHA functional class | 0.12 (0.04 to 0.19) | <0.001 | 0.66 (0.26 to 1.02) | <0.001 | 0.15 (0.01 to 0.28) | 0.027 |
| 2-yr mortality or cardiac transplantation |              |         |              |         |                             |         |
| Mayo vs. Mayo + diuretic dose | 0.06 (0.01 to 0.12) | 0.020 | 0.64 (0.00 to 0.90) | 0.047 | 0.08 (0.00 to 0.16) | 0.027 |
| Mayo + diuretic dose vs. Mayo + diuretic + NYHA functional class | 0.05 (0.01 to 0.08) | <0.001 | 0.84 (0.44 to 1.10) | <0.001 | 0.07 (0.03 to 0.10) | <0.001 |
| Mayo vs. Mayo + diuretic + NYHA | 0.11 (0.05 to 0.17) | <0.001 | 0.66 (0.34 to 0.92) | <0.001 | 0.15 (0.03 to 0.22) | <0.001 |
| UK vs. UK + diuretic dose | 0.07 (-0.00 to 0.13) | 0.053 | 0.66 (-0.08 to 0.90) | 0.12 | 0.13 (-0.01 to 0.17) | 0.073 |
| UK + diuretic dose vs. UK + diuretic + NYHA functional class | 0.05 (0.01 to 0.09) | 0.007 | 0.88 (0.38 to 1.16) | 0.007 | 0.07 (0.02 to 0.11) | 0.001 |
| UK vs. UK + diuretic dose + NYHA functional class | 0.13 (0.05 to 0.20) | <0.001 | 0.62 (0.22 to 0.92) | 0.007 | 0.16 (0.02 to 0.24) | 0.013 |

*For diuretic dosing, 0 points were assigned for 0 mg/kg, 1 point for >0 to 0.5 mg/kg daily dose, 2 points for >0.5 to 1 mg/kg daily dose, and 3 points for >1 mg/kg daily dose.*

**TABLE 6** Internal Validation With Bootstrapping: Optimism-Adjusted Survival-Based AUC

|                     | All-Cause Mortality AUC (95% CI) | Death or Cardiac Transplantation AUC (95% CI) |
|---------------------|----------------------------------|---------------------------------------------|
| Mayo model | 0.691 (0.633-0.745) | 0.687 (0.632-0.738) |
| Mayo + diuretic dose | 0.765 (0.711-0.815) | 0.751 (0.696-0.801) |
| Mayo + diuretic dose + NYHA functional class | 0.795 (0.742-0.843) | 0.783 (0.731-0.831) |
| UK model | 0.698 (0.638-0.755) | 0.678 (0.619-0.733) |
| UK + diuretic | 0.776 (0.724-0.826) | 0.753 (0.700-0.803) |
| UK + diuretic dose + NYHA functional class | 0.806 (0.757-0.853) | 0.787 (0.783-0.834) |
| Diuretic dose only | 0.715 (0.657-0.771) | 0.705 (0.653-0.757) |
| SHFM | 0.785 (0.734-0.836) | 0.774 (0.724-0.822) |

*For diuretic dosing, 0 points were assigned for 0 mg/kg, 1 point for >0 to 0.5 mg/kg daily dose, 2 points for >0.5 to 1 mg/kg daily dose, and 3 points for >1 mg/kg daily dose.*

*Abbreviations as in Tables 1 to 3.*
deposition, where medical therapy may no longer be effective and when patients should be considered for advanced HF options or palliative care. Future studies with stratification by disease severity or responder analysis in already completed trials based on these risk models should be considered, particularly if treatment cost remains prohibitively high for many patients (27).

STUDY LIMITATIONS. Our cohort included both wtATTR and hATTR. It is possible that risk markers may be differentially predictive in these distinct cohorts because natural disease progression is more aggressive with hATTR, and there may also be differences between mutation types. Of note, the Mayo risk score (2) was derived in only wtATTR; the UK risk model (3) included both wtATTR and hATTR. However, we did not separate the data according to these 2 groups due to the limited sample size. With respect to sample size, although our cohort was not large, it is still one of the larger cohorts of ATTR-CM patients studied to date. The study involved a single referral center with primarily NYHA functional class I to III, so its generalizability to other centers, and to those with pre-clinical or end stage disease needs to be tested. Additionally, we did not externally validate our models, given the lack of readily accessible datasets, but plan to do so in future studies. We also did not create separate derivation and validation cohorts secondary to sample size, but we did perform internal validation with bootstrapping. The laboratory values in our dataset included both BNP and NT-proBNP and both troponin-T and troponin-I, which were each combined into a single variable. Ideally, all patients should have the same laboratory test performed at baseline. However, the need to combine different assays arguably reflects real-world practice where different institutions will use different troponin and BNP assays.

CONCLUSIONS

In the current study, we demonstrate that diuretic dose and NYHA functional class are strong independent predictors of all-cause mortality and the composite outcome of all-cause mortality or cardiac transplantation. We validate the Mayo and UK ATTR-CM risk scores, demonstrating that each of these has moderate discriminatory ability in our ATTR-CM cohort. When added to either the Mayo or UK risk scores for ATTR-CM, diuretic dose and NYHA functional class provide incremental predictive and discriminative utility, while maintaining calibration. Given that diuretic dose and NYHA functional class are easily obtainable data points, these should be considered when risk stratifying patients with ATTR-CM in the clinical setting.

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COMPETENCY IN MEDICAL KNOWLEDGE: The current Mayo and UK risk models for ATTR-CM provide moderate discriminatory ability in our ATTR-CM cohort. Diuretic dose and NYHA functional class are easily obtainable clinical parameters that provide incremental predictive and discriminative value to the existing Mayo and UK risk scores for ATTR-CM.

TRANSLATIONAL OUTLOOK: Further research is needed to validate these findings in larger cohorts and determine if they apply similarly to hATTR and wtATTR. Optimized risk stratification tools can also be used to aid in decision making for targeted therapy in ATTR-CM.

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KEY WORDS cardiac amyloidosis, heart failure, transthyretin

APPENDIX For an expanded Methods section and supplemental tables and figures, please see the online version of this paper.
The Incremental Value of Diuretic Dose in Staging Systems for Transthyretin Cardiac Amyloid

Keep It Simple*

Martha Grogan, MD

Transthyretin (TTR)-type cardiac amyloidosis (ATTR-CM) is increasingly recognized as an important cause of heart failure (1). The acceptance of technetium-labeled nuclear scintigraphy for nonbiopsy diagnosis has revolutionized the diagnosis of this condition (2). Until recently, the only treatment options for ATTR-CM were organ transplantation or supportive care. The ATTR-ACT study (3) demonstrated that the TTR stabilizer, tafamidis, decreased mortality and heart failure hospitalizations and ushered in a new era of awareness of ATTR-CM. Prognostic staging systems for ATTR are important in patient counseling, determining treatment options, including timing of advanced therapies for heart failure or palliative care.

We proposed the Mayo Clinic cardiac biomarker staging for patients with wild-type ATTR-CM (ATTRwt) using thresholds for troponin T and N-terminal pro-B-type natriuretic peptide (NT-proBNP) to define 3 stages of disease (4). Gilmore et al. (5) reported the UK staging system using NT-proBNP and estimated glomerular filtration rate. Both systems reported a threshold of NT-proBNP of 3,000 pg/ml. The Mayo model uses a troponin T threshold of 0.05 ng/ml, whereas the UK model uses an estimated glomerular filtration rate of <45 ml/min/1.73 m². The UK model included patients with both ATTRwt and hereditary ATTR-CM.

In this issue of JACC: CardioOncology, Cheng et al. (6) report the incremental value of diuretic dose and New York Heart Association (NYHA) functional class added to the Mayo and UK staging systems. Their study included 309 patients with ATTR-CM (66% ATTRwt) from a single center, conducted between 2002 and 2018 (Columbia University, New York). The authors reported that diuretic dose and NYHA functional class were independent predictors of mortality when added to the previously reported staging systems. The Seattle Heart Failure Model (SHFM) was able to stratify this cohort according to risk, but the 5-year predicted survival using SHFM was higher at 53.7% than the observed 36.7%. The authors noted that the “modified” Mayo and UK staging systems (adding diuretic dose and NYHA functional class) provided discriminatory results similar to the SHFM yet were easier to calculate with less input of variables.

The current study does not include echocardiographic findings in the analysis. In developing the Mayo staging system, multiple echocardiographic variables were studied, with the exception of left ventricular strain, which was not available in many in our cohort. Only reduced left ventricular ejection fraction remained independently predictive of survival in the multivariable model including cardiac biomarkers. The only echocardiographic variables reported by the UK group were interventricular septal thickness and left ventricular ejection fraction, again...
only the latter remained significant in multivariable analysis. Cardiovascular diagnosis usually includes imaging; thus cardiologists and patients have a tendency to focus on those results. Although cardiovascular imaging has revolutionized the diagnosis of cardiac amyloidosis, the role in prognosis is less clear. When the addition of imaging variables to the staging systems is considered, the availability, reproducibility, and incremental value must also be considered.

In contrast to the current study, NYHA functional class was not independently predictive of survival in the UK staging system. The discordant results of NYHA functional class as a predictor of mortality is not discussed by the authors but may be reflective of a smaller cohort evaluated by a small number of providers with less variability. Limitations of the interobserver variation in determining the NYHA functional class may limit the use of this variable in larger cohorts.

The report by Cheng et al. (6) validates the Mayo and UK staging systems and demonstrates the incremental value of adding diuretic dose and NYHA functional class. Most clinicians incorporate a variety of factors in assessing prognosis. Ideally the staging systems are used as reference points and then tailored accordingly, using the overall clinical assessment. This study, along with the other reports cited, suggests that complex models are probably not needed for prognosis in ATTR-CM. Simple models are easily remembered and incorporated into patient visits.

We have now entered a new era of therapy for ATTR-CM. Although the original and proposed “modified” Mayo and UK staging systems provide prognostic estimates based on natural history, these models are not applicable to the majority of newly diagnosed patients who will receive therapy. Our new challenges to prognosis of ATTR-CM are 3-fold: 1) determining prognosis in treated patients; 2) assessing response to therapy; and 3) determining outcomes for patients with noncardiac TTR amyloid deposition, such as those found in carpal tunnel tissue and spinal stenosis.

Analysis of the ATTR-ACT study and ongoing cardiac trials of TTR-silencer therapy provides an opportunity to evaluate prognostic staging systems in the modern treatment era and to determine markers of therapeutic response. It is hoped that these analyses will be forthcoming, although sponsors may be reluctant to report detailed predictors of nonresponse after drug approval. Patients almost uniformly seek information regarding treatment response. As tafamidis slows the progression of disease, we do not currently have a metric to assess response. Although stabilization or only mild progression of cardiac biomarkers, diuretic dose, hospitalizations, and other simple clinical variables may be reassuring, those variables do not necessarily answer the patient’s question: “Is this drug helping me?”

Due to the focus on imaging, patients often inquire about the results of follow-up studies, usually wondering if there has been regression of myocardial thickening. Given the challenges of determining prognosis using echocardiographic variables, caution is needed in enthusiasm for using these measures to assess therapeutic response. Part of the challenge lies in the pathophysiology of ATTR-CM, which is likely complex and remains incompletely understood. Dr. Rodney Falk appropriately coined the term “toxic-infiltrative cardiomyopathy” in reference to the toxicity of circulating light chains (AL) to cardiomyocytes in AL (7). Similar mechanisms of cardiomyocyte toxicity due to TTR oligomeric intermediates have been reported in ATTR (8,9).

Cardiac amyloidosis is not a simple infiltrative condition, even though most cardiologists tend to think of it that way. Although intraventricular septal thickness was one of the first prognostic markers described, that variable is not independently predictive of survival in AL or ATTR. Measurements of overall cardiac function and physiologic adaptation may be more important in assessing response to therapy than simple structural variables. The time frame between the onset of noncardiac manifestations such as carpal tunnel syndrome and spinal stenosis suggests that ATTR-CM may develop very slowly which perhaps allows for physiologic adaptation of the heart, lungs, and peripheral vasculature. If treatment is successful in slowing progression or promoting regression of disease, the changes may be difficult to detect with current cardiac imaging techniques, at least in the short term.

The next frontier in determining prognosis in ATTR lies in the assessment of patients found to have TTR deposition in noncardiac tissues. Many centers are performing clinical or research studies to detect the presence of TTR in ligaments from carpal tunnel release or surgery for spinal stenosis. Although patients with ATTR-CM often have a history of carpal tunnel syndrome or spinal stenosis, it is not known if all patients with TTR amyloid in these tissues will develop ATTR-CM.
in noncardiac tissues for whom we could truly prevent the development of cardiac disease? Perhaps some very early stage ATTR-CM patients may benefit from standard heart failure therapy directed at modulating systemic responses, despite the observation that those with advanced ATTR-CM usually do not tolerate those medications well. More research into the clinical course and response to therapy of ATTR-CM is needed to identify factors that will allow us to create staging models for the current era to determine prognosis, guide therapy, and change the natural history of this devastating disease.

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KEY WORDS cardiac amyloidosis, heart failure, transthyretin
Direct Oral Anticoagulants in Patients With Active Cancer
A Systematic Review and Meta-Analysis

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ABSTRACT

BACKGROUND Many patients with cancer have a hypercoagulable state and an increased risk of developing venous thromboembolism (VTE), arterial occlusion, and pulmonary emboli. Patients with cancer may also have an increased risk of bleeding with anticoagulant treatment. Recent trials have reported that direct oral anticoagulants (DOACs) are non-inferior to the low-molecular-weight heparin, dalteparin, in preventing VTE, but have a higher bleeding rate.

OBJECTIVES This study compared the efficacy and risks of DOACs versus dalteparin in patients with cancer-related VTEs across all randomized controlled trials (RCTs).

METHODS This study performed a systematic analysis of RCTs published in PubMed, SCOPUS, and Google Scholar from September 1, 2007 through March 31, 2020 that reported clinical outcomes of treatment with DOACs versus dalteparin in patients with cancer with acute VTE. Two investigators independently performed study selection and data extraction. Extracted data were recorded and exported to statistical software for all analyses (OpenMetaAnalyst).

RESULTS This study included 4 randomized trials (N = 2,907). Compared with DOACs, dalteparin was associated with higher VTE recurrence (risk ratio [RR]: 1.55; 95% confidence interval [CI]: 1.19 to 2.03; p = 0.001), whereas clinically relevant nonmajor bleeding (CRNMB) was significantly less frequent with dalteparin than that with DOACs (RR: 0.68; 95% CI: 0.54 to 0.86; p = 0.001). The risk of CRNMB was largely observed with patients with gastrointestinal malignancies. No significant differences were observed in major bleeding (RR: 0.74; 95% CI: 0.52 to 1.06; p = 0.11).

CONCLUSIONS DOACs were noninferior to dalteparin in preventing VTE recurrence in patients with cancer without a significantly increased risk of major bleeding. However, DOACs were associated with higher rates of CRNMB compared with dalteparin, primarily in patients with gastrointestinal malignancies. (J Am Coll Cardiol CardioOnc 2020;2:428–40) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Cardiovascular care of patients with cancer can be complex. Patients can have a persistent hypercoagulable state and an increased risk of recurrent venous thromboembolism (VTE). Furthermore, patients with cancer have an increased bleeding risk, which may be further exacerbated by antithrombotic treatments. Patients with cancer may also develop complications of cancer treatments that further increase the risk of thrombotic and bleeding events, including: invasive diagnostic and surgical procedures; toxicity secondary to radiotherapy, anti-angiogenic agents, hormonal therapies, immunotherapy and/or chemotherapy; hepatotoxicity; renal injury; tumor friability; or thrombocytopenia (1,2).

The CLOT (Randomized Comparison of Low-Molecular-Weight Heparin versus Oral Anticoagulant Therapy for the Prevention of Recurrent Venous Thromboembolism in Patients with Cancer) Investigators trial established low-molecular-weight heparin (LMWH) as the guideline-recommended first-line therapy over vitamin K antagonists (VKAs) for patients with cancer-related acute VTEs, based on data that showed a lower risk of VTE recurrence in patients who were treated with low-molecular-weight heparin (LMWH) (3,4). However, direct oral anticoagulants (DOACs), including dabigatran, apixaban, rivaroxaban, and edoxaban, demonstrated increased safety and efficacy in comparison with VKAs for management of VTE in the general population (5–8). Recently, randomized controlled trials (RCTs) compared different DOACs, including edoxaban (Hokusai VTE Cancer [Edoxaban for the Treatment of Cancer-Associated Venous Thromboembolism]) (9), rivaroxaban (SELECT-D [Comparison of an Oral Factor Xa Inhibitor With Low Molecular Weight Heparin in Patients With Cancer With Venous Thromboembolism: Results of a Randomized Trial]) (10), and apixaban (ADAM VTE [Apixaban and Dalteparin in Active Malignancy Associated Venous Thromboembolism: The ADAM VTE Trial]; CARAVAGGIO [Apixaban for the Treatment of Venous Thromboembolism]) (11,12) with dalteparin with regard to efficacy and safety in preventing recurrent VTEs in patients with cancer. A previous meta-analysis (13), which evaluated results from the Hokusai VTE Cancer, SELECT-D, and ADAM VTE trials, observed a trend for reduced VTE recurrence in DOAC-treated patients, in which DOACs were found to be noninferior to LMWH in preventing VTE recurrence in patients with cancer, but were associated with an increased risk of major bleeding and clinically relevant nonmajor bleeding (CRNMB). The results of these trials were somewhat conflicting, which might have been due to differences in trial design and enrollment criteria. The recently published results of the CARAVAGGIO trial, the largest trial to date in this specific clinical setting, reported that oral apixaban therapy in patients with cancer was associated with significantly lower VTE recurrence, low rates of bleeding, and reported enhanced quality of life outcome measures compared with dalteparin (12). Applying these newly available data, we performed a meta-analysis to evaluate the efficacy and safety of DOACs compared with dalteparin in patients with cancer-related acute VTEs.

METHODS

DATA SOURCES AND SEARCHES. We searched PubMed, SCOPUS, and Google Scholar electronic databases from September 1, 2007 through March 31, 2020, because the first journal article was published in 2007 regarding clinical trial testing with a DOAC for the prevention of VTEs (14). We used the following keywords and the corresponding MeSH terms: “venous thromboembolism,” “cancer,” “DOAC/NOAC.” We also reviewed the reference lists of eligible studies and screened scientific abstracts and relevant websites (i.e., www.clinicaltrialresults.org; www.escardio.org; www.tctmd.com; https://accscientificsession.acc.org; and https://exhibitatsessions.org).

STUDY SELECTION. Two investigators (J.S., S.D.R.) independently screened search records to identify eligible trials. There were no disagreements. RCTs were included if they compared a DOAC versus dalteparin in patients with cancer and acute VTE. Additional inclusion criteria included the following outcomes: recurrent VTE, and major bleeding or CRNMB. Exclusion criteria consisted of duplicate publications and journal articles in which trial results were published in a language other than English and/or did not report the pre-specified endpoint measure.

DATA EXTRACTION AND QUALITY ASSESSMENT. Two reviewers (J.S., S.D.R.) independently extracted data concerning study characteristics and event rates from full text journal articles. Two investigators (J.S., S.D.R.) independently assessed study quality using the Cochrane Risk of Bias Tool (https://methods.cochrane.org/bias/resources/rob-2-revised-cochrane-risk-bias-tool-randomized-trials). In particular, the assessment considered: randomization method; allocation concealment; blinding of patient, investigator, and outcome adjudication committee; reporting bias; attrition bias; and any other potential sources of bias.
such as those related to trial designs or the risk for contamination or crossover between the groups.

**DATA SYNTHESIS AND ANALYSIS.** We extracted data from the original primary publications (9-12). Efficacy outcomes of interest consisted of recurrent VTEs that included deep vein thrombosis (DVT) and pulmonary embolism (PE). Safety outcomes consisted of major bleeding and CRNMB. In addition, death as an outcome was analyzed as a secondary endpoint. We used the risk ratio (RR) with 95% confidence interval (CI) as the summary measure (15). Heterogeneity was assessed using Cochrane’s Q test, and p values <0.10 were considered indicative for heterogeneity. $I^2$ values were calculated for estimation of variation among studies attributable to heterogeneity (16). A fixed effect was used to compute estimates for the summary effect in case of low heterogeneity ($I^2 < 45\%$ and Cochrane’s Q test = NS); otherwise, a random-effects model was applied (17). Meta-analysis results were reported graphically using forest plots: the measure of effect (RR) was represented by a square, with the area being proportional to study weight, as previously described (18). A p value <0.05 was considered significant. Subgroup and sensitivity analyses were conducted using fixed effect and random effects models alternatively (15-18). Publication bias was assessed using funnel plots. Analyses were performed using OpenMetaAnalyst 10 (Brown University, Providence, Rhode Island) and Review Manager 5.3 (Cochrane, London, United Kingdom).
RESULTS

LITERATURE SEARCH AND STUDY SELECTION. The literature search retrieved a total of 2,860 articles, after removing duplicates. By screening titles and abstracts, we identified 2,835 citations not relevant to our study aims. After a full review of the remaining manuscripts, 4 randomized controlled trials that included 2,907 patients with cancer and acute VTEs, were included in the systematic review and meta-analysis (9–12). The study flowchart is described in Figure 1.

The 4 included studies were designed to compare the efficacy of DOACs with dalteparin for the treatment of cancer-related acute VTEs (9–12). Main trial characteristics are outlined in Table 1. Treatment duration was at least 6 months for all trials (9,11,12); the Hokusai VTE trial included data from 12 months’ follow-up, and patients in the SELECT-D trial were also eligible for randomization to a further 6 months of rivaroxaban or placebo. Data from the SELECT-D trial used for calculation in this meta-analysis only refer to the 6-month follow-up with comparison to dalteparin (10). Each study reported data on VTE recurrence, major bleeding, and CRNMB. The definitions of major bleeding, CRNMB, and active cancer were homogeneous among the included studies, whereas VTE definitions were more heterogeneous. The ADAM VTE trial was the only study that included thromboembolism of the upper extremities in the inclusion criteria as a qualifying event for participating in the study. Furthermore, this study also included any arterial thromboembolism in the endpoint definition of recurrent thromboembolism (11). A detailed description of key trial definitions is included in Tables 2 and 3. The trials also represented populations with slight differences in the distribution of cancer types, as reported in Table 2.

Risk of bias assessments is reported in Supplemental Figure 1. Overall, the risk for selection

| Study | First Author (Ref. #); Year | N | Mean Age (yrs) | Design | Intervention | Control | Outcome |
|-------|-----------------------------|---|---------------|--------|-------------|---------|---------|
| CARAVAGGIO | Agenelli et al. (12); 2020 | 1,155 | 67 | Open-label RCT (non-inferiority) | Apixaban | Dalteparin | Primary efficacy outcome: VTE recurrence. Primary safety outcome: major bleeding |
| SELECT-D | Young et al. (10); 2018 | 406 | 67 | Open-label RCT (pilot trial) | Rivaroxaban | Dalteparin | Primary outcome: thromboembolic recurrence. Secondary outcome: major bleeding and CRNMB |
| Hokusai VTE Cancer | Roskab et al. (9); 2018 | 1,046 | 64 | Open-label RCT (non-inferiority) | Edoxaban | Dalteparin | Primary outcome: composite of recurrent VTE or major bleeding |
| ADAM-VTE | McBane et al. (11); 2020 | 300 | 64 | Open-label RCT (superiority) | Apixaban | Dalteparin | Primary outcome: major bleeding. Secondary outcome: VTE recurrence |

ADAM VTE – Apixaban and Dalteparin in Active Malignancy Associated Venous Thromboembolism: The ADAM VTE Trial; CARAVAGGIO – Apixaban for the Treatment of Venous Thromboembolism trial; CRNMB – clinically relevant non-major bleeding; Hokusai VTE Cancer – Edoxaban for the Treatment of Cancer-Associated Venous Thromboembolism; RCT – randomized clinical trial; SELECT-D – Comparison of an Oral Factor Xa Inhibitor With Low Molecular Weight Heparin in Patients With Cancer With Venous Thromboembolism: Results of a Randomized Trial; VTE – venous thromboembolism.

| Study | First Author (Ref. #); Year | VTE (Qualifying Event) | VTE Recurrence |
|-------|-----------------------------|-----------------------|----------------|
| CARAVAGGIO | Agenelli et al. (12); 2020 | Newly diagnosed symptomatic or incidental proximal lower limb DVT or PE | Proximal DVT of the lower limbs (symptomatic or incidental), symptomatic DVT of the upper limbs, or PE (symptomatic, incidental, or fatal) occurring during the 6-month trial period |
| SELECT-D | Young et al. (10); 2018 | Symptomatic lower extremity proximal DVT, or symptomatic PE, or incidental PE | Recurrent proximal DVT, or recurrent PE (symptomatic or incidental), or fatal PE, or other sites of venous thrombosis (e.g., subclavian vein, hepatic vein, or inferior vena cava) |
| Hokusai VTE Cancer | Roskab et al. (9); 2018 | Acute, symptomatic or incidentally detected DVT involving the popliteal, femoral, or iliac vein or the inferior vena cava; acute symptomatic PE that was confirmed by means of diagnostic imaging; or incidentally detected PE involving segmental or more proximal pulmonary arteries | Symptomatic new DVT or PE, incidental (detected by means of imaging tests performed for other reasons) new DVT, or PE involving segmental or more proximal pulmonary arteries, or fatal PE or unexplained death for which PE could not be ruled out as the cause |
| ADAM-VTE | McBane et al. (11); 2020 | Acute lower or upper extremity (jugular, innominate, subclavian, axillary, brachial) DVT, PE, splanchic (hepatic, portal, splenic, mesenteric, renal, gonadal), or cerebral vein thrombosis confirmed by appropriate cross-section imaging. | Any thromboembolic recurrence including DVT, PE, fatal PE, or arterial thromboembolism. A recurrent event was a new filling defect evident on the second study not appreciated on the original images, or when an interval study clearly showed thrombus resolution. An arterial thromboembolism could include myocardial infarction, stroke, transient ischemic attack, or peripheral arterial embolism. |

DVT = deep vein thrombosis; PE = pulmonary embolism; VTE = venous thromboembolism; other abbreviations as in Table 1.
bias, detection bias, attrition bias, and reporting bias was judged as low. All trials used appropriate randomization and allocation concealment. All studies were open label; hence, the risk for performance bias could not be completely excluded. However, endpoint adjudication committees were blinded to the treatment strategy in all trials, with the exception of the ADAM VTE study.

MEASURES OF EFFICACY. Recurrence of VTE. Among 2,907 patients included in the analysis, 132 (9.1%) experienced VTE recurrence in the dalteparin treatment group, and 82 patients (5.7%) had VTE recurrence in the DOAC treatment groups, which resulted in a significantly higher incidence with dalteparin compared with DOACs (RR: 1.55; 95% CI: 1.19 to 2.03; p = 0.001) (Figure 2A). Heterogeneity was low with respect to this outcome (Q = 3.93; p = 0.270; I² = 24%). No difference was found between the subgroup of studies using a once or twice daily DOAC administration regimen. No evidence for publication bias was present at funnel plot inspection (Figure 3A).

Recurrence of PE. Among 2,907 patients included in the analysis, 70 (4.8%) developed PE recurrence in the dalteparin treatment group, and 50 (3.4%) experienced PE recurrence in the DOAC treatment groups, with no statistically significant difference between the treatment arms (RR: 1.38; 95% CI: 0.96 to 1.97; p = 0.080) (Figure 2B). No relevant heterogeneity was evident for this outcome (Q = 2.59; p = 0.459; I² = 0%). No evidence for publication bias was present at funnel plot inspection (Figure 3B).

Mortality. All-cause mortality was determined in 408 patients (28.0%) who developed VTE recurrence in the dalteparin treatment groups and in 412 patients (28.4%) in the DOAC treatment groups, with no significant difference between the 2 treatment arms (RR: 0.95; 95% CI: 0.73 to 1.24; p = 0.714) (Figure 2C). Heterogeneity was determined to be high for this outcome (Q = 11.25; p = 0.010; I² = 73%). No evidence for publication bias was found on funnel plot inspection (Figure 3C).

MEASURES OF SAFETY. Major bleeding. Major bleeding occurred in 52 patients (3.6%) in the dalteparin treatment groups and in 69 patients (4.8%) in the DOAC treatment groups (RR: 0.74; 95% CI: 0.52 to 1.06; p = 0.110) (Figure 4A). Heterogeneity was low with respect to this outcome (Q = 4.08; p = 0.253; I² = 26%). A significantly higher rate of major bleeding was evident with DOACs, until publication of the recent CARAVAGGIO trial findings, which changed the results of this analysis. No evidence for publication bias was determined at funnel plot inspection (Figure 3D).
Gastrointestinal bleeding. Data on gastrointestinal (GI) bleeding were available from 3 studies (2,9,10), involving 2,607 patients. GI bleeding was reported in 20 patients (1.5%) in the dalteparin treatment groups and in 39 patients (3.0%) in the DOAC treatment groups (RR: 0.53; 95% CI: 0.31 to 0.84; p = 0.003) (Figure 4B). No evidence for publication bias was found at funnel plot inspection (Figure 3E).

CRNMB. CRNMB was reported in 107 patients (7.3%) in the dalteparin treatment group and in 161 patients (11.1%) in the DOAC treatment groups (RR: 0.68; 95% CI: 0.54 to 0.86; p = 0.001) (Figure 4C). No evidence for publication bias was determined at funnel plot inspection (Figure 3F). Some heterogeneity was evident with respect to this outcome (Q = 5.27; p = 0.153; I² = 43%). We performed a sensitivity analysis to assess the consistency of our findings. Sensitivity analysis found the most heterogeneity related to the SELECT-D study. Accordingly, removal of this trial resulted in a substantial reduction of heterogeneity (Q = 0.36; p = 0.836; I² = 0%), with no impact on the results of the meta-analysis (RR: 0.73; 95% CI: 0.58 to 0.94; p = 0.013). Sensitivity analysis also showed that meta-analysis results were not primarily determined by the 2 largest trials, because removal of either the Hokusai VTE Cancer study (RR: 0.59; 95% CI: 0.42 to 0.84; p = 0.003) or the CARAVAGGIO trial (RR: 0.68; 95% CI: 0.51 to 0.91; p = 0.008) had a modest numerical impact on the RR, but did not significantly change the interpretation of our findings. Meta-regression analysis revealed that DOACs were associated with higher rates of CRNMB events that were primarily observed in patients with GI cancers (p = 0.027) (Figure 5A). Regarding the specific localization of GI cancer, meta-regression confirmed a significant increase of CRNMB events with upper GI cancers (p = 0.032) (Figure 5B) and a borderline increase of such events with lower GI cancers (p = 0.052) (Figure 5C).

DISCUSSION

Over the past few years, the use of DOACs has revolutionized anticoagulation treatment. Clinical trials and secondary real-world data have served to endorse DOACs as preferred therapy over VKAs for the treatment of VTEs in patients without cancer (5-8).

Although patients with active cancer were excluded from most critical DOAC efficacy trials, and the CLOT trial resulted in guidelines that recommended LMWH as first-line therapy in patients with active cancer and VTE recurrence, clinical observations from real-life experience suggested that the risks of long-term treatment with LMWH might outweigh the benefits. This resulted in recent trials comparing DOACs with LMWH in preventing VTEs in patients with cancer.

Recent studies suggested that DOACs might result in an analogous or lower incidence of recurrent VTEs compared with that of dalteparin, but were also associated with an increased risk of bleeding (9-12). Because the number of events observed in each study was limited, these results..
appeared to be conflicting and continue to be debated. A previous meta-analysis (13), that included results from the Hokusai VTE Cancer, the SELECT-D, and the ADAM VTE trials demonstrated a nonstatistically significant relative risk reduction of VTE recurrence with DOACs in patients with cancer versus patients treated with LMWH. However, DOACs were also found to be associated with an increased risk of major bleeding and CRNMB. As a consequence, most recent guidelines continue to recommend dalteparin as an initial standard treatment for the prevention of VTEs in patients with active cancer, with DOAC treatment recommended as an option only (19,20).

FIGURE 2 Measures of Efficacy

### A Recurrent venous thromboembolism

| Study                      | Dalteparin | DOACs |
|----------------------------|------------|-------|
|                            | Events     | Total | Events     | Total | P value | Risk Ratio |
| Hokusai VTE Cancer         | 59         | 524   | 27        | 522   | 0.063   | 1.43 [0.98, 2.10] |
| SELECT-D                   | 18         | 203   | 8         | 203   | 0.050   | 2.25 [1.00, 5.06] |
| ADAM VTE                   | 9          | 150   | 1         | 150   | 0.036   | 9.00 [1.15, 70.16] |
| CARAVAGGIO                 | 46         | 579   | 32        | 576   | 0.108   | 1.43 [0.92, 2.21] |

Total (95% CI) 132, 1,456 82, 1,451 0.001 1.55 [1.19, 2.03]

Heterogeneity: $\chi^2 = 3.93$, df = 3 ($P = 0.270$); $I^2 = 24$

### B Recurrent pulmonary embolism

| Study                      | Dalteparin | DOACs |
|----------------------------|------------|-------|
|                            | Events     | Total | Events     | Total | P value | Risk Ratio |
| Hokusai VTE Cancer         | 28         | 524   | 27        | 522   | 0.090   | 1.03 [0.62, 1.73] |
| SELECT-D                   | 9          | 203   | 4         | 203   | 1.171   | 2.25 [0.70, 7.19] |
| ADAM VTE                   | 1          | 150   | 0         | 150   | 0.503   | 3.00 [0.12, 73.06] |
| CARAVAGGIO                 | 32         | 579   | 19        | 576   | 0.069   | 1.68 [0.96, 2.92] |

Total (95% CI) 70, 1,456 50, 1,451 0.08 1.38 [0.96, 1.97]

Heterogeneity: $\chi^2 = 2.59$, df = 3 ($P = 0.459$); $I^2 = 0$

### C All-cause death

| Study                      | Dalteparin | DOACs |
|----------------------------|------------|-------|
|                            | Events     | Total | Events     | Total | P value | Risk Ratio |
| Hokusai VTE Cancer         | 192        | 524   | 206       | 522   | 0.348   | 0.93 [0.80, 1.08] |
| SELECT-D                   | 56         | 203   | 48        | 203   | 0.364   | 1.17 [0.84, 1.63] |
| ADAM VTE                   | 7          | 150   | 23        | 150   | 0.004   | 0.30 [0.13, 0.69] |
| CARAVAGGIO                 | 153        | 579   | 135       | 576   | 0.241   | 1.13 [0.92, 1.38] |

Total (95% CI) 408, 1,456 412, 1,451 0.714 0.95 [0.73, 1.24]

Heterogeneity: $\tau^2 = 0.05$; $\chi^2 = 11.25$, df = 3 ($P = 0.010$); $I^2 = 73$

Forest plots illustrating results of meta-analysis on the rate of recurrent venous (A) thromboembolism, (B) pulmonary embolism, and (C) all-cause death. CI = confidence interval; DOAC = direct oral anticoagulants; $I^2$ = inconsistency index.
Funnel plots for the assessment of publication bias are reported for all outcomes: (A) recurrent venous thromboembolism, (B) recurrent pulmonary embolism, (C) all-cause death, (D) major bleeding, (E) gastrointestinal bleeding, and (F) clinically relevant nonmajor bleeding. log[RR] = logarithm of risk ratio.
In our meta-analysis, DOACs were shown to be noninferior to dalteparin in preventing VTE recurrence in patients with cancer (Central Illustration). The RR of recurrent VTE was 1.55-fold greater with dalteparin compared with that of DOACs. Furthermore, although DOACs were associated with an increased risk of CRNMB compared with that of dalteparin, the risk of major bleeding was similar across the 2 treatment groups and not significantly different (p = 0.110). The additional data reported by the recently published CARAVAGGIO trial, which is the largest trial to date, provided significant evidence regarding the safety and efficacy of DOAC treatment in decreasing the risk of VTEs in patients with cancer.

### FIGURE 4 Measures of Safety

#### A Major bleeding

| Study                  | Dalteparin | DOACs | Risk Ratio |
|------------------------|------------|-------|------------|
| Hokusai VTE Cancer     | 21         | 524   | 36         | 522 | 0.043 | 0.58 [0.34, 0.98] |
| SELECT D               | 6          | 203   | 11         | 203 | 0.223 | 0.55 [0.21, 1.45] |
| ADAM VTE               | 2          | 150   | 0          | 150 | 0.296 | 5.00 [0.24, 103.28] |
| CARAVAGGIO             | 23         | 579   | 22         | 576 | 0.893 | 1.04 [0.59, 1.84] |
| Total (95% CI)         | 52         | 1,456 | 69         | 1,451 | 0.110 | 0.74 [0.52, 1.06] |

Heterogeneity: Chi² = 4.08, df = 3 (P = 0.253); I² = 26%

#### B Gastrointestinal bleeding

| Study                  | Dalteparin | DOACs | Risk Ratio |
|------------------------|------------|-------|------------|
| Hokusai VTE Cancer     | 6          | 524   | 20         | 522 | 0.009 | 0.30 [0.12, 0.74] |
| SELECT D               | 4          | 203   | 8          | 203 | 0.251 | 0.50 [0.15, 1.63] |
| CARAVAGGIO             | 10         | 579   | 11         | 576 | 0.816 | 0.90 [0.39, 2.11] |
| Total (95% CI)         | 20         | 1,306 | 39         | 1,301 | 0.020 | 0.53 [0.31, 0.92] |

Heterogeneity: Chi² = 3.07, df = 2 (P = 0.21); I² = 35%

#### C Clinically relevant nonmajor bleeding (CRNMB)

| Study                  | Dalteparin | DOACs | Risk Ratio |
|------------------------|------------|-------|------------|
| Hokusai VTE Cancer     | 58         | 524   | 76         | 522 | 0.093 | 0.76 [0.55, 1.05] |
| SELECT D               | 7          | 203   | 25         | 203 | 0.002 | 0.28 [0.12, 0.63] |
| ADAM VTE               | 7          | 150   | 8          | 150 | 0.791 | 0.88 [0.33, 2.35] |
| CARAVAGGIO             | 35         | 576   | 52         | 576 | 0.057 | 0.67 [0.45, 1.02] |
| Total (95% CI)         | 107        | 1,453 | 161        | 1,451 | 0.001 | 0.68 [0.54, 0.86] |

Heterogeneity: Chi² = 5.27, df = 3 (P = 0.153); I² = 43%

Forest plots illustrating results of meta-analysis on the rate of (A) major bleeding, (B) GI bleeding, and (C) clinically relevant nonmajor bleeding (CRNMB). Abbreviations as in Figures 2 and 3.
This evidence was welcome information following the reported results of the 3 previous smaller clinical trials, which had notable differences in trial design and enrollment criteria.

The data from the CARAVAGGIO trial concerning the risk of major bleeding were particularly noteworthy. It appears that this finding was not related to any differences in the definition of major bleeding, because this was relatively consistent across trials (Table 3). In contrast, the differences in reported major bleeding risk might have resulted from the heterogeneity in enrolled populations. Patients with primary brain tumors, brain metastases, and acute leukemia were excluded from the CARAVAGGIO trial.
This might have affected the occurrence of major bleeding in patients in the treatment groups, although the proportion of patients with brain tumors or acute leukemia was also low in the remaining studies (Table 4).

Meta-regression analysis showed that the increased risk of CRNMB events observed with DOAC treatment in patients with cancer was observed with a higher proportion of GI bleeding in single studies, and the risk of CRNMB was greater in those studies with a larger proportion of patients with GI cancers (Figure 4). Although these results were not consistently statistically significant, they concurred with the 2-fold increase in GI bleeding with DOACs compared with that of dalteparin and with previous evidence. These data suggested that patients with GI cancers represented a slightly different clinical category, in which oral anticoagulation might result in a higher bleeding risk. Again, it should be noted that the CARAVAGGIO trial included fewer patients with upper GI cancer than the other studies. The observation that the risk of CRNMB events was higher with upper GI cancers compared with lower GI cancers suggested that the known effect of DOACs on upper GI bleeding might have played a role (21). It is reassuring that this phenomenon was apparently limited to CRNMB and was not associated with major bleeding. However, particular caution should be used in managing anticoagulation, because absorption of oral anticoagulants might be affected in patients with GI cancer or there might be toxicity. Caution should also be used in those who have undergone surgery of the upper GI tract (22). In addition, it is crucial to note that uncertainty remains regarding drug–drug interaction with oral anticoagulants and cancer therapies. Patients treated with powerful inducers and/or inhibitors of CYP3A4 or P-glycoprotein were primarily excluded from trial participation, and few patients treated with checkpoint inhibitors or other newly approved therapies were included in these trials.

It was reassuring that the data reported from the CARAVAGGIO trial served to confirm that DOACs were noninferior to dalteparin in preventing VTE recurrence. Despite the many differences among the 4 trials, their results concerning the prevention of recurrent VTEs in patients with cancer were consistent, with the estimated risk for recurrent VTEs in patients with cancer was consistent, with the estimated risk for recurrent VTE being increased with dalteparin. Furthermore, although the CARAVAGGIO trial excluded patients with brain tumors and included few patients with upper GI cancer, the ADAM VTE trial adopted a broader definition of the qualifying VTE event, encompassing upper extremity thrombosis. This difference, along with the smaller sample size and the slightly different distribution of cancer types compared with
the other trials, might explain the lower mortality rate reported in the ADAM VTE trial.

**STUDY LIMITATIONS.** As with all meta-analyses, the present analysis had limitations. Although all included studies were high quality, there was some heterogeneity among the trials. Because we were unable to define the extent to which this heterogeneity might be related to differences in trial designs and the enrolled populations, we could not completely exclude bias. Nevertheless, the meta-analysis results were consistent using both fixed-effect and random-effects calculation models, as well as across study subgroups at sensitivity analysis. The included studies encompassed all DOACs available on the market, with the exception of dabigatran, because no study evaluated the use of dabigatran against LMWHs in this clinical context. Therefore, we do not know whether these results would also apply to dabigatran. Finally, our analysis was limited to the use of dalteparin as the comparator drug, because none of the other LMWHs was selected as the control treatment arm in any of the included studies. This might be because dalteparin was tested in the CLOT study (3).

**CONCLUSIONS**

We demonstrated that DOACs are noninferior to LMWH in preventing VTE recurrence in patients with active cancer, although such treatment leads to an increased risk of CRNMB, which was primarily observed in patients with GI malignancy. Results of this meta-analysis of 4 randomized trials provided compelling evidence for prescribing DOACs with more confidence and perhaps to an increased number of patients with cancer who are at risk for recurrent VTEs. Initial caution was exercised with DOAC use in this setting, which was primarily related to uncertainty regarding the clinical relevance of drug interactions with anticancer treatments and concerns regarding bleeding. Nevertheless, the new evidence of noninferiority in preventing recurrent VTEs and the findings that increased bleeding risk does not involve major bleeding and is primarily related to the enrollment of patients with GI cancer should affect current clinical practice in this setting.

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**PERSPECTIVES**

**COMPETENCY IN MEDICAL KNOWLEDGE:** DOACs are noninferior to dalteparin in preventing VTE recurrence in patients with active cancer. There is no significantly increased risk of major bleeding, but there is an elevated risk of CRNMB events, primarily related to GI cancers.

**TRANSLATIONAL OUTLOOK:** The new evidence of noninferiority of DOACs in protection from recurrent VTEs, along with the lack of increased major bleeding, should affect current clinical management for patients with active cancer and VTEs. Careful selection of anticoagulants and continued development of evidence-based management strategies to include DOACs are needed to help ensure the continued careful care of the growing cancer population.

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**KEY WORDS** cancer, direct oral anticoagulants, DOACs, hypercoagulable state, venous thromboembolism

**APPENDIX** For a supplemental figure, please see the online version of this paper.
People with cancer are much more prone to experience venous thromboembolism (VTE) and its consequences, which include increased risk of short- and long-term mortality, need for urgent care and hospitalization, and increased health resource utilization (1,2). For the need for urgent care and hospitalization, and increased risk of short- and long-term mortality, VTE (3). However, concerns had been raised about considered the standard of care for cancer-associated (LMWH) monotherapy for at least 6 months has been past decade and a half, low-molecular-weight heparin this standard given lack of full con.

non-major bleeding (CRNMB) (6,7). Current risk of major bleeding as well as clinically relevant this setting with DOACs but at the cost of an increased

generated a potential reduced risk of recurrent VTE in gesting data from initial randomized trials sug-
treat and prevent recurrent VTE in cancer patients. Promising data from initial randomized trials sug-
gested a potential reduced risk of recurrent VTE in this setting with DOACs but at the cost of an increased

risk of major bleeding as well as clinically relevant non-major bleeding (CRNMB) (6,7). Current

guidelines therefore include both LMWHs and DOACs as options but caution against the use of the latter in patients at high risk for bleeding, particularly the subgroup of patients with gastrointestinal malignancies (8-10).

In this issue of JACC: CardioOncology, Sabatino et al. (11) provide a new meta-analysis of data of the latest 4 randomized controlled trials (RCTs) comparing the safety and efficacy of DOACs versus LMWH in the treatment of cancer-associated VTE. These 4 included RCTs comprising 2,907 patients evaluated different DOACs: edoxaban in the Hokusai VTE Cancer trial (7), rivaroxaban in the Select-D trial (6), and apixaban in the ADAM-VTE (Apixaban and Dalteparin in Active Malignancy Associated Venous Thromboembolism) and Caravaggio trials (12,13). All 4 RCTs utilized dalteparin, the only approved LMWH for this indication, as the control arm. An important strength of this meta-analysis is the inclusion of the very recently published Caravaggio study, which substantially increases sample size relative to prior such analyses.

The clinical utility of anticoagulation rests on the net benefit to individual patients when evaluating both efficacy and safety. In terms of efficacy, this meta-analysis favored DOACs, finding that dalteparin was associated with higher risk of recurrent VTE (risk ratio [RR]: 1.55; 95% confidence interval [CI]: 1.19 to 2.03; p = 0.001). In terms of safety, no significant differences were observed in major bleeding (RR: 0.74; 95% CI: 0.52 to 1.06; p = 0.11). However, there was a higher risk of CRNMB with DOACs (RR: 0.68 favoring dalteparin; 95% CI: 0.54 to 0.86; p = 0.001), particularly in patients with gastrointestinal malignances with an increased incidence of CRNMB in upper GI cancers (p = 0.032) compared with lower GI cancers (p = 0.052). Gastrointestinal bleeding was less frequent in the dalteparin group (RR: 0.53; 95% CI: 0.31 to 0.92; p = 0.020). Certainly, there are limitations to this meta-analysis. There is heterogeneity in

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the JACC: CardioOncology author instructions page.
the included clinical trials because they differ in their primary outcomes, design, and selection of included patients regarding the cancer type and stage. Moreover, this meta-analysis is not able to address major knowledge gaps in this setting. Which cancer patients are most likely to bleed on anticoagulation? What is the appropriate duration of anticoagulation? Should all incidentally discovered thrombi (e.g., visceral vein thrombi) be treated with full-dose anticoagulation? Many more studies are needed to appropriately answer these questions.

What are the clinical implications of this meta-analysis? First, the findings reaffirm that DOACs are at least as effective (and possibly more effective) than LMWH monotherapy for preventing recurrent VTE. Safety concerns raised by the initial RCTs regarding risk of bleeding, particularly in patients with gastrointestinal malignancies, are reiterated by this meta-analysis. In this context, the findings of the Caravaggio trial are of interest. In this study, there were no significant differences between patients randomized to apixaban or dalteparin either related to major bleeding outcomes (3.8% with apixaban and 4.0% with dalteparin; \( p = 0.60 \)) or CRNMB (9.0% with apixaban and 6.0% with dalteparin). Clinicians may be tempted to draw the conclusion that apixaban is safer than other DOACs in patients with gastrointestinal malignancies. However, it should be noted that the Caravaggio trial (13) included a smaller proportion of patients with upper gastrointestinal cancers (the population most likely to have major bleeding) (4% on apixaban and 5.4% on dalteparin) compared with other included trials (6,12,14). Given this heterogeneity, it is premature to conclude that apixaban is superior to other DOACs until direct comparison studies are conducted.

Overall, however, the findings of this meta-analysis confirm that the introduction of DOACs is a major step forward toward the evolving management of anticoagulant therapy in cancer patients with VTE. It is important to recognize successes in medicine when they occur: the overwhelming majority of patients treated with DOACs for acute cancer-related VTE will not experience either recurrent VTE or major bleeding. This is important for people with cancer who already carry a major burden of illness. Selection of patients for DOAC versus LMWH therapy needs to be individualized, keeping in mind risk of bleeding, drug-drug interactions, financial cost, toxicity, and above all, patient preferences and values.

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**KEY WORDS** cancer, direct oral anticoagulant, venous thromboembolism, VTE

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Comprehensive Characterization of the Vascular Effects of Cisplatin-Based Chemotherapy in Patients With Testicular Cancer

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ABSTRACT

BACKGROUND Cisplatin-based chemotherapy increases the risk of cardiovascular and renal disease.

OBJECTIVES We aimed to define the time course, pathophysiology, and approaches to prevent cardiovascular disease associated with cisplatin-based chemotherapy.

METHODS Two cohorts of patients with a history of testicular cancer (n = 53) were recruited. Cohort 1 consisted of 27 men undergoing treatment with: 1) surveillance; 2) 1 to 2 cycles of bleomycin, etoposide, and cisplatin (BEP) chemotherapy (low-intensity cisplatin); or 3) 3 to 4 cycles of BEP (high-intensity cisplatin). Endothelial function (percentage flow-mediated dilatation) and cardiovascular biomarkers were assessed at 6 visits over 9 months. Cohort 2 consisted of 26 men previously treated 1 to 7 years ago with surveillance or 3 to 4 cycles BEP. Vasomotor and fibrinolytic responses to bradykinin, acetylcholine, and sodium nitroprusside were evaluated using forearm venous occlusion plethysmography.

RESULTS In cohort 1, the percentage flow-mediated dilatation decreased 24 h after the first cisplatin dose in patients managed with 3 to 4 cycles BEP (10.9 ± 0.9 vs. 16.7 ± 1.6; p < 0.01) but was unchanged from baseline thereafter. Six weeks after starting 3 to 4 cycles BEP, there were increased serum cholesterol levels (7.2 ± 0.5 mmol/l vs. 5.5 ± 0.2 mmol/l; p = 0.01), hemoglobin A1c (41.8 ± 2.0 mmol/l vs. 35.5 ± 1.2 mmol/l; p < 0.001), von Willebrand factor antigen (62.4 ± 5.4 mmol/l vs. 45.2 ± 2.8 mmol/l; p = 0.048) and cystatin C (0.91 ± 0.07 mmol/l vs. 0.65 ± 0.09 mmol/l; p < 0.01). In cohort 2, intra-arterial bradykinin, acetylcholine, and sodium nitroprusside were evaluated using forearm venous occlusion plethysmography.

CONCLUSIONS Cisplatin-based chemotherapy induces acute and transient endothelial dysfunction, dyslipidemia, hyperglycemia, and nephrotoxicity in the early phases of treatment. Cardiovascular and renal protective strategies should target the early perchemotherapy period. (Clinical Characterisation of the Vascular Effects of Cis-platinum Based Chemotherapy in Patients With Testicular Cancer [VECTOR], NCT03557177; Intermediate and Long Term Vascular Effects of Cisplatin in Patients With Testicular Cancer [INTELLECT], NCT03557164) (J Am Coll Cardiol CardioOnc 2020;2:443–55) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Testicular cancer is the most common malignancy in men aged 20 to 40 years, and cisplatin-based chemotherapy with bleomycin, etoposide, and cisplatin (BEP) achieves a cure for almost all patients (1,2). However, this treatment is associated with cardiovascular disease (CVD), including myocardial infarction, thrombosis, and nephrotoxicity (3,4). Adverse effects on the endothelium appear to be a central pathophysiological mechanism (5), and perturbations in metabolic and inflammatory parameters may also be important (6,7). A retrospective epidemiological study of >15,000 patients demonstrated a 5-fold increase in standardized cardiovascular mortality following cisplatin-based chemotherapy that was confined primarily to the first year following treatment (1). This challenges the preconception that cardiovascular risk in testicular cancer survivors is a late phenomenon (3,8).

Historically, the prospective evaluation of vascular effects of cisplatin-based chemotherapy has been hindered by assessments in heterogeneous groups with differing cardiovascular risk, cancer types, and treatment regimens. Furthermore, examination of immediate effects has been limited and usually without longitudinal assessment (9-11). Understanding the time course and pathophysiological basis of cisplatin-induced vascular and renal injury is critical to inform surveillance and trials of treatment and prevention strategies.

Thus, we assessed the effects of cisplatin-based chemotherapy on endothelial function, metabolic parameters, fibrinolytic factors, and cardiovascular and renal biomarkers in the immediate peri-treatment phase followed by prospective, longitudinal assessments over 9 months in men with testicular cancer. In a further series of studies, we used forearm venous occlusion plethysmography, the gold standard method to evaluate endothelial function (12), to assess endothelial vasomotor and endogenous fibrinolytic function in testicular cancer survivors treated 1 to 7 years prior. We also assessed the in vitro effects of cisplatin on stress kinase signaling and thrombosis pathways in human aortic endothelial cell (HAEC) culture.

**METHODS**

The studies (NCT03557177 and NCT03557164) were approved by the West of Scotland Research Ethics Committee 4 and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

**COHORT 1: EARLY EFFECTS OF CISPLATIN-BASED CHEMOTHERAPY. Study participants.** Patients were recruited from the Beatson West of Scotland Cancer Centre between January 2016 and July 2017. Inclusion criteria included diagnosis of testicular/retroperitoneal germ cell cancer with orchidectomy ≤8 weeks prior and scheduled for cisplatin-based chemotherapy or surveillance. Participants were categorized into 3 groups: 1) surveillance; 2) 1 to 2 cycles BEP; or 3) 3 to 4 cycles BEP. Exclusion criteria included: carboplatin treatment; age <18 or >65 years; clinical trial participation; antiplatelet/lipid-lowering therapy; recreational drug use; inflammatory/infective/autoimmune disease; another malignancy in the previous 5 years; previous thrombosis; and inability to provide informed consent.

**Chemotherapy regimens.** Cisplatin-based chemotherapy regimens included BEP or etoposide and cisplatin (EP). Each treatment cycle lasted 21 days, with cisplatin administered on days 1 and 2 (cisplatin dose 50 mg/m²/day) or days 1 to 5 (cisplatin dose 20 mg/m²/day), such that the cumulative dose of cisplatin in each cycle of treatment was 100 mg/m². Patients attended outpatient bleomycin administration on days 8 and 15 (each dose 30,000 IU). Patients were treated with etoposide 165 mg/m² on days 1 to 3 if receiving 1, 3, or 4 cycles of BEP; or etoposide 120 mg/m² on days 1 to 3 if receiving 2 cycles of BEP. Patients with stage 1 disease received 1 to 2 cycles of BEP, a low-intensity cisplatin regimen. Patients with metastatic disease received 3 or 4 cycles of BEP, a high-intensity cisplatin regimen. Patients with metastatic disease or with a contraindication to bleomycin received 4 cycles of EP, with cisplatin

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organizations were not involved in the study design or conduct; data collection, analysis and interpretation; nor the preparation, review, or approval of the manuscript. The authors have reported that they have no relationships relevant to the contents of this paper to disclose. The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the JACC: CardioOncology author instructions page.

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administered over days 1 to 5 (cisplatin dose 20 mg/m²/day) to achieve cumulative cisplatin dose 100 mg/m². All patients received hydration containing potassium and magnesium before and after each cisplatin dose.

**Study assessments.** Initial assessments were performed ≥8 weeks after orchidectomy and ≥2 weeks pre-chemotherapy. In patients managed with surveillance, subsequent assessments were 1 to 2 weeks, 6 weeks ± 3 days, 3 months ± 1 week, 6 months ± 1 week, and 9 months ± 1 week after the initial assessment. In patients managed with chemotherapy, subsequent assessments were within 24 h of cisplatin administration and 6 weeks ± 3 days, 3 months ± 1 week, 6 months ± 1 week and 9 months ± 1 week (Figure 1). Participants fasted (with the exception of water) for 8 h and abstained from exercise, caffeine, and tobacco for 4 h before each assessment. Height, weight, and blood pressure (BP) were assessed at each visit.

**Primary outcome: endothelial function.** The primary outcome was change in endothelial function relative to baseline, assessed using the AngioDefender system (Everist Genomics, Ann Arbor, Michigan). This portable device allows bedside assessment of endothelial vasomotor function. It calculates percentage flow-mediated dilatation (%FMD) using a proprietary algorithm deriving changes in brachial artery diameter from pulse wave amplitude data before and after brachial artery occlusion with an upper arm cuff (13). Maximal post-occlusion change in brachial artery diameter relative to baseline is calculated and expressed as %FMD. The repeatability of AngioDefender %FMD is similar to traditional brachial artery ultrasound (BAUSS) assessment of %FMD (coefficient of variation 25.9% and 25.1%, respectively, according to personal communication with investigative teams (Everist Health, March 2015) for NCT02641197 and NCT02682576. AngioDefender quantification of %FMD is similar to BAUSS (Pearson’s correlation coefficient, r_p = 0.75; p < 0.0001) and correlates more strongly with 10-year Framingham risk score (r_p = −0.38; p < 0.001 AngioDefender; r_p = −0.24; p = 0.03 BAUSS) (14).

**Secondary outcomes: cardiovascular and renal biomarkers.** Fasting venous blood was collected at each visit. Serum creatinine, lipid profile, glycated hemoglobin A1c (HbA1C), and urine albumin to creatinine ratio (ACR) were measured in the local clinical laboratory. Estimated glomerular filtration rate (eGFR) was calculated using the 2009 Chronic Kidney Disease Epidemiology Collaboration
**TABLE 1** Cohort 1 (Early Effects Study): Baseline Characteristics in Groups Treated With Different Chemotherapy Strategies

|                      | Surveillance (n = 10) | 1 to 2 Cycles of BEP | 3 to 4 Cycles of BEP |
|----------------------|-----------------------|----------------------|----------------------|
|                      | Low-Intensity Cisplatin (n = 7) | High-Intensity Cisplatin (n = 10) |                      |
| Age, yrs             | 39 ± 3               | 31 ± 2               | 34 ± 2               |
| Height, m            | 1.79 ± 0.02          | 1.78 ± 0.03          | 1.77 ± 0.03          |
| Weight, kg           | 93.5 ± 7.0           | 90.4 ± 5.8           | 88.7 ± 3.8           |
| Body mass index, kg/m² | 28.9 ± 2.0           | 28.3 ± 1.4           | 28.3 ± 1.1           |
| Systolic blood pressure, mm Hg | 122.9 ± 5.3 | 134.1 ± 3.4 | 131.7 ± 4.3 |
| Diastolic blood pressure, mm Hg | 73.1 ± 4.2 | 76.7 ± 2.9 | 79.0 ± 3.0 |
| Heart rate, beats/min | 66.7 ± 0.3 | 70.9 ± 5.3 | 64.7 ± 2.8 |
| Cholesterol, mmol/l  | 5.2 ± 0.3            | 5.1 ± 0.5            | 5.5 ± 0.2            |
| Triglycerides, mmol/l | 1.2 ± 0.2            | 1.1 ± 0.2            | 1.9 ± 0.5            |
| LDL cholesterol, mmol/l | 3.3 ± 0.3         | 3.4 ± 0.4            | 3.5 ± 0.2            |
| HDL cholesterol, mmol/l | 1.2 ± 0.1           | 1.3 ± 0.1            | 1.8 ± 0.4            |
| Glucose, mmol/l      | 4.9 ± 0.1            | 5.1 ± 0.2            | 5.0 ± 0.2            |
| HbA1C, mmol/mol      | 34.7 ± 1.1           | 33.1 ± 1.0           | 35.5 ± 1.2           |
| vWF-Ag, %            | 44.3 ± 4.8           | 38.4 ± 3.4           | 45.2 ± 2.8           |
| Log urine ACR, mg/l  | −0.18 ± 0.10         | −0.23 ± 0.08         | −0.14 ± 0.05         |
| Histological diagnosis |                     |                      |                      |
| Seminoma             | 7 (70)               | —                    | 2 (20)               |
| Nonseminoma/mixed    | 3 (30)               | 7 (100)              | 8 (80)               |
| Performance status   |                      |                      |                      |
| O                    | 10 (100)             | 7 (100)              | 6 (60)               |
| I                    | —                    | —                    | 4 (40)               |
| Medical history      |                      |                      |                      |
| Hypertension         | 1 (10)               | —                    | 1 (10)               |
| Diabetes             | —                    | —                    | —                    |
| Smoker               | 1 (10)               | —                    | —                    |
| Medications          |                      |                      |                      |
| Alpha-blocker        | —                    | —                    | 1 (10)               |
| Angiotensin-II receptor blocker | —     | —                    | 1 (10)               |

Values are mean ± SEM or n (%). Units reported in mmol/l can be converted to mg/dl through the following conversion factors: cholesterol mmol/l to mg/dl = 38.6; triglyceride mmol/l to mg/dl = 88.5; glucose mmol/l to mg/dl = 18. ACR = albumin to creatinine ratio; BEP = bleomycin, etoposide, and cisplatin; HDL = high-density lipoprotein; LDL = low-density lipoprotein; vWF-Ag = von Willebrand factor antigen.

BEP or active surveillance 1 to 7 years previously were recruited. Exclusion criteria included ongoing clinical trial participation; vascular disease; asthma; chronic obstructive pulmonary disease; diabetes; atrial fibrillation/flutter; anticoagulation; tobacco/recreational drug use; inflammatory, infectious, or autoimmune disease; another malignancy within 7 years; and prior thrombosis. **Forearm venous occlusion plethysmography.** Studies were performed with the patient supine in a quiet, temperature-controlled room. Participants fasted for 4 h, abstained from alcohol for 24 h, and did not consume medications for 3 days before each study. Bilateral venous cannulae were inserted into large anteceubital fossa veins for venous sampling. Brachial artery cannulation was performed using a 27-standard-gauge steel needle for intra-arterial administration of locally active doses of drugs. Forearm blood flow (FBF) was measured in the infused and noninfused arms by venous occlusion plethysmography. Supine heart rate (HR) and BP were monitored (12,16).

**Pharmaceutical agents.** Pharmaceutical-grade bradykinin (BK) (Bachem, Bubendorf, Switzerland), acetylcholine (ACh) (Novartis Pharmaceuticals, London, United Kingdom), and sodium nitroprusside (SNP) (UL Medicines, Surrey, United Kingdom) were dissolved in physiological saline. BK is an endothelium-dependent vasodilator that provokes endothelial release of tissue plasminogen activator (t-PA). ACh is an endothelium-dependent vasodilator that does not provoke t-PA release. SNP is an endothelium-independent vasodilator. **Outcomes.** The primary outcome was change in BK-induced vasodilation. Secondary outcomes were change in ACh- and SNP-induced vasodilation, BK-induced tPA and plasminogen activator inhibitor (PAI)-1 release, and between-group differences in cardiovascular biomarkers. **Intra-arterial drug administration.** After a 20-min intra-arterial 0.9% saline infusion, participants received ascending doses of BK (100, 300, and 1,000 pmol/min), ACh (5, 10, and 20 μg/min), and SNP (2, 4, and 8 μg/min) for 6 min at each dose, with a 20-min 0.9% saline washout between agents. The infusion rate was maintained at 1 ml/min and the infusion order was randomized for each volunteer. **Blood sampling.** Venous blood was collected at baseline for lipid profile, HbA1C, vWF antigen, and ICAM-1 concentration (Vacuette, Kremsmünster, Austria). Full blood count, renal function, liver function, lipid profile, glucose, and HbA1C concentrations were measured in local clinical laboratories. Blood samples were simultaneously drawn from each...

**creatinine equation** (15). Enzyme-linked immunosorbent assays were performed to measure serum tissue plasminogen activator (Asserachrom, Stago, Reading, United Kingdom), plasminogen activator inhibitor-1 (Asserachrom), von Willebrand factor (vWF) (Asserachrom), and intracellular adhesion molecule (ICAM)-1 (Quantikine, R&D Systems, Abingdon, United Kingdom). Cystatin-C was measured using a particle enhanced turbidimetric immunoassay (Tinaquant, Roche, Germany). Serum high-sensitivity C-reactive protein and lipoprotein(a) were measured (Roche c311 analyzer) and high-sensitivity troponin-I was measured (Abbot, Architect i1000SR). Urine was collected for assessment of interleukin (IL)-18 (Quantikine, R&D Systems).

**COHORT 2: MEDIUM-TERM EFFECTS OF CISPLATIN-BASED CHEMOTHERAPY. Participants.** Testicular cancer survivors age 18 to 50 years managed with 3 to 4 cycles of...
arm at the end of equilibration and each BK dose into acidified buffered citrate (TrinILIZE Stabilyte, Co., Wicklow, Ireland) for t-PA assays and citrate (Vacuette) for analysis of PAI-1 (the major endogenous inhibitor of t-PA). Enzyme-linked immunosorbent assays were performed as described in the previous text to determine concentrations of t-PA antigen, PAI-1 antigen, vWF and ICAM-1, and PAI-1 activity (2B Scientific, Oxfordshire, United Kingdom).

**HUMAN AORTIC ENDOTHELIAL CELLS.** HAECs (Life Technologies, Paisley, United Kingdom) were cultured in endothelial cell growth medium (Promocell, Heidelberg, Germany) supplemented with 15 ml SupplementMix (Promocell) and penicillin/streptomycin 50 µg/ml. Confluent cells were rendered quiescent by serum starvation for 2 h in low-serum medium with 0.5% fetal bovine serum. Cells were stimulated with cisplatin (1, 3, or 15 µg/ml) (Accord Healthcare, Devon, United Kingdom) or vehicle (phosphate-buffered saline) for 5 min, 15 min, and 24 h.

**Immunoblotting.** HAECs were homogenized in lysis buffer and proteins (30 µg) were separated by electrophoresis as described previously (17). Membranes were probed with antiphosphorylated Akt (Cell Signalling [Danvers, Massachusetts] 4060, 1:1,000) and antiphosphorylated extracellular signal-regulated kinases 1/2 (ERK 1/2) (Cell Signalling 9101, 1:1,000). Protein phosphorylation levels were normalized to α-tubulin (Abcam ab4074, 1:1,0000) and expressed as percentage of the respective time point control, which was taken as 100%.

**Quantitative real-time polymerase chain reaction.** mRNA expression of t-PA (QT00075761, Qiagen, Manchester, United Kingdom) and PAI-1 (QT00062496, Qiagen) was assessed by qPCR. Total RNA was extracted using TRIzol (Qiagen) as previously described (17). Data are expressed as target gene/GAPDH housekeeping gene (Sense: GAGTCAACGGATTTGGTCGT; Anti-Sense: TTGATTTGGAGGGATCTCG; Eurofins Genomics, Ebersberg, Germany). Relative gene expression was calculated by the 2−ΔΔCt method, and results were compared with control.

**DATA ANALYSIS AND STATISTICS.** **Cohort 1: early effects of cisplatin-based chemotherapy.** Data were analyzed using 2-way analysis of variance (ANOVA) with repeated measures based on the general linear model and Dunnett’s correction for multiple comparisons. Power calculations determined that, at a significance of 5%, 10 subjects/group would provide 90% power of detecting 1.6% difference in % FMD between visits by paired Student’s t-test with SD of paired difference of 1.4 (13). Serum lipoprotein(a), urine ACR, and urine IL-18 (adjusted for urine creatinine concentration) were logarithmically transformed to ensure normality.

**Cohort 2: medium-term effects of cisplatin-based chemotherapy.** Forearm plethysmographic data were analyzed as described previously (18). Net t-PA and PAI-1 release were defined as the product of the infused forearm plasma flow and the concentration difference between infused and noninfused arms (18). Previous studies demonstrated that 8 subjects per group provides sufficient power to detect an approximately 20% change in FBF at 5% significance (19,20). The influence of a range of factors on FBF responses have been reported in similar sample sizes (21–24). Analysis was by repeated measures based on the general linear model or 1-way ANOVA.

**ENDOTHELIAL CELL CULTURE.** Data were analyzed by 1-way ANOVA with Dunnett’s correction for multiple comparisons (Western Blot analyses) or unpaired Student’s t-test (mRNA expression). Relative mRNA expression values were logarithmically transformed to ensure normality.

Variables are reported as mean ± SEM. Statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, California) with statistical significance at 5%.
TABLE 2  Cohort 1 (Early Effect): Cardiovascular Biomarkers

|                | Surveillance (n = 10) | 1-2 cycles of BEP (low-intensity cisplatin) (n = 7) | 3-4 cycles of BEP (high-intensity cisplatin) (n = 10) |
|----------------|-----------------------|-----------------------------------------------------|-----------------------------------------------------|
|                | Chol (mmol/l)         | HDL-C (mmol/l)                                      | LDL-C (mmol/l)                                      |
|                | 5.2 ± 0.3             | 1.2 ± 0.1                                           | 3.3 ± 0.3                                           |
| 1-2 weeks      | 5.1 ± 0.3             | 1.3 ± 0.1                                           | 3.2 ± 0.2                                           |
| 6 weeks        | 5.0 ± 0.3             | 1.2 ± 0.1                                           | 3.2 ± 0.2                                           |
| 3 months       | 4.7 ± 0.3             | 1.2 ± 0.1                                           | 3.0 ± 0.2                                           |
| 6 months       | 4.7 ± 0.3             | 1.2 ± 0.1                                           | 2.9 ± 0.2                                           |
| 9 months       | 4.8 ± 0.4             | 1.2 ± 0.1                                           | 3.0 ± 0.2                                           |

Values are mean ± SEM. *p < 0.001; †p < 0.05.

BL = baseline; Chol = cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; gluc = glucose; hs-CRP = high-sensitivity C-reactive protein; hs-TNI = high-sensitivity troponin I; ICAM = intracellular adhesion molecule; PAI = plasminogen activator inhibitor; TG = triglyceride; t-PA = tissue plasminogen activator; vWF:Ag = von Willebrand factor antigen.

TABLE 3  Cohort 2 (Medium-Term Effects): Baseline Characteristics in Groups Treated With Different Chemotherapy Strategies

|                | Surveillance (n = 14) | 3 to 4 Cycles of BEP (n = 12) |
|----------------|-----------------------|-------------------------------|
| Age, yrs       | 38 ± 2                | 36 ± 2                        |
| Body mass index, kg/m² | 25.6 ± 0.9             | 26.9 ± 0.8                    |
| Systolic blood pressure, mm Hg | 122.6 ± 3.7           | 128.4 ± 3.1                   |
| Diastolic blood pressure, mm Hg | 74.9 ± 2.7             | 74.1 ± 2.7                    |
| Heart rate, beats/min | 60.1 ± 2.5              | 60.9 ± 3.3                    |
| Cholesterol, mmol/l | 5.1 ± 0.3              | 4.9 ± 0.3                     |
| Triglycerides, mmol/l | 1.2 ± 0.2              | 1.4 ± 0.2                     |
| LDL cholesterol, mmol/l | 3.2 ± 0.3              | 3.1 ± 0.2                     |
| HDL cholesterol, mmol/l | 1.4 ± 0.1              | 1.3 ± 0.1                     |
| Glucose, mmol/l | 4.7 ± 0.1              | 4.5 ± 0.4                     |
| HbA1C, mmol/mol | 32.7 ± 0.7             | 33.1 ± 0.8                    |
| vWF:Ag, %         | 40.1 ± 2.2             | 43.0 ± 1.1                    |
| Histological diagnosis | 8 (57)              | 2 (17)                        |
| Seminoma          | 8 (57)                | 2 (17)                        |
| Nonseminoma/mixed | 6 (43)                | 10 (83)                       |
| Performance status | 0 (100)              | 12 (100)                       |

Values are mean ± SEM or n (%). Units reported in mmol/l can be converted to mg/dl through the following conversion factors: cholesterol mmol/l = mg/dl × 0.0259; triglyceride mmol/l = mg/dl × 0.0113; glucose mmol/l = mg/dl × 0.0555.

RESULTS

COHORT 1: EARLY EFFECTS OF CISPLATIN-BASED CHEMOTHERAPY. Vascular function. Participant characteristics are presented in Table 1. %FMD decreased 24 h after the first dose of cisplatin in the 10 patients managed with 3 to 4 cycles of BEP (16.7 ± 1.6 at baseline vs. 10.9 ± 0.9 at 24 h after cisplatin; p = 0.003) (Figure 2). At 6 weeks, %FMD had returned to baseline (15.7 ± 2.1; p = 0.97 vs. baseline) (Figure 2, Supplemental Table 1). %FMD was unchanged compared with baseline at all other times (Figure 2) (all p > 0.05). In the 7 patients managed with 1 to 2 cycles of BEP or surveillance, %FMD was not significantly different from baseline at any point (Figure 2) (p > 0.05). %FMD data were available for 157 of 162 study visits. Resting BP and HR were unchanged during treatment and follow-up in all groups (p = NS for all; data not shown).

Cardiovascular biomarkers. In patients managed with 3 to 4 cycles of BEP, serum cholesterol increased at 6 weeks (7.2 ± 0.5 mmol/l vs. 5.5 ± 0.2 mmol/l at baseline; p = 0.012) (Table 2). This remained numerically greater than baseline thereafter, but was not statistically significant. There were trends toward...
TABLE 2  Continued

| t-PA (ng/ml) | PAI-1 (ng/ml) | hs-CRP (mg/l) | ICAM-1 (ng/ml) | hs-TNI (pg/ml) | Log Urine ACR (mg/l) |
|-------------|--------------|--------------|----------------|----------------|----------------------|
| 11.9 ± 2.5  | 111.2 ± 4.3  | 2.1 ± 1.0    | 408.1 ± 37.4   | 0.24 ± 0.16    | −0.18 ± 0.10         |
| 13.5 ± 3.1  | 116.7 ± 3.3  | 2.1 ± 0.7    | 389.3 ± 47.7   | 0.19 ± 0.19    | −0.30 ± 0.05         |
| 12.1 ± 2.4  | 113.0 ± 3.1  | 1.7 ± 0.7    | 387.6 ± 39.1   | 0.47 ± 0.35    | −0.25 ± 0.06         |
| 12.8 ± 2.5  | 111.3 ± 5.3  | 2.0 ± 0.8    | 417.4 ± 32.1   | 0.13 ± 0.13    | −0.30 ± 0.04         |
| 13.7 ± 2.6  | 110.9 ± 3.9  | 3.4 ± 1.8    | 454.9 ± 58.9   | 0.15 ± 0.11    | −0.18 ± 0.07         |
| 16.7 ± 2.4  | 101.8 ± 6.2  | 1.8 ± 0.8    | 460.4 ± 23.4   | 0.31 ± 0.21    | −0.22 ± 0.07         |
| 9.0 ± 1.2   | 105.6 ± 8.0  | 3.8 ± 1.5    | 353.6 ± 37.5   | 0.19 ± 0.19    | −0.23 ± 0.08         |
| 6.1 ± 0.9   | 108.9 ± 6.7  | 2.2 ± 0.5    | 355.1 ± 32.6   | 0.43 ± 0.29    | 0.13 ± 0.04†         |
| 7.2 ± 0.9   | 109.5 ± 2.8  | 4.4 ± 1.9    | 437.8 ± 57.7   | 1.21 ± 0.50    | −0.21 ± 0.05         |
| 10.5 ± 2.4  | 111.1 ± 3.7  | 3.4 ± 1.3    | 436.9 ± 49.7   | 0.24 ± 0.24    | −0.21 ± 0.15         |
| 15.4 ± 3.6  | 105.0 ± 5.9  | 1.6 ± 0.5    | 452.7 ± 41.3   | 0.94 ± 0.51    | −0.29 ± 0.10         |
| 13.4 ± 3.2  | 101.7 ± 8.0  | 4.0 ± 2.1    | 425.6 ± 45.0   | 0.27 ± 0.27    | −0.09 ± 0.18         |
| 13.4 ± 1.3  | 114.4 ± 2.8  | 2.7 ± 1.5    | 411.1 ± 28.9   | 0.83 ± 0.55    | −0.14 ± 0.05†        |
| 13.5 ± 1.3  | 110.7 ± 2.9  | 1.4 ± 0.5    | 382.2 ± 32.1   | 0.12 ± 0.12    | 0.20 ± 0.07†         |
| 14.6 ± 2.7  | 115.5 ± 3.7  | 3.1 ± 1.6    | 460.8 ± 43.1   | 1.39 ± 0.63    | 0.20 ± 0.16          |
| 15.1 ± 2.0  | 104.5 ± 3.5  | 3.7 ± 1.9    | 495.2 ± 20.5   | 1.41 ± 0.70    | −0.23 ± 0.08         |
| 16.1 ± 2.6  | 108.0 ± 3.2  | 1.5 ± 0.4    | 392.2 ± 62.4   | 0.47 ± 0.24    | −0.28 ± 0.07         |
| 15.3 ± 2.5  | 110.4 ± 3.9  | 3.4 ± 1.5    | 440.8 ± 40.1   | 1.22 ± 0.40    | −0.17 ± 0.08         |

FIGURE 3  Cohort 1 (Early Effects Study): Renal Biomarkers

Changes in renal biomarkers among patients managed with surveillance (purple line), 1 to 2 cycles BEP (blue line), and 3 to 4 cycles BEP (red line): (A) Log urine ACR; (B) eGFR; (C) serum cystatin C; (D) log urine IL-18 per urine creatinine. Shaded areas indicate chemotherapy. The p values represent 2-way analysis of variance with correction for multiple comparisons. *p < 0.05; **p < 0.01; ***p < 0.001. ACR = albumin to creatinine ratio; Cr = creatinine; eGFR = estimated glomerular filtration rate; IL = interleukin; other abbreviations as in Figure 2.
increased triglycerides and low-density lipoprotein (LDL) cholesterol in patients managed with 3 to 4 cycles of BEP (p = 0.076 and p = 0.285 vs. baseline). This pattern was not seen in the other groups. Serum fasting glucose increased within 24 h in patients managed with 1 to 2 or 3 to 4 cycles of BEP (both p < 0.0001 vs. baseline), but did not change in patients managed with surveillance. There was a rise in HbA1c in the patients receiving 3 to 4 cycles of BEP at 6 weeks (p < 0.001), but thereafter it was not elevated versus baseline. vWF antigen increased at 6 weeks versus baseline in the 3 to 4 cycles of BEP group (p = 0.048) but was not different from baseline at any other time point or in any other group. High sensitivity troponin-I, ICAM-1, and high-sensitivity C-reactive protein were unchanged throughout (Table 2).

Renal biomarkers. In patients managed with 3 to 4 cycles of BEP, urine ACR increased at 24 h and 6 weeks (p = 0.011 and p = 0.014) and returned to baseline thereafter (Figure 3). Urine IL-18 increased at 24 h (p = 0.023) and returned to baseline by 6 weeks. Serum cystatin C increased at 24 h (p = 0.012) and 6 weeks (p = 0.004) and returned to baseline thereafter. eGFR was unchanged throughout (Figure 3). Urine ACR and IL-18 increased 24 h after 1 to 2 cycles of BEP (p = 0.038 and p = 0.039) and returned to baseline by 6 weeks. There were no other significant changes in this group or patients managed with surveillance.

Cohort 2: medium-term effects of cisplatin-based chemotherapy. Forearm arterial vasomotor function. Baseline characteristics are presented in Table 3. Intra-arterial BK, ACh, and SNP evoked dose-dependent vasodilation in all participants (all p < 0.0001) (Figure 4). Vasodilator responses to BK, ACh, and SNP were not different in patients managed with 3 to 4 cycles of BEP (n = 12) versus surveillance (n = 14) (p = 0.811, 0.866, and 0.938, respectively (Figure 4, Supplemental Table 2). BK-induced vasodilation data were available in 11 of 12 subjects managed with 3 to 4 cycles of BEP and all 14 subjects (100%) managed with surveillance.

BK-induced release of fibrinolytic factors. BK evoked dose-dependent net t-PA antigen release in patients managed with surveillance (−1.8 ± 10.6 mg/100 ml/min [baseline] vs. 316.4 ± 57.3 ng/100 ml/min [BK 1,000 ng/min]; p < 0.001) and 3 to 4 cycles of BEP (−7.7 ± 7.7 ng/100 ml/min [baseline] vs. 263.9 ± 67.7 ng/100 ml/min [BK 1,000 ng/min]; p < 0.001). There was no differences between groups (p = 0.285). BK did not evoke changes in net PAI-1 antigen in patients managed with surveillance (p = 0.524) or 3 to 4 cycles BEP (p = 0.502) and responses were not different between groups (p = 0.946).

In vitro effects of cisplatin on thrombotic and stress kinase pathways. HAEC exposure to cisplatin 3 μg/ml for 15 min increased Akt phosphorylation compared with control (n = 5; p = 0.032) (Figure 5). No changes were observed with other concentrations or periods of cisplatin exposure. Similar results were found for ERK 1/2 phosphorylation (n = 5; p = 0.026). t-PA mRNA expression decreased in cells exposed to cisplatin (n = 7; p = 0.014), whereas PAI-1 mRNA expression was unchanged (n = 7; p = 0.122) (Figure 5).

Discussion

Cisplatin-based chemotherapy is associated with endothelial vasomotor dysfunction, hypercholesterolemia, hyperglycemia, and renal dysfunction in testicular cancer patients (Central Illustration). These effects are confined to the immediate peri-chemotherapy period. Our observations suggest the early time period post-cisplatin treatment is one that is potentially of increased renal and cardiovascular risk, and one during which attention should be given to risk reduction with cardiovascular protection strategies.

A strength of the study is the early assessment of endothelial vasomotor function within 24 h following chemotherapy. Prior studies evaluating vascular function after cisplatin-based chemotherapy are limited by infrequent assessments performed months or years following treatment (6,9), potentially missing the period of maximum vascular injury and dysfunction. We demonstrate that, following 3 to 4 cycles of BEP, endothelial function is impaired at this acute and vulnerable time. Although endothelial function returned to baseline values at the subsequent 6-week assessment, it is biologically plausible that a similar transient deterioration in endothelial function is induced with each treatment cycle (5). Endothelial dysfunction is a key feature in de novo thrombosis and rupture of pre-existing atherosclerotic plaque (25). As such, it is an important component to the risk of thrombotic cardiovascular events in the early period following cisplatin-based chemotherapy.

In our cell-based study, exposure of HAECs to cisplatin was associated with decreased t-PA mRNA expression and activation of Akt and ERK 1/2. In previous work, cisplatin has been shown to reduce endothelial cell survival and induce apoptosis in human dermal microvascular endothelial cells (HMEC-1)
and in the vasa nervorum in rats (27). These data support the hypothesis that cisplatin induces direct endothelial toxic effects resulting in increased stress kinase signaling and a propensity for thrombosis via reduction in the capacity for endogenous fibrinolysis.

Although adverse thrombotic effects are most frequently observed in the early period following chemotherapy, cisplatin is detectable in serum for several years after treatment (28). This chronic exposure may provoke low-grade endothelial stimulation and a consequent pro-atherogenic environment (28,29). Our subsequent assessment was with forearm venous occlusion plethysmography, the gold standard for assessing endothelial function (12). In patients treated 1 to 7 years previously, there was no difference in endothelial vasomotor function between patients managed with orchidectomy and cisplatin-based chemotherapy versus those managed with orchidectomy alone. Furthermore, BK-induced t-PA release was not different in patients managed with or without cisplatin-based chemotherapy. The capacity for endothelial t-PA release is a sensitive, and mechanistically relevant, marker for the prediction of patients at risk of cardiovascular events (30).

Serum cholesterol and HbA1C increased transiently at 6 weeks after 3 to 4 cycles of BEP and similar trends were seen for LDL cholesterol and triglycerides. Although cisplatin-based chemotherapy may have contributed to these observations, we cannot conclude that this is the only explanation. Indeed, a reduction in tumor burden may have contributed to increases in cholesterol (31). Furthermore, glucocorticoid antiemetics may have contributed to changes in lipid profile and glycemia. However, we do not believe glucocorticoids are the sole explanation for the change in lipid parameters. Indeed, in a randomized study in which dexamethasone or placebo were given to healthy male volunteers, dexamethasone did not affect endothelial function, triglycerides, or LDL cholesterol, albeit with a different dexamethasone regimen to our study (32).

Most studies evaluating the risk of CVD after cisplatin-based chemotherapy have focused on the later period after treatment (3,8,33,34). In the 10 to 19 years following treatment, a 5- to 7-fold increased risk of CVD has been reported compared with patients managed with surveillance or the general population (8,33). Larger studies of up to 2,700 patients demonstrate a more modest 1.5- to 2-fold increased risk of CVD at 10 to 18 years after treatment (3,34). However, the incidence of thrombosis appears to be highest early after treatment. Between 9% and 11% have a thrombotic event within 1 year following 3 or more cycles of cisplatin-based chemotherapy (9,35).

Importantly, a more granular assessment of 15,006 patients revealed a 5-fold increased risk of cardiovascular death in the first year after treatment. This risk fell dramatically thereafter and was not significant after 1 year (1). Therefore, in keeping with our observations, the early period after treatment with 3 or more cycles of cisplatin-based chemotherapy.
appears to be the time period of increased cardiovascular risk. While these effects predominantly occur in the early period after treatment, cisplatin is detectable in serum several years after treatment (28). It remains possible that chronic exposure to low levels of cisplatin may cause low-grade endothelial stimulation that contributes to the pathophysiology underlying cardiovascular events occurring more than a decade after initial exposure (8,28).

Nephrotoxicity is a major, dose-limiting side effect of cisplatin that affects 20% to 40% of patients (4,36). We found evidence of early nephrotoxicity following 3 to 4 cycles of BEP as illustrated by increased serum cystatin C (37). Importantly, this returned to baseline by 3 months and, although there was an absolute decrease in eGFR, the change in this less-sensitive measure did not reach statistical significance.

Increased urinary ACR in the immediate period following chemotherapy supports the hypothesis that cisplatin induces renovascular endothelial and proximal tubular dysfunction and inflammation as evidenced by the rise in urinary IL-18 (4,36,38). We hypothesize that this profile of renal injury is compatible with widespread endothelial activation and injury.

**STUDY LIMITATIONS.** To our knowledge this is one of the most comprehensive, longitudinal evaluations of changes in vascular and renal function after cisplatin-based chemotherapy. However, there are some limitations that should be acknowledged. The number of patients in cohort 1 is small, particularly the group managed with 1 to 2 cycles of BEP, and it is not possible to conclude whether associations in this group did not reach statistical significance because of reduced power or because greater cancer burden potentiates the toxic effect of cisplatin-based chemotherapy. Furthermore, we did not adjust for potential confounders that may have contributed, such as the baseline elevated BMI in all patients.
groups, acute hyperglycemia perhaps due to steroids, and an acute inflammatory response to tumor cell death.

Vascular function was assessed using different approaches in the acute and long-term studies. The use of portable equipment allowed assessment of endothelial function at the patient’s bedside in the immediate post-chemotherapy period. We reserved the use of forearm venous occlusion plethysmography for studies in survivors after 1 year. These techniques primarily examine function at different levels of the arterial tree, and we accept this as a limitation. We did not use forearm venous occlusion plethysmography to assess the early vascular effects of cisplatin because this requires arterial cannulation in the context of a potentially pro-thrombotic state and is impractical in the peri-chemotherapy period. Serial assessment of %FMD each day during chemotherapy provides insight to the duration of effects from cisplatin on endothelial function; it would be helpful to assess %FMD in the period at least 1 year after treatment, but we limited assessments to maximize recruitment and retention of patients undergoing intensive treatment regimes. Although AngioDefender %FMD correlates with Framingham risk score (14), it has
not been demonstrated that AngioDefender %FMD is independently predictive of cardiovascular risk. Although we demonstrate deleterious effects of cisplatin-based chemotherapy in the early phase that are not evident in the medium-term, these findings could be further strengthened by the inclusion of a larger number of participants and the assessment of survivors treated >7 years previously. Heightened risk of CVD is reported up to 20 years after cisplatin-based chemotherapy (8), although endothelial function has not been assessed beyond 7 years after treatment, and it remains possible that endothelial dysfunction is also detectable in longer-term survivors.

CONCLUSIONS

Acute and transient endothelial toxicity, dyslipidemia, hyperglycemia, and nephrotoxicity are apparent in the early period following cisplatin-based chemotherapy, when cardiovascular risk is greatest (1). Our data highlight the early perichemotherapy period as an important window for focused surveillance of cardiovascular and renal health during which baseline and emergent cardiovascular risk factors should be treated aggressively. The evaluation of short-term preventative strategies, such as statins and antithrombotic therapies, is warranted in this group to allow cancer survivorship to come at the minimum cardiovascular cost.

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KEY WORDS germ cell tumors, platinum therapy, testicular cancer, thrombosis

APPENDIX For supplemental tables, please see the online version of this paper.
Cardiovascular Toxicity With Cisplatin in Patients With Testicular Cancer

Looking for Something Heavier Than Heavy Metal*

Joerg Herrmann, MD

It is said that two-thirds of anticancer drugs have their origin in serendipity, and this is most certainly true for cisplatin (1). Fifty-five years ago, Barnett Rosenberg inquired about the influence of an electrical field on cell division and found that the division of Escherichia coli showed an on-off effect depending on the power state (2). Two years later came the realization that the antiproliferative phenomenon had nothing to do with electricity but rather the release of a heavy metal, platinum, from the electrodes. Translating this knowledge to cancer cells was seemingly more straightforward and rewarding, and clinical trials commenced in 1972, leading to the approval of cisplatin for testicular cancer in 1978. Since then platinum drugs have been a game changer in the treatment of testicular cancer in particular, with a decrease in death rates by two thirds. In combination with other drugs, most commonly etoposide or bleomycin and etoposide (BEP), and orchidectomy and radiation therapy, cure rates for testicular cancer have exceeded 90%.

One of the most prominent toxicities noted in the original experimental studies was renal failure, and this concern has persisted. Other side effects noted relatively early in clinical practice included vascular events. These were primarily of two kinds: vaso-spasm, especially Raynauds, and venous and arterial thrombosis (3). Of interest, atherosclerotic plaques do not seem to be a prerequisite for acute coronary events in patients undergoing platinum-based therapies (4,5). This may suggest that induction of endothelial apoptosis with an erosion-type acute coronary syndrome might be the underlying mechanism (or thromboembolism from an alternate source) (6–8). Of further interest, even after the active treatment period, patients with testicular cancer may remain at higher risk for vascular disease and related events (9). Defining the excess cardiovascular risk, however, has not been easy, especially treatment-related cardiovascular mortality (9). In addition to the debate on the magnitude and duration of excess cardiovascular risk, there is the debate of whether cisplatin, alone or in combination with other therapies, even plays a causal role. Metabolic changes and the adverse cardiovascular effects of hypogonadism are well described in testicular cancer survivors (9).

The study by Cameron et al. (10) in this issue of JACC: CardioOncology aimed to address the nebulous landscape of cisplatin vascular toxicity in an elegant investigation of two cohorts of patients with testicular cancer. The first group included those with active disease for the assessment of early effects. Patients were stratified based on disease severity and related treatment strategy (active surveillance; 1 to 2 cycles of BEP; and 3 to 4 cycles of BEP). The second group included testicular cancer survivors 1 to 7 years from diagnosis, stratified by their management approach at the time: active surveillance or 3 to 4 cycles of BEP therapy. The key observations were as follows: in the early effects cohort, a decrease in flow-mediated dilation (FMD) of the brachial artery was recognized in the first 24 h of treatment in both BEP therapy
groups, but the effect was more profound and statistically significant in those receiving 3 to 4 cycles. Of note, the absolute value of FMD was not different between the 1 to 2 and 3 to 4 cycle groups, rather the latter group started with a higher baseline value. The duration of the effect was said not to differ between the two groups, but a recurrent decrease was noted at 9 months in the 3 to 4 cycle group. Von Willebrand factor (vWF) antigen levels increased significantly from baseline to 6 weeks in the 3 to 4 cycles of BEP group but not otherwise. High-sensitive troponin-I, intercellular adhesion molecule–1, and high-sensitivity C-reactive protein remained unchanged in all groups at all time points. The albumin-creatinine ratio increased in both treatment groups within 24 h and this increase persisted for 6 weeks in the 3 to 4 cycle group. An increase in urine interleukin-18 was seen in both treatment groups at 24 h, whereas an increase in Cystatin C was seen only in the 3 to 4 cycle group, with a lower level. In the survivor cohort, based on forearm blood flow response to bradykinin, acetylcholine and sodium nitroprusside (by venous occlusion plethysmography) showed no differences between those on surveillance and those who had received BEP. Likewise, bradykinin induced a dose-dependent net tissue plasminogen activator (but not plasminogen activator inhibitor-1) antigen release in both survivor groups. In human aortic endothelial cells, tissue plasminogen activator and plasminogen activator inhibitor-1 messenger RNA expression decreased significantly and nonsignificantly in cells exposed to cisplatin. An increase in protein kinase B and extracellular signal-regulated kinase 1/2 phosphorylation was seen seemingly with a defined optimum for dose and time constellation (maximal at an intermediate cisplatin dose and 15-min stimulation).

The results, as reported, echo prior studies in the field and thereby consolidate the evidence. For instance, in women with ovarian and endometrial

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**FIGURE 1** Risk of Vascular Disease and Events in Patient With Testicular Cancer

Patients with testicular cancer are at risk of vascular disease and events. Those with higher-dose cisplatin therapy face a higher acute and potentially long-term risk. Cardiovascular risk factors (CVRFs) and cardiovascular diseases (CVD) set a baseline risk level, and the acute risk with cancer therapy remains variable in extent and duration. Conceivably some patients show complete reversibility to baseline levels but others do not. The long-term risk is illustrated as a linear risk model, but may differ in reality and is influenced by various factors in the individual patient. Vasoreactivity testing in particular seems to be well suited to follow patients across this continuum of care and possibly to gauge the need and benefit of therapeutic interventions. CV = cardiovascular; vWF Ag = von Willebrand factor antigen.
cancer an acute decrease in FMD was seen as early as with the first cycle of carboplatin and paclitaxel chemotherapy (11). In conjunction with the current findings, one would conclude that the common denominator is platinum drugs and that there is no gender-related difference. Furthermore, one would conclude that platinum drugs seemingly induce relatively acute changes in endothelial cells that translate into a reduction in flow-mediated vasodilation and alterations in nitric oxide bioavailability. Indeed, a reduction of inducible nitric oxide production was shown in human umbilical vein cells along with a reduction in inducible protein kinase B and endothelial nitric oxide synthase phosphorylation (11). It is of interest that FMD decreased again in the 3 to 4 BEP cycle group at 9 months, although reportedly not to a significant degree. Nevertheless, it would be of interest to see if this recurrent decrease relates to any clinical events, and, in general, if long-term serial assessment of vascular reactivity, possibly even with other devices such as the EndoPAT (Itamar Medical, Caesarea, Hefa, Israel), can inform and guide management.

A high-risk vascular fingerprint for patients with testicular cancer undergoing cisplatin therapy was recently proposed, consisting of ≥3 of the following: body mass index >25 kg/m², current smoking, blood pressure >140/90 mm Hg (or treated), hyperlipidemia (or treated), and elevated fasting plasma glucose (12). vWF levels increased much more during therapy in the high-risk fingerprint group, which included 3 of 4 patients with arterial ischemic events. The dynamics of vWF levels were recapitulated in the current study by Cameron et al. (10), and it will remain important to confirm if such increases identify those at risk of (arterial) thrombotic events. Whether high-risk patients should be started on antiplatelet therapy is unknown at present and suitable for testing in a clinical trial.

Interestingly, a prior study in testicular cancer survivors showed that those patients who were exposed to cisplatin-based chemotherapy nearly 3 to more than 20 years ago had a more severe reduction in FMD and higher levels of circulating endothelial cells than those not exposed (13). The fact that cisplatin levels are detectable even nearly 30 years from therapy supports the theory of long-term exposure and endothelial injury (14). A difference in vascular reactivity between cisplatin-exposed and cisplatin-nonexposed was not seen in the current study by Cameron et al. (10). It is important, however, to realize that the vascular response was blunted in both groups, even in comparison with patients with metabolic syndrome (considered to account for the vascular outcomes in testicular cancer survivors as mentioned) (15). Such results can also not be unequivocally attributed to hypogonadism (16,17). Accordingly, more studies are needed to define the nature of the reduced vasoreactivity in patients with testicular cancer as well as the therapeutic and prognostic implications.

In summary, the authors are to be congratulated for their efforts and their contributions to the field. Their work raises awareness for the development of renal and endothelial dysfunction in patients with testicular cancer, which can progress to acute and chronic renal failure and acute and chronic vascular events. The risk of the individual patient, however, is still hard to predict. Of the various parameters, vascular reactivity testing is well suited to serve as a parameter of cardiovascular health and its modification by therapies and lifestyle interventions (Figure 1). All in all, patients with testicular cancer are a prime example of individuals who benefit from being followed in a cardio-oncology approach. Optimal management of cardiovascular risk factors and cardiovascular disease entities (as well as any renal disease) is as important as their most optimal oncology treatment approach. After all, cardiovascular toxicity with cisplatin use in patients with testicular cancer is a reality with the potential for something heavier than heavy metal serendipity.

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Left Ventricular Systolic Function in Long-Term Survivors of Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

BACKGROUND Allogeneic hematopoietic stem cell transplantation (allo-HSCT), a potentially curative therapy for malignant and nonmalignant diseases, is being increasingly used in younger patients. Although allo-HSCT survivors have an established increased risk of cardiovascular disease, there is limited knowledge of the long-term effects on cardiac function in survivors.

OBJECTIVES The purpose of this study was to describe left ventricular (LV) systolic function in long-term allo-HSCT survivors treated in childhood, adolescence, or early adulthood.

METHODS Our cross-sectional cohort study included 104 patients (56% women), age 18 ± 10 years at time allo-HSCT with 17 ± 6 years of follow-up. Echocardiography included 2-dimensional (2D) and 3-dimensional (3D) analyses and speckle tracking imaging. In total, 55 healthy control subjects with a similar age, sex, and body mass index were used for comparison. Left ventricular systolic dysfunction (LVSD) was defined as reduced 2D left ventricular ejection fraction (LVEF) of <52% in men and <54% in women, and/or a reduced global longitudinal strain (GLS) of ≤−17%. Multivariable linear regression was used to determine independent predictors of 2D-LVEF and GLS.

RESULTS Allo-HSCT survivors had significantly reduced LV systolic function compared with control subjects: 2D-LVEF (55.2 ± 5.8% vs. 59.0 ± 2.9%; p < 0.001), 3D LVEF (54.0 ± 5.1% vs. 57.6 ± 2.7%; p < 0.001), and GLS (−17.5 ± 2.2% vs. −19.8 ± 1.4%; p < 0.001). LVSD was found in 44.2%, of whom 28.3% were symptomatic. Clinical factors independently associated with 2D-LVEF and/or GLS included anthracyclines, graft versus host disease (GVHD), heart rate, and hypertension. In the 45% of survivors pre-treated with anthracyclines, the effect of anthracyclines on 2D-LVEF and GLS was dose-dependent.

CONCLUSIONS LVSD is common in long-term survivors of allo-HSCT treated in their youth. Pre-HSCT therapies with anthracyclines, age, heart rate, hypertension, and graft versus host disease are associated with measures of LV function. (J Am Coll Cardiol CardioOnc 2020;2:460–71) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a complex and potentially curative therapy for malignant and nonmalignant diseases. It is increasingly being used, particularly in younger individuals (1). Improvements in protocols and supportive therapy have resulted in improved initial survival rates (2). However, long-term survivors of allo-HSCT face high rates of life-debilitating complications (3–6). In patients that survive beyond 5 years, mortality rates are 4 to 9 times higher than the general population, corresponding with a 30% shorter life expectancy regardless of age at transplantation (7). The incidence of heart failure (HF) has been reported to be between 5.6% to 10.8% in those who have survived at least 10 years after HSCT (4,5,8,9), and the risk of cardiovascular (CV) related mortality is 2 to 4 times higher in HSCT survivors than in the general population (4). The relative role of different risk factors in the development of left ventricular systolic dysfunction (LVSD) in long-term allo-HSCT survivors is not well defined, especially in those treated during childhood, adolescence, and young adulthood. Furthermore, few studies have comprehensively evaluated heart function in long-term survivors of allo-HSCT. This observational study aimed to evaluate cardiac function in a population recruited nationwide who underwent allo-HSCT at a young age. Modern echocardiography techniques, including 3-dimensional (3D) imaging and speckle tracking echocardiography, were used to assess left ventricular (LV) systolic function.

METHODS

STUDY DESIGN AND INCLUSION CRITERIA. This Norwegian cross-sectional study included all survivors of allo-HSCT performed at Oslo University Hospital who were <30 years of age at HSCT, were age 16–18 years (born prior to August 1998) at inclusion, and had a minimum of 5 years of follow-up after allo-HSCT. Our hospital has been the single national center for allo-HSCT, and a complete nationwide cohort was identified by browsing the quality registry. Indications for allo-HSCT included malignant and nonmalignant diseases. A total of 11 individuals with mucopolysaccharidosis type 1 (Hurler syndrome) were excluded, as these patients may have multiorgan pathology as part of their primary disease. Medical history was documented retrospectively, including: disease type, pre-/post-transplantation therapies, other medical illness, risk factors, symptoms, and current medication. Anthracycline cumulative dosage was converted to isotoxic doses of doxorubicin (10). Written informed consent was obtained from all participants, and the study was approved by the Regional Committee for Medical and Health Research Ethics.

CLINICAL ASSESSMENT. Dyspnea was graded according to the New York Heart Association (NYHA) functional classification (11). Blood pressures (BPs) were acquired after echocardiography (>30 min), in the supine position as the average of 3 measurements. Blood samples were collected after overnight fasting and were analyzed at the hospital laboratory. N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations were determined by an electrochemiluminescence immunoassay (Roche proBNP II, Roche Diagnostics, Basel Switzerland), and troponin was measured using a high-sensitive immunoassay (Roche hs-TnT). Manufacturer’s recommendations were used for classifying elevated NT-proBNP according to the age- and sex-specific cutoffs, and for classifying elevated troponin as >14 ng/l. Hypertension was defined as current use of antihypertensive drugs, systolic BP >140 mm Hg, or diastolic BP >90 mm Hg. Diabetes mellitus type II was identified by hemoglobin HbA1c >6.5% (48 mmol/mol), or use of glucose-lowering medication. Hypothyroidism was defined by the use of thyroid replacement medication or serum TSH >4 mg/l and fT4 <9 pmol/l. Hypercholesterolemia was defined as LDL >4.1 mmol/l (160 mg/dl) or use of lipid-lowering medication. Acute and chronic graft versus host disease (GVHD) were graded by the Glucksberg and Schumlan scales, respectively (12,13).

ECHOCARDIOGRAPHY. Transthoracic echocardiography studies were performed using Vivid E9 scanners and dedicated software (Echo-PAC version 113.1.3, GE-Vingmed Ultrasound, Horten, Norway). All echocardiograms were acquired and analyzed by the same experienced investigator (R.J.M) en-bloc, and in
random order after the last patient inclusion. After acquisition and prior to analyses, all echocardiograms were de-identified, and the investigator was blinded to all of the patient’s medical records. Two-dimensional (2D) left ventricular ejection fraction (LVEF), 3D-LVEF, and global longitudinal strain (GLS) were measured on separate occasions to reduce bias. Studies followed current guidelines for evaluation of LV function and cardiotoxicity (14,15). Measurements were averaged from 3 consecutive heart cycles.

2D-LVEF was manually traced using the modified Simpson’s biplane method (14). 3D-LVEF was calculated with semiautomated software for endocardial detection, and was subsequently manually adjusted. A 3D pyramid volume acquisition was obtained from 4 to 6 cardiac cycles, adjusting for depth and sector width (60° to 70°), resulting in an average volume rate of 39 frames/s (range 29 to 51 frames/s). Sex-specific cutoffs for 2D-LVEF were used as recommended (14). GLS was performed on the 3 standard apical views of good acoustic quality, with similar frame rates (average 62 frames/s), sector depth, and heart rate. The region of interest was manually adjusted to correctly define the endocardial borders, apex, and mitral valve plane. The AFI software from GE calculated GLS as the average of peak systolic strain values in a 17-segment model. We considered GLS ≥−17% to be abnormal in young individuals, based on the lower limit of the 95% confidence interval (CI) found in our control group. LVSD was defined as reduced 2D-LVEF (men <52%, women <54%) and/or abnormal GLS (≥−17%).

Valve stenosis or regurgitation was categorized as mild, moderate, or severe according to guidelines (16). Pericardial thickness was assessed after careful adjustments of gain settings, and nonharmonic ultrasound set at 2.5 MHz. Pathology was defined as increased pericardial fluid >0.5 cm at end-diastole, and/or presence of abnormal thickening or fusion of the visceral and parietal membranes.

### MEASUREMENT ACCURACY AND INTRAOBSERVER VARIABILITY

In total, 25 recordings for 2D-LVEF and GLS from patients and control subjects were randomly selected for blinded intraobserver variability assessment. For intraobserver variability, the same images were analyzed >3 months apart by the same observer and software, blinded to the previous result. The average value of 3 repeated measurements was used to calculate intraclass correlation coefficient. The Cronbach’s alpha and intraclass correlation coefficient from average measures, using 2-way mixed and absolute agreement for 2D-LVEF, was 0.95 (95% CI: 0.89 to 0.98) and GLS 0.96 (95% CI: 0.89 to 0.99).

### CONTROL GROUP

A control group was recruited from healthy volunteers responding to advertisements. The sample size was estimated to detect differences between allo-HSCT patients and control subjects for the normally distributed variables 2D-LVEF and GLS. With 104 patients, an expected 10% SD in-group and α of 0.05, it was determined that 52 control subjects were required at a power of 0.83 to demonstrate a 5% difference in 2D-LVEF between groups. We included 55 control subjects to allow for a small buffer and still obtain a power >0.80. Control individuals were selected to obtain comparative group characteristics for race, ethnicity, sex, age, height, and body mass index (BMI). The only exclusion criterion was established CV disease.
Continuous data are presented as mean ± SD if normally distributed, or as median (25th, 75th percentile) if asymmetrically distributed. Categorical data are presented as numbers and percentages. Allo-HSCT survivors and control subjects were compared with the Student’s t-test for continuous data, and chi-square or Fisher exact test for categorical data. To adjust for any differences between the allo-HSCT group and control subjects when analyzing cardiac function, inverse-probability weighting (propensity scoring) was performed. In general linear regression analyses, the observations were weighted in each group by the inverse of the probability of being in that group with a given set of covariates: age, BMI, heart rate, and diastolic blood pressure (DBP). Multivariable linear regression analyses were conducted to determine significant predictors for the primary outcome variables of 2D-LVEF and GLS in survivors. Covariates included: age, height, BMI, heart rate, cumulative anthracycline dosage, sex, mediastinal radiation, total body irradiation, malignancy, hypertension, diabetes mellitus, hypercholesterolemia, hypothyroidism, smoking, and GVHD (acute and/or chronic). All continuous variables were standardized, and p values, beta, and corresponding 95% confidence intervals were reported. The final regression models considered multicollinearity, and contained all variables with p < 0.20 in a univariable regression and/or variables considered as clinically important risk factors for LVSD. Likewise, deletion was used for the data omissions: 2 for smoking in the univariable test, and 2 for hypercholesterolemia (high triglycerides). To evaluate the effects of anthracycline dosage, patients were allocated into 3 groups according to cumulative anthracycline dosage: none, moderate (<300 mg/m²), and high (≥300 mg/m²). Chi-square, Kruskal-Wallis test, and 1-way analysis of covariance with Bonferroni post hoc analyses were used to identify group differences. Analysis of covariance was used to adjust for age, BMI, heart rate, and DBP. Statistical analyses used SPSS version 25 (SPSS Inc., Chicago, Illinois), and a p value <0.05 was considered significant.

RESULTS

PATIENT DEMOGRAPHICS AND ALLO-HSCT TREATMENT CHARACTERISTICS. Patients characteristics are shown in Table 1. In total, 290 patients were treated during the time period specified for inclusion. Of these, 131 died prior to study start (total deaths 45.2%) and 2 were excluded due to incomplete patient files. Non-participants (n = 53) were younger (age 27.7 years vs. 34.3 years; p = 0.001), had shorter follow-up time (13.2 years vs. 16.5 years; p < 0.001), and were more likely men (37 men vs. 16 women; p = 0.005). A total of 104 participants (66.2% of eligible survivors) accepted the study invitation, provided informed consent, and completed clinical examinations (Figure 1). Malignant disease occurred in 74% (55.8% women), and was the indication for anthracycline chemotherapy in 45.2% (59.6% women) and mediastinal radiotherapy in 2 (1.9%). Median isotoxic cumulative anthracycline dosage was 270 mg/m² (range 45 to 585 mg/m²). Stem cells were obtained from the
bone marrow in 84.6%, and 29.8% had unrelated donors. In total, 95 (91.3%) received a myeloablative conditioning regimen consisting of busulfan in combination with cyclophosphamide. The total dosage of busulfan was 4 to 5 mg/kg/day administered orally over 4 days, combined with 50 mg/kg/day of cyclophosphamide administered intravenously over 4 days or 60 mg/kg/day over 2 days. In total, 23 (22.1%) received antilymphocyte globulin and 7 (6.7%) fractionated total body irradiation during conditioning. Cyclosporine was administered in 99% as part of standard GVHD prophylaxis. Resolved GVHD was identified in 29 (43.3%) of those with prior acute and/or chronic GVHD.

**Clinical Assessment and Risk Factors.** Table 2 presents clinical assessment findings. Of 104 allo-HSCT survivors, LVSD was detected in 46 (44.2%) survivors, of whom 13 (12.7% of all survivors) had NYHA functional class II or III symptoms, and 33 (32.4% of all survivors) were asymptomatic (NYHA functional class I). NYHA functional class II or III symptoms were present in 28 (27.5%) of all survivors. Elevated NT-proBNP was more prevalent among survivors than control subjects (17 vs. 2 participants; p = 0.029), and was found in 10 (58.8%) with LVSD. Cardiovascular medications (predominantly antihypertensive therapies) were prescribed in 19 (41.3%) survivors with LVSD. Compared with survivors without hypertension, those with hypertension (n = 42) were older (age 42 years vs. 30 years; p < 0.001), had longer follow-up time (19.1 years vs. 16.0 years; p = 0.004), had higher BMI (26.2 kg/m² vs. 23.4 kg/m²; p = 0.007), and were more likely to be on cardiac medications (76.2%). Compared with survivors without hypercholesterolemia, survivors with hypercholesterolemia had higher BMI (27.9 vs. 23.9 kg/m²; p = 0.004), were older (age 45 years vs. 33 years; p < 0.001), and had longer follow-up time (19.5 years vs. 16.6 years; p = 0.043). Among survivors, hypothyroidism was more frequent in women (9 women vs 1 man; p = 0.016). In comparing survivors with control subjects, no sex differences were observed for the risk factors: hypertension (p = 0.064), hypercholesterolemia (p = 0.374), GVHD (p = 0.206), or obesity (p = 0.375). At examination, allo-HSCT survivors had a slightly higher DBP (72 mm Hg vs. 66 mm Hg; p = 0.002) compared with the healthy control subjects.

### Table 2: Clinical Assessment

| Risk Factor | Allo-HSCT (n = 104) | Control Subjects (n = 55) | p Value |
|-------------|---------------------|--------------------------|---------|
| NYHA functional class | | | |
| I | 74 (72.5) | 55 (100) | <0.001 |
| II | 16 (15.7) | 0 (0.0) | 0.002 |
| III | 12 (11.8) | 0 (0.0) | 0.009 |
| Comorbidities | | | |
| Hypertension | 42 (40.4) | 1 (1.8) | <0.001 |
| Diabetes mellitus | 3 (2.9) | 0 (0.0) | 0.552 |
| Hypothyroidism | 10 (9.6) | 0 (0.0) | 0.016 |
| Hypercholesterolemia | 16 (15.4) | 0 (0.0) | 0.002 |
| Smoking (current/previous) | 10 (9.8)/18 (17.6) | 2 (3.6)/11 (20.0) | 0.015/0.717 |
| Cardiovascular medications | 33 (31.7) | 0 (0.0) | <0.001 |
| Statins | 4 (3.8) | 0 (0.0) | – |
| Calcium-channel blockers | 13 (12.5) | 0 (0.0) | – |
| Beta-blockers | 13 (12.5) | 0 (0.0) | – |
| Angiotensin-converting enzyme inhibitors | 7 (6.7) | 0 (0.0) | – |
| Angiotensin receptor blockers | 13 (12.5) | 0 (0.0) | – |
| Laboratory parameters | | | |
| Troponin T >14 ng/l | 3 (2.9) | 0 (0.0) | 0.551 |
| Elevated NT-proBNP | 17 (16.3) | 2 (4.0) | 0.029 |
| HDL cholesterol, mmol/l | 1.5 ± 0.4 | 1.7 ± 0.5 | 0.051 |
| LDL cholesterol, mmol/l | 3.1 ± 0.8 | 3.0 ± 0.9 | 0.433 |

Values are n (%) or mean ± SD. *2 absences in findings. t1 = 50. Elevated age = age 18 to 44 years: men = 86 ng/l, women = 130 ng/l; age 45 to 54 years: men = 121 ng/l, women = 249 ng/l. §Conversion rate to mg/dl = mmol/l × 38.66 (Roche Diagnostics, Basel, Switzerland).

allo-HSCT = allogeneic hematopoietic stem cell transplantation; HDL = high-density lipids; LDL = low-density lipids; NT-proBNP = N-terminal pro-B-type natriuretic peptide.
Univariable and multivariable linear regression analyses are shown in Tables 4 and 5. Statistically significant independent predictors for 2D-LVEF were age, cumulative anthracycline dosage, and GVHD. Significant independent predictors for GLS were age, cumulative anthracycline dosage, and hypertension. Anthracycline therapy was found to be a strong predictor of impaired LVEF and GLS. Further analysis confirmed a dose dependent relationship between anthracycline dose and reduction in 2D-LVEF and GLS, after adjusting for age, BMI, heart rate, and DBP (Table 6). Those who received higher anthracycline dosages tended to have greater evidence of cardiac dysfunction and adverse remodeling.

**Other Pathology.** Valvular heart disease was rare, and no lesions were severe. Pericardial pathology was found in 8 (7.7%) of the allo-HSCT survivors and none in the control group. In total, 7 cases of pericardial pathology occurred in those with acute and/or chronic GVHD (n = 67), although the difference was not statistically significant (p = 0.254).

**Discussion**

Our study is unique for several reasons. Allo-HSCT regimens in Norway have remained standardized without much radiation exposure. We have a complete hospital registry, long observation time (average 17 years), and applied contemporary echocardiographic techniques. To our knowledge, no prior studies have evaluated LV systolic function with comprehensive echocardiography in very long-term survivors of allo-HSCT in children, adolescents, and young adults. We found a high rate of LVSD, occurring in 44.2%, indicating that cardiac dysfunction in this patient group is more prevalent than previously documented. Symptomatic LVSD (NYHA functional class II or III) was found in 12.7% of the entire study population, which is higher than previously reported in other HSCT studies (4,5,8,9). An equally important finding was the high frequency of asymptomatic LVSD (NYHA functional class I), which was found in 32.3% of survivors that over time is likely to manifest as overt HF. In comparison, asymptomatic HF has

| Table 3: Echocardiography Assessment |
|-------------------------------------|
| **Allo-HSCT** (n = 104) | **Control Subjects** (n = 55) | **Inverse-Probability Weighting Beta (Standard Error). p Value** |
| IVSd, mm | 0.86 ± 0.17 | 0.89 ± 0.14 | 0.274 |
| IVSd >12 mm | 4 (3.8) | 2 (3.6) | 0.957 |
| Indexed LV mass index, g/m²† | 70.5 ± 18.0 | 73.5 ± 12.5 | 0.230 |
| Relative wall thickness | 0.30 ± 0.06 | 0.29 ± 0.05 | 0.263 |
| Indexed LVDD, cm/m² | 2.68 ± 0.32 | 2.69 ± 0.25 | 0.984 |
| Indexed LVIDs, cm/m² | 1.87 ± 0.30 | 1.84 ± 0.20 | 0.395 |
| Indexed 2D-LVEDV, ml/m² | 63.6 ± 13.3 | 71.9 ± 13.9 | 0.000 |
| Indexed 2D-LVESV, ml/m² | 28.8 ± 8.3 | 29.6 ± 6.5 | 0.562 |
| Indexed 3D-LVEDV, ml/m² | 71.5 ± 13.4 | 75.2 ± 12.8 | 0.083 |
| Indexed 3D-LVESV, ml/m² | 33.0 ± 8.6 | 31.7 ± 7.7 | 0.374 |
| 3D sphericity index | 0.33 ± 0.07 | 0.33 ± 0.06 | 0.652 |
| Cardiac index, l/min/m² | 2.62 ± 0.44 | 2.76 ± 0.47 | 0.082 |
| Fractional shortening, % | 30.6 ± 5.7 | 31.6 ± 4.0 | 0.210 |
| 2D-LVEF, % | 55.2 ± 5.8 | 59.0 ± 2.9 <0.001 | 3.80 (0.74), <0.001 |
| 2D-LVEF <52%, % <54% | 33 (31.7) | 0 (0.0) <0.001 |
| 2D-LVEF <50% | 17 (16.3) | 0 (0.0) <0.001 |
| 3D-LVEF, % | 54.0 ± 5.1 | 57.6 ± 2.7 <0.001 | 3.42 (0.68), <0.001 |
| 3D-LVEF <52%, % <54% | 29 (27.9) | 0 (0.0) <0.001 |
| MAPSE, mm | 12.9 ± 2.1 | 14.9 ± 2.2 <0.001 | 2.02 (0.34), <0.001 |
| s' velocity, cm/s | 8.0 ± 1.7 | 8.9 ± 1.7 0.002 | 7.72 (2.66), 0.004 |
| GLS, % | −17.5 ± 2.2 | −19.8 ± 1.4 <0.001 | 2.13 (0.30), <0.001 |
| GLS ≥ −17% | 34 (32.7) | 0 (0.0) <0.001 |
| Pericardial pathology | 8 (7.7) | 0 (0.0) 0.051 |

Values are mean ± SD or n (%), unless otherwise indicated. Indexed values to BSA. *Inverse-probability weighting, covariates: age, heart rate, body mass index, and diastolic blood pressure. †ASE cube formula (14).

<ref>TABLE 3. Echocardiography Assessment</ref>
previously been reported in 5.1% of long-term survivors of lymphoma when treated with autologous HSCT (9). Our study differed by defining LVSD with sex-specific 2D-LVEF cutoffs and GLS. Moreover, in our cohort, transplants were primarily of stem-cell origin, and although there were heterogeneous reasons for allo-HSCT, there were also few cases of radiotherapy. The lack of symptoms (NYHA functional class II or III) in a greater proportion of those with LVSD is unexpected. However, symptoms of dyspnea and fatigue are commonly accepted side effects of chemotherapy, possibly accounting for reduced recognition and lack of awareness. The absence of symptoms may also explain the infrequent prescription of cardioprotective medications in our cohort.

Plausible explanations for the high frequency of LVSD in this study population include exposure time, age at HSCT, pre-transplantation therapies, and post-transplant risk factors. Few previous studies have as long follow-up time as this present study; this is an important difference, because cardiac dysfunction after chemotherapy may worsen in parallel with observation time (4,8,17,18). However, this study cannot discern the precise timing of LVSD onset, and one could potentially speculate that cardiac injury occurred at time of therapy, with a further worsening in LVSD over time. Another possible explanation is that young patients are at higher risk of heart disease due to organ immaturity and growth disturbances caused by cardiotoxic therapies (19). This may explain the smaller LV size in survivors compared with control subjects, even after consideration for CV disease and confounders. Sex has previously been implicated as a risk factor for cardiotoxicity (8,19–21), but this association was not found in our study.

Cyclophosphamide has historically been noted to have cardiotoxic effects (22). However, published data linking alkylating agents to long-term heart disease is scarce. In contrast, anthracycline exposure is known to cause myocyte depletion, and is shown to increase the risk of HF by 5-fold in long-term

### CENTRAL ILLUSTRATION

**Left Ventricular Systolic Function in Long-Term Survivors Treated as Children, Adolescents, and Young Adults With Allo-HSCT**

| 2D-LVEF | 3D-LVEF | GLS | MAPSE (mm) |
|---------|---------|-----|------------|
| Controls | Allo–HSCT | Controls | Allo–HSCT |
| p < 0.001 | p < 0.001 | p < 0.001 | p < 0.001 |

Massey, R.J. et al. *J Am Coll Cardiol CardioOnc.* 2020;2(3):460–71.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) survivors were found to have significantly reduced left ventricular systolic function with echocardiography compared with healthy control subjects. Most cases were described as mild to moderate global hypokinesis with few cases of valve disease. Significant independent predictors of left ventricular systolic function were age, anthracyclines, graft versus host disease, heart rate, and hypertension. 2D = 2-dimensional; 3D = 3-dimensional; LVEF = left ventricular ejection fraction; GLS = global longitudinal strain; MAPSE = mitral annular plane systolic excursion.
survivors treated in their youth (23). The cardiotoxic effect of anthracycline was also confirmed in this study; anthracycline treatment was a significant independent predictor for the reduction of 2D-LVEF and GLS. Moreover, the reduction in systolic function and adverse LV remodeling were dose-dependent. These observations are in agreement with other studies that have used similar thresholds of cumulative anthracycline dosage (>250 to 300 mg/m²) (8,9,19,21,23).

In addition to cardiotoxic therapies, we found that traditional CV risk factors have a potential role in modifying the risk for LVSD in long-term survivors of allo-HSCT. CV risk factors are commonly reported in HSCT survivors, occurring more frequently in allo-HSCT compared with autologous-HSCT survivors, resulting in a higher prevalence of heart conditions in allo-HSCT survivors (6,18,24–26). Survivors in this study had a high prevalence of CV risk factors, but at a level comparable to other studies (6,18,20,21,24–26).

### TABLE 4 Linear Regression Analysis for Predictors of 2D-LVEF in allo-HSCT Survivors (n = 104)

|                          | Univariable | Multivariable |
|--------------------------|-------------|---------------|
|                          | β           | 95% CI        | p Value | β           | 95% CI        | p Value |
| Sex                      | 0.05        | –0.34 to 0.44 | 0.809   | –0.06       | –0.42 to 0.30 | 0.739   |
| Age, yrs*                | 0.27        | 0.09 to 0.46  | 0.005   | 0.29        | 0.07 to 0.52  | 0.011   |
| Height                   | –0.08       | –0.28 to 0.17 | 0.423   | –0.06       | –0.14 to 0.26 | 0.548   |
| Body mass index, kg/m²   | 0.18        | –0.01 to 0.38 | 0.062   | 0.06        | –0.01 to 0.38 | 0.952   |
| Heart rate, beats/min    | –0.01       | –0.20 to 0.19 | 0.952   | –0.1        | –0.12 to 0.91 | 0.527   |
| Mediastinal radiation    | –0.64       | –2.07 to 0.77 | 0.370   | –0.22       | –0.66 to 0.23 | 0.339   |
| Cumulative anthracycline dosage, mg/m² | –0.41 | –0.59 to –0.23 | 0.001 | –0.46 | –0.63 to –0.28 | 0.001 |
| Total body irradiation   | 0.26        | –0.52 to 1.03 | 0.517   | –0.20       | –0.91 to 0.51 | 0.580   |
| Malignancy               | –0.22       | –0.66 to 0.23 | 0.339   | –0.46       | –0.80 to 0.34 | 0.393   |
| Hypertension             | 0.13        | –0.27 to 0.53 | 0.511   | –0.18       | –0.60 to –0.24 | 0.393 |
| Diabetes mellitus        | –0.68       | –1.84 to 0.48 | 0.248   | –0.22       | –0.74 to –0.07 | 0.537   |
| Hypercholesterolemia     | 0.06        | –0.48 to 0.61 | 0.832   | 0.12        | –0.57 to 0.78 | 0.727   |
| Hyperthyroidism          | 0.12        | –0.55 to 0.78 | 0.727   | 0.12        | –0.55 to 0.78 | 0.727   |
| Smoking, current/previous| 0.11        | –0.34 to 0.55 | 0.632   | 0.11        | –0.34 to 0.55 | 0.632   |
| Graft versus host disease, acute and/or chronic GVHD | 0.29 | –0.12 to 0.69 | 0.164 | 0.64 | 0.26 to 1.03 | 0.001 |

Linear regression was conducted with standardized continuous variables, including 2-dimensional left ventricular ejection fraction (2D-LVEF) and dichotomous variables. *Age at examination.

### TABLE 5 Linear Regression Analysis for Predictors of GLS in allo-HSCT Survivors (n = 98)

|                          | Univariable | Multivariable |
|--------------------------|-------------|---------------|
|                          | β           | 95% CI        | p Value | β           | 95% CI        | p Value |
| Sex                      | 0.38        | –0.06 to 0.73 | 0.092   | 0.22        | –0.16 to 0.59 | 0.249   |
| Age, yrs*                | –0.06       | –0.26 to 0.14 | 0.548   | –0.24       | –0.45 to –0.02 | 0.031   |
| Height                   | 0.07        | –0.14 to 0.27 | 0.523   | –0.24       | –0.45 to –0.02 | 0.031   |
| Body mass index, kg/m²   | 0.01        | –0.21 to 0.22 | 0.964   | 0.25        | 0.07 to 0.44  | 0.009   |
| Heart rate, beats/min    | 0.33        | 0.13 to 0.52  | 0.001   | 0.15        | 0.53 to 0.93  | 0.001   |
| Mediastinal radiation    | 0.68        | –0.74 to 2.10 | 0.343   | 0.20        | –0.64 to 1.04 | 0.638   |
| Cumulative anthracycline dosage, mg/m² | 0.19 | –0.01 to 0.39 | 0.059 | 0.19 | –0.01 to 0.39 | 0.059 |
| Total body irradiation   | 0.40        | –0.44 to 1.24 | 0.345   | 0.20        | –0.64 to 1.04 | 0.638   |
| Malignancy               | 0.38        | –0.07 to 0.82 | 0.095   | 0.50        | 0.04 to 0.96  | 0.035   |
| Hypertension             | 0.48        | 0.08 to 0.87  | 0.019   | 0.50        | 0.04 to 0.96  | 0.035   |
| Diabetes mellitus        | 0.49        | 0.24 to 1.91  | 0.493   | 0.44        | –0.14 to 1.02 | 0.134   |
| Hypercholesterolemia     | 0.66        | 0.11 to 1.22  | 0.020   | 0.44        | –0.14 to 1.02 | 0.134   |
| Hyperthyroidism          | –0.37       | –1.03 to 0.29 | 0.271   | 0.44        | –0.14 to 1.02 | 0.134   |
| Smoking, current/previous| 0.20        | –0.25 to 0.65 | 0.372   | 0.44        | –0.14 to 1.02 | 0.134   |
| GVHD, acute and/or chronic GVHD | –0.02 | –0.44 to 0.39 | 0.919 | –0.29 | –0.69 to 0.11 | 0.158   |

Linear regression was conducted with standardized continuous variables (including global longitudinal strain [GLS]) and dichotomous variables. GLS is a negative value. Increases in GLS (e.g., with hypertension) reflect worsening in longitudinal shortening (systolic function). *Age at examination.

Abbreviations as in Table 4.
Any potential differences in reported CV risk factor prevalence are likely associated with cardiotoxic exposures, recipient age, and observation time. In our study, hypertension and hypercholesterolemia were more frequently observed in survivors with older age and longer survival time, suggesting that risk profiles may alter with aging after HSCT. One concerning finding from our cross-sectional analyses was that hypertension was prevalent in 40.4%, but the use of antihypertensive therapy in only 30.8%. As similarly high prevalence of hypertension (24% to 45%) has been reported in long-term survivors (>10 years) treated with HSCT at various ages (5,9,20,21,24,25). The significance of hypertension in contributing to prevailing cardiac disease in HSCT survivors has previously been shown (5,21). However, in our study, given that the onset and duration of hypertension is unknown, it is unclear if the cumulative consequences of hypertension were fully manifested. Indeed, the echocardiograms showed little evidence of concentric remodeling beyond a small but significant difference in indexed LV end-diastolic volume.

However, hypertension is known to cause myocardial fibrosis that leads to reduced longitudinal shortening of the heart (27). This may explain why hypertension was found to be an independent predictor of GLS.

Hypothyroidism has been reported in 30% of long-term survivors of HSCT with busulfan conditioning (28). Hypothyroidism was found in 9.6% in our cohort, although no association with LVSD was found in our data. Dyslipidemia has been reported in 13% to 52% of survivors of HSCT (5,6,18,20,24,25). Hypercholesterolemia occurred in 15% of our patients, and a trend between elevated cholesterol level and reduced GLS was observed. Overall, the contribution of CV risk factors to LVSD in allo-HSCT survivors stresses the importance of preventive strategies for reduction of risk factors and promotion of a healthy lifestyle. This is especially important in younger patients with potentially longer life expectancy.

In our study, GVHD was found to be a highly prevalent complication of allo-HSCT survivors. There is limited evidence that GVHD directly mediates myocardial damage. However, active chronic GVHD

### Table 6: Dose-Related Responses to Anthracycline Used in Pre-Treatment Therapies in allo-HSCT Survivors

| Response | None (n = 57) | Low Dosage (≤ 300 mg/m²) | High Dosage (> 300 mg/m²) | p Value |
|----------|--------------|-------------------------|---------------------------|---------|
| Cumulative anthracycline dosage, mg/m² | 0 (0, 0) | 170 (75, 200) | 435 (354, 464) | <0.001† |
| Total body irradiation | 4 (7.0) | 1 (4.0) | 2 (9.1) | 0.779 |
| Female | 28 (50.0) | 14 (56.0) | 14 (63.6) | 0.495 |
| Age, yrs | 35.1 ± 11.9 | 36.4 ± 11.7 | 32.8 ± 11.3 | 0.571 |
| Body mass index, kg/m² | 25.0 ± 5.7 | 25.2 ± 4.6 | 22.7 ± 3.6 | 0.146 |
| Systolic blood pressure, mm Hg | 124 ± 19 | 127 ± 20 | 112 ± 15 | 0.016* |
| Diastolic blood pressure, mm Hg | 73 ± 12 | 77 ± 14 | 66 ± 12 | 0.015* |
| Heart rate, beats/ min | 70 ± 11 | 70 ± 12 | 67 ± 11 | 0.511 |
| Hypertension | 23 (40.4) | 12 (48.0) | 7 (31.8) | 0.529 |
| Malignancy | 30 (52.6) | 25 (100.0) | 22 (100.0) | <0.001† |
| GVHD (acute and/or chronic GVHD) | 32 (56.1) | 15 (60.0) | 20 (90.9) | 0.013* |
| New York Heart Association functional class II or III | 10 (18.2) | 10 (40.0) | 8 (36.4) | 0.073 |
| NT-proBNP, ng/l | 33 (18, 58) | 52 (25, 148) | 110 (57, 182) | <0.001† |
| IVOD, cm | 0.90 ± 0.2 | 0.86 ± 0.1 | 0.79 ± 0.1 | 0.089§ |
| LVId, cm | 4.8 ± 0.5 | 4.9 ± 0.5 | 5.1 ± 0.4 | 0.002§ |
| LVİds, cm | 3.2 ± 0.5 | 3.5 ± 0.6 | 3.7 ± 0.4 | <0.001§ |
| LV mass, g/m² | 131.5 ± 49.9 | 133.1 ± 29.0 | 125.2 ± 36.2 | 0.264§ |
| Fractional shortening, % | 32.8 ± 4.7 | 29.2 ± 6.5 | 26.6 ± 4.4 | <0.001§ |
| 2D-LVEF, % | 57.0 ± 4.7 | 54.6 ± 7.0 | 51.1 ± 5.0 | <0.001§ |
| 3D-LVEF, % | 55.9 ± 3.9 (n = 48) | 52.7 ± 6.2 (n = 21) | 50.8 ± 4.4 (n = 19) | 0.001§ |
| s’ velocity, cm/s | 8.2 ± 1.4 | 8.0 ± 1.9 | 7.4 ± 2.0 | 0.039§ |
| MAPSE, mm | 13.2 ± 2.1 | 12.6 ± 2.1 | 12.5 ± 2.1 | 0.242§ |
| GLS, % | –17.9 ± 2.1 (n = 55) | –17.1 ± 2.1 (n = 24) | –16.8 ± 2.2 (n = 21) | 0.023§ |

Values are median (25th, 75th percentiles), n (%), or mean ± SD. *Significant difference between anthracycline ≤300 mg/m² and >300 mg/m² in Bonferroni post hoc analysis (<0.05). †Significant difference between both treatment groups (anthracycline ≤300 mg/m² and >300 mg/m²) with no anthracycline group in Bonferroni post hoc analysis (<0.05). §No significant difference between treatment groups in Bonferroni post hoc analysis. ¶Echocardiographical parameters are adjusted for covariates of age, heart rate, body mass index, and diastolic blood pressure. |
has been shown to be associated with a higher risk of CV death (5). It is thought that the chronic inflammation processes instigated by GVHD results in endothelial damage leading to accelerated atherosclerosis (29). We did not find GVHD (acute or chronic) to be consistently associated with LV systolic function, and found a very modest association with increased LVEF (β = 0.64; 95% CI: 0.26 to 1.03) and no association with GLS. GVHD may have an indirect role in altering cardiac function through the development of CV risk factors. Moreover, a higher proportion of pericardial pathology was observed in patients with GVHD. Although not statistically significant, it is numerically striking, may have clinical relevance possibly affecting systolic function, and supports the few previous reports on this matter (30). This is an area that deserves further study.

Echocardiography is a noninvasive, cost-effective, and readily available technique with a traditional reliance on 2D-LVEF to define systolic dysfunction. The limitations of traditional 2D-LVEF are well known, leading to 3D and speckle tracking echocardiography being recommended by expert consensus panels (15,31). The necessity for HSCT studies to use modern echocardiographic techniques to evaluate structural and functional changes in survivors has been promoted by oncology experts (32). Our study placed an emphasis on high-quality echocardiography with incorporation of modern techniques to ensure conclusive evidence of LVSD. We found a consistent difference between allo-HSCT survivors and control subjects in our measures of LV systolic function. These newer techniques can potentially lead to a higher reported incidence of cardiac dysfunction, but can also identify more patients at risk of developing HF. As is well-established, a relationship between LVEF and GLS exists, although these 2 parameters measure slightly different properties of systolic function. GLS reflects impaired longitudinal shortening and has been described as an earlier marker of myocardial damage, whereas reductions in LVEF (radial and circumferential contraction) are often first seen in more advanced remodeling (15,33).

A remaining challenge with GLS is the lack of consensus in defining absolute cutoffs for dysfunction, variability, and intervendor differences (27). The GLS cutoff used in our analysis was based on our normal data, using the same equipment and operator. This value (–17%) is conservative when compared with a meta-analysis performed by Yingchoncharoen et al. (27), which found the 95% CI for normal GLS to be –18.9% to –20.4% (27). The use of linear regression with a continuous outcome variable, however, allowed identification of predictors of LV function without dependency on cutoff values, and provided a specific analysis of incremental change.

**STUDY STRENGTHS AND LIMITATIONS.** The cross-sectional design of this study describes associations and not causality. This study cannot determine the timing of initial myocardial damage or conclude if deterioration of LV function is a part of a progressive continuum. The number of deaths attributed to solely CV reasons prior to study start is not known, as the CV status of nonstudy participants had not been recorded. Registry data showed nonparticipants to be younger with shorter follow-up, possibly resulting in overestimation of LVSD in our sample. Potential selection bias from recruitment of control subjects without CV disease was addressed by adjusting for baseline differences, and subgroup analyses focused on those without CV disease.

The strengths of this study are completeness of the cohort, nationwide patient inclusion, long follow-up, and standardized transplantation regimens with minimum confounding from radiation. We consider the data collection to be comprehensive, of high quality, and contemporary. All echocardiograms were performed and analyzed by the same investigator eliminating interobserver bias, were conducted blinded to medical status, and had excellent reproducibility.

**CONCLUSIONS**

In our study of 104 long-term allo-HSCT survivors treated in their youth, LV systolic function was found to be significantly reduced when compared with a healthy control group. We found 44% experienced LVSD, of whom 28.3% were symptomatic. Variables independently associated with LV systolic function were anthracyclines, age, heart rate, hypertension, and GVHD. Anthracyclines were found to have a significant dose-dependent effect on LV systolic function.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Long-term survivors treated in their youth with allo-HSCT are at increased risk of LVSD. Anthracyclines, age, heart rate, hypertension, and GVHD were found to be significant independent predictors of LV systolic function. A high prevalence of cardiac dysfunction and CV risk factors in young survivors after allo-HSCT suggests potential benefits from surveillance regimens that include echocardiography with strain imaging, and monitoring of modifiable risk factors.

TRANSLATIONAL OUTLOOK: Larger, prospective studies with contemporary imaging technologies are needed to understand the full impact of allo-HSCT on cardiac function.

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**KEY WORDS** anthracyclines, cardiovascular risk factors, echocardiography, graft versus host disease, heart failure, left ventricular systolic function

**APPENDIX** For a supplemental table, please see the online version of this paper.
EDITORIAL COMMENT

Not So Young at Heart*
Long-Term Cardiac Dysfunction in Young Adult Hematopoietic Cell Transplantation Survivors

Navneet S. Majhail, MD, MS,a Neel S. Bhatt, MBBS, MPHb

Cardiomyopathy and heart failure are well-described late complications of allogeneic hematopoietic cell transplantation (HCT). By the time they clinically manifest, significant and irreversible damage to the myocardium has already occurred with resultant substantial morbidity and mortality. A standardized screening tool that may identify early harbingers of cardiac injury in high-risk HCT recipients is currently lacking. Based on studies in childhood cancer survivors where subtle abnormalities have been shown to be associated with subsequent heart failure, echocardiography is frequently used for monitoring pediatric HCT survivors, although this modality is not widely used for the follow-up of adult recipients. More advanced technology such as 3-dimensional imaging and speckle tracking echocardiography may lead to greater sophistication in detecting early cardiac dysfunction, which may possibly lead to earlier interventions to mitigate risks of clinically manifest heart failure.

In this issue, Massey et al. (1) present a cross-sectional study of cardiovascular function in a cohort of long-term allogeneic survivors with malignant and nonmalignant disorders who underwent HCT as children, adolescents, and young adults. Cardiovascular function was measured through clinical, laboratory, and transthoracic echocardiography assessments in 104 survivors and 55 healthy controls irrespective of their cardiovascular risk-factor profile. At a median time of 17.2 years since HCT, survivors had significantly higher burden of left ventricular systolic dysfunction (LVSD), including decrease in 2-dimensional left ventricular ejection fraction and in global longitudinal strain compared to controls. Left ventricular ejection fraction and global longitudinal strain impairments correlated with greater cumulative anthracycline exposure. The investigators also found significantly higher prevalence of modifiable risk factors such as hypertension and hypercholesterolemia among survivors.

Most prior studies on cardiomyopathy and heart failure have primarily focused on either childhood cancer survivors or older adults with malignancy undergoing autologous or allogeneic HCT, and their extrapolation to adolescent and young adult HCT survivors including patients with nonmalignant diseases is challenging. Moreover, self-reported or registry-level data have been mostly used to study an overt and severe phenotype of cardiomyopathy/heart failure. Hence, this study is novel as it helps us understand the magnitude of subclinical LVSD among HCT survivors including recipients with nonmalignant diseases. They show a higher prevalence of LVSD with advanced echocardiographic imaging technology than what has been previously reported; especially in a cohort of HCT recipients who did not receive total body irradiation (TBI)-based conditioning regimens. Importantly, nearly three-

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the JACC: CardioOncology author instructions page.

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quarters of survivors with LVSD were asymptomatic. As noted by the investigators, the actual magnitude of the problem may be different because only 66% of study-eligible survivors completed the assessments; additionally, nearly 45% of HSCT recipients had died before the study initiation. Notwithstanding the limitations, these findings have several implications in survivorship care and warrant further discussion.

First, this study highlights the role of cardiovascular surveillance using echocardiograms in HCT survivors. Heart failure is recognized as a progressive disorder where patients move from subclinical dysfunction to overt heart failure over a variable period (2). It is important to detect and intervene during an earlier stage because the course is largely irreversible once heart failure has progressed to an advanced stage. However, there is no clear guidance regarding the need for routine screening, its frequency, or duration among HCT survivors. Routine use of serial echocardiograms in childhood, adolescent, and young adult cancer survivors exposed to anthracyclines and/or radiation with potential impact to the heart is endorsed by the Children’s Oncology Group long-term follow-up guidelines and International Late Effects of Childhood Cancer Guideline Harmonization Group. In contrast, the international guidelines for screening and preventive practices for HCT survivors do not provide any specific recommendations regarding echocardiographic surveillance (3). Recent publications have questioned the cost-effectiveness and reproducibility of echocardiograms in surveillance (4,5). This is especially relevant when considering cost- and resource-based challenges associated with performing routine serial echocardiograms for the entire and increasing numbers of HCT survivor population. The investigators do not tell us whether better technology adds substantially to the time-tested and widely available 2D echocardiography in identifying LVSD. Also, this study was not designed to tell us how and when to use echocardiography to screen for early cardiac dysfunction, and how often and in which patients these early changes progress to overt heart failure. However, the high prevalence of LVSD, especially in asymptomatic patients, makes the case for continued discovery of interventions to identify and mitigate progression of early cardiomyopathy.

This study provides an opportunity to discuss approaches to prevent occurrence of cardiomyopathy or heart failure in HCT survivors. Since anthracyclines and chest radiation have known association with these complications (6), several primary preventive interventions are under study to reduce their exposure in pediatric and young adult oncology population. The use of dexrazoxane as a cardioprotective agent has shown promise in mitigating anthracycline-induced cardiotoxicity in the short-term and warrants longer follow-up to understand its effects on reducing late cardiovascular dysfunction (7). Ongoing trials are investigating less cardiotoxic chemotherapy regimens (e.g., liposomal preparation of daunorubicin and cytarabine [CPX-351]) and radiation-free preparative regimens. For survivors with prior exposure to cardiotoxic therapies, similar to those in current study, emphasis must be placed on secondary preventive interventions. Angiotensin-converting enzyme inhibitors and beta-blockers have been used for this purpose in cancer survivors with variable degree of success and require further work in HCT population. It is also important to understand the role of modifiable risk factors such as hypertension, dyslipidemia, diabetes mellitus, and obesity in the development of late cardiac toxicity, for which HCT survivors are at increased risk due to conditioning regimen exposure and graft-versus-host disease (8). This study also highlights the question about why patients with similar HCT-related exposures have varying risks for developing LVSD and heart failure. A growing body of literature has shown the role of genetic risk factors in development of cardiotoxicity in childhood cancer survivors (9).

How can we apply the findings of this study and existent literature to pragmatically care for young adult allogeneic HCT survivors while we await high-quality evidence on appropriate screening and preventive interventions against cardiac late effects? First and foremost, these patients need life-long follow-up and high vigilance for early symptoms of cardiomyopathy and heart failure. Surveillance must be individualized with a focus on those who are particularly at high-risk (e.g., exposure to high anthracycline doses and TBI). This study shows that HCT survivors who get non-TBI-based conditioning but have prior anthracycline exposure also have a high prevalence of LVSD. Young-adult survivors can be screened with echocardiography using the schedule suggested by pediatric-focused guidelines. If resources do not allow screening of all survivors, imaging can be focused on patients with high-risk exposures. Routine screening and aggressive management of modifiable risk factors and counseling to...
adopt healthy diet, regular exercise, and minimize high-risk behaviors should be performed in clinical survivorship care, and probably has greater impact in reducing cardiac morbidity than general screening with imaging.

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KEY WORDS cardiac dysfunction, cardiac late effects, guidelines, hematopoietic cell transplantation, screening, survivorship
Doxorubicin-Induced Oxidative Stress and Endothelial Dysfunction in Conduit Arteries Is Prevented by Mitochondrial-Specific Antioxidant Treatment

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ABSTRACT

BACKGROUND Doxorubicin (DOXO) chemotherapy increases risk for cardiovascular disease in part by inducing endothelial dysfunction in conduit arteries. However, the mechanisms mediating DOXO-associated endothelial dysfunction in (intact) arteries and treatment strategies are not established.

OBJECTIVES We tested the hypothesis that DOXO impairs endothelial function in conduit arteries via excessive mitochondrial reactive oxygen species (ROS) and that these effects could be prevented by treatment with a mitochondrial-targeted antioxidant (MitoQ).

METHODS Endothelial function (endothelium-dependent dilation [EDD] to acetylcholine) and vascular mitochondrial ROS were assessed 4 weeks following administration (10 mg/kg intraperitoneal injection) of DOXO. A separate cohort of mice received chronic (4 weeks) oral supplementation with MitoQ (drinking water) for 4 weeks following DOXO.

RESULTS EDD in isolated pressurized carotid arteries was 55% lower 4 weeks following DOXO (peak EDD, DOXO: 42 ± 7% vs. sham: 94 ± 3%; p = 0.006). Vascular mitochondrial ROS was 52% higher and manganese (mitochondrial) superoxide dismutase was 70% lower after DOXO versus sham (p = 0.0008). Endothelial function was rescued by administration of the mitochondrial-targeted antioxidant, MitoQ, to the perfusate. Exposure to plasma from DOXO-treated mice increased mitochondrial ROS in cultured endothelial cells. Analyses of plasma showed differences in oxidative stress-related metabolites and a marked reduction in vascular endothelial growth factor A in DOXO mice, and restoring vascular endothelial growth factor A to sham levels normalized mitochondrial ROS in endothelial cells incubated with plasma from DOXO mice. Oral MitoQ supplementation following DOXO prevented the reduction in EDD (97 ± 1%; p = 0.002 vs. DOXO alone) by ameliorating mitochondrial ROS suppression of EDD.

CONCLUSIONS DOXO-induced endothelial dysfunction in conduit arteries is mediated by excessive mitochondrial ROS and ameliorated by mitochondrial-specific antioxidant treatment. Mitochondrial ROS is a viable therapeutic target for mitigating arterial dysfunction with DOXO. (J Am Coll Cardiol CardioOnc 2020;2:475–88) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Approximately 650,000 people undergo chemotherapy treatment annually (1). In many cases, these treatments are effective in treating the cancer, but severely damage the cardiovascular system of the surviving patients (2). As a result, cardiovascular disease is a leading cause of later morbidity and mortality among chemotherapy-treated cancer survivors (3).

Anthracyclines are first-line chemotherapy agents for several common cancers, including leukemias, breast cancer, and lymphomas, that exert particularly toxic effects on the cardiovascular system (4). Doxorubicin (DOXO) is the most commonly used anthracycline (5), and its use markedly increases the risk of subsequent cardiovascular disease (6). As such, elucidating the mechanisms underlying the adverse cardiovascular effects of DOXO is an ongoing research priority, as it may help identify therapeutic approaches.

To date, the majority of research on DOXO has focused on its effects on the heart and, specifically, cardiomyocytes. Mechanistic studies in preclinical models implicate excessive bioactivity of reactive oxygen species (ROS) such as superoxide and consequent oxidative stress in DOXO-induced damage to cardiomyocytes (7,8). The excessive ROS bioactivity in cardiomyocytes after DOXO treatment appears to be mediated by some combination of increased ROS production and suppression of endogenous antioxidant enzymes (8,9). Several sources of excessive ROS production have been identified, including the mitochondria (10). Indeed, DOXO directly induces mitochondrial dysfunction and stimulates superoxide production in cardiomyocytes (11,12).

Recent work suggests an important role of the vascular endothelium in DOXO-associated cardiovascular effects (13). DOXO is administered systemically, and vascular endothelial cells are exposed to circulating DOXO before the compound is taken up by cardiomyocytes and other tissues. In vitro studies performed in endothelial cell culture indicate that DOXO induces mitochondrial dysfunction, down-regulates antioxidant enzymes, and increases ROS bioactivity (14,15). Consistent with these observations, endothelial function of intact arteries assessed by endothelium-dependent dilation (EDD) is impaired in DOXO-treated animal models (16,17) and patients compared with untreated control subjects (18). However, the mechanisms by which DOXO induces this endothelial dysfunction in vivo have not been established, nor is there evidence that therapeutic strategies based on these mechanisms can preserve endothelial function after DOXO.

Here, we systematically investigated the underlying mechanisms by which DOXO impairs endothelial function in intact (conduit) arteries and show that a mitochondrial-specific antioxidant can prevent these effects.

**METHODS**

Detailed descriptions of all procedures and statistical analyses are provided in the Supplemental Material. Studies were approved by the University of Colorado Boulder Institutional Care and Use Committee (Protocol# 2618) and conformed to the Guide for the Use of Laboratory Animals.

Male C57BL6/J mice (4 months of age) were randomly assigned to receive DOXO (10 mg/kg intraperitoneal injection; n = 4) or sham (intraperitoneal injection of saline; n = 4). This method of administration causes cardiovascular dysfunction in 4-month-old C57BL6/J mice (19). An additional cohort of mice received DOXO (n = 4) or DOXO + the mitochondrial-targeted antioxidant mitoquinol mesylate (10-[2,5-dihydroxy-3,4-dimethoxy-6-methylphenyl] decyl) (MitoQ) (250 μmol/l in drinking water; n = 5) for 4 weeks (20), initiated on the same day immediately following DOXO. Data are presented separately for each cohort for vascular endothelial function, but DOXO samples were combined for biochemical experiments. Estrogen may be protective against DOXO-induced cardiac dysfunction (21); thus, only male mice were used in the present study to determine the mechanism by which DOXO causes vascular endothelial dysfunction without the confounding effects of female sex hormones.

All mice were euthanized 4 weeks following treatment via cardiac puncture (under inhaled isoflurane anesthesia). Blood was spun and plasma was isolated and saved for cell culture experiments (22) and for assessment of circulating factors, as described in the Supplemental Appendix. Following sacrifice, carotid arteries were excised and cannulated on glass pipettes for assessment of endothelium-dependent and -independent dilation, as described in the Supplemental Appendix. Then, the thoracic aorta was dissected and cleaned, and 1-mm rings were used for assessment of total (22) and mitochondrial-specific ROS (20) or were flash frozen, as described in the Supplemental Appendix. All data are reported as mean ± SEM.
RESULTS

ANIMAL CHARACTERISTICS. Young (4-month-old) male C57BL6/J mice were administered with a single intraperitoneal injection of sterile 1× phosphate-buffered saline (Sham) or DOXO (10 mg/kg in sterile 1× phosphate-buffered saline) and sacrificed 4 weeks later. As observed in previous studies (16,23), body mass at the time of sacrifice was lower (−12%) in the group administered with DOXO (Table 1). Lower body mass with DOXO was associated with lower heart, quadriceps, gastrocnemius, and epididymal white adipose tissue mass, food consumption (−17%), and water intake (−26%) (Table 1). There were no significant differences in the mass of other organs (Supplemental Table 1).

EFFECT OF DOXO ON VASCULAR ENDOTHELIAL FUNCTION. Carotid artery resting (DOXO: 419 ± 10 μm vs. sham: 412 ± 11 μm; p = 0.702) and maximal (DOXO: 449 ± 12 μm vs. sham: 457 ± 11 μm; p = 0.653) diameters did not differ between groups. Carotid artery EDD, measured by the increase in luminal diameter in response to increasing concentrations of acetylcholine, was 57% lower in DOXO versus sham control mice (peak EDD: DOXO, 40 ± 7% vs. sham, 94 ± 3%; p = 0.006), indicating a marked impairment in vascular endothelial function with DOXO treatment (Figure 1A). To determine how DOXO impairs EDD, we first measured endothelium-independent dilation as the dilatory response to sodium nitroprusside, a nitric oxide (NO) donor. We found no difference between groups (peak: DOXO, 40 ± 7% vs. sham, 40 ± 3%; p = 0.653), indicating that impaired EDD with DOXO is likely due to excessive ROS-mediated EDD (Figure 1B). Next, to determine if this excess ROS had a functional role in the impaired EDD of DOXO-treated mice, we assessed EDD in isolated carotid arteries with and without prior incubation with the superoxide dismutase (SOD) mimetic/ROS scavenger, TEMPOL. We found that TEMPOL administration completely restored peak EDD in the DOXO-treated mice to sham levels (Figures 2B and 2C), indicating that impaired EDD with DOXO is likely due to excessive vascular ROS bioactivity. Moreover, we found no differences in the abundance of the cytosolic (SOD1) and extracellular (SOD3) isoforms of SOD, a major endogenous antioxidant enzyme, in the aorta of the DOXO and sham animals (SOD1: p = 0.157; SOD3: p = 0.854) (Figures 2D and 2E). These observations indicate that DOXO-associated EDD impairment is likely due to excessive bioactivity of ROS without stimulating expected compensatory increases in SOD1 or SOD3.

ROLE OF MITOCHONDRIAL-SPECIFIC ROS IN DOXO-INDUCED ENDOTHELIAL DYSFUNCTION. DOXO is reported to induce excess bioactivity of mitochondrial ROS in endothelial cells in vitro (25), but the effects of DOXO on mitochondrial ROS in intact arteries has not been investigated. Accordingly, we next assessed mitochondrial ROS bioactivity in the aorta. We found that aortic mitochondrial ROS was 52% greater (p < 0.001) in DOXO-treated mice compared with sham (Figure 3A). To determine if this excess mitochondrial ROS plays a role in DOXO-induced endothelial dysfunction, we incubated carotid arteries from DOXO-treated mice with the mitochondrial-targeted antioxidant, MitoQ, prior to assessing EDD. Incubation with MitoQ fully restored peak EDD (%) in DOXO treated mice to levels of DOXO versus sham: p = 0.002) in the DOXO- than Sham-treated mice (Figure 2A).

Table 1: Body Mass, Tissue Mass, Energy Intake, and Water Intake

|          | Sham (n = 4) | DOXO (n = 4) | p Value |
|----------|-------------|-------------|---------|
| Body mass, g | 28.5 ± 0.9  | 25.2 ± 1.0  | 0.017   |
| Heart, mg    | 151.5 ± 15.1| 123.5 ± 4.5 | 0.042   |
| Quadriceps, mg | 389.8 ± 18.8| 290.0 ± 14.8| 0.003   |
| Gastrocnemius, mg | 309.0 ± 4.3 | 233.8 ± 20.4| 0.029   |
| Epididymal white adipose tissue, mg | 468.5 ± 19.9 | 187.1 ± 25.7 < 0.001 |
| Food intake, kcal/day | 12.6 ± 0.4 | 10.5 ± 0.3 | 0.024   |
| Water intake, ml/day | 3.8 ± 0.1 | 2.8 ± 0.1 | 0.003   |

Values are mean ± SEM. DOXO = doxorubicin.

pathophysiological states (24). To determine the effect of DOXO on vascular ROS bioactivity, we first assessed ROS in the aorta using electronic paramagnetic resonance spectroscopy and found it was 40% greater (p = 0.002) in the DOXO- than Sham-treated mice (Figure 2A). Next, to determine if this excess ROS had a functional role in the impaired EDD of DOXO-treated mice, we assessed EDD in isolated carotid arteries with and without prior incubation with the superoxide dismutase (SOD) mimic/ROS scavenger, TEMPOL. We found that TEMPOL administration completely restored peak EDD in the DOXO-treated mice to sham levels (Figures 2B and 2C), indicating that impaired EDD with DOXO is likely due to excessive vascular ROS bioactivity. Moreover, we found no differences in the abundance of the cytosolic (SOD1) and extracellular (SOD3) isoforms of SOD, a major endogenous antioxidant enzyme, in the aorta of the DOXO and sham animals (SOD1: p = 0.157; SOD3: p = 0.854) (Figures 2D and 2E). These observations indicate that DOXO-associated EDD impairment is likely due to excessive bioactivity of ROS without stimulating expected compensatory increases in SOD1 or SOD3.
observed in sham control mice (Figures 3B to 3C). To determine if increased vascular mitochondrial ROS after DOXO treatment developed in conjunction with reduced endogenous mitochondrial antioxidant defenses, we also assessed aortic protein abundance of manganese SOD, the mitochondrial isoform of SOD (SOD2). We found that aortic SOD2 abundance was 70% lower in DOXO versus sham (p < 0.001) (Figure 3D). Together, these results indicate that the greater total bioactivity of vascular ROS and tonic ROS-related suppression of EDD in DOXO-compared with sham-treated mice is mediated by excessive bioactivity of mitochondrial ROS in the absence of appropriate compensatory up-regulation mitochondrial SOD antioxidant defenses.

**STIMULATION OF TOTAL AND MITOCHONDRIAL ROS BIOACTIVITY BY DOXO TREATMENT IS INDUCED BY SYSTEMIC CIRCULATING FACTORS.** We have recently shown that in vivo pharmacological treatments can influence ROS bioactivity in cultured endothelial cells by inducing changes in the circulating milieu that persist after the treatment compound has been cleared from the circulation (22). We hypothesized that this mechanism might be involved in excessive ROS stimulation after DOXO treatment, as DOXO and its primary metabolite, doxorubicinol, are cleared from plasma within 24 to 96 h of administration (26). To test this hypothesis, we used an ex vivo model in which human umbilical vein endothelial cells (HUVECs) are treated for 24 h with ROS with plasma obtained from DOXO and sham-treated mice (upon sacrifice) after which ROS was assessed (22).

We first sought to establish that total ROS bioactivity was increased in HUVECs exposed to plasma from DOXO-treated mice compared with sham control mice. To do so, we assessed total cellular ROS using the CellROX fluorescent probe (Thermo Fisher [Waltham, Massachusetts]; catalog# C10422) and found that ROS bioactivity was greater in HUVECs incubated with plasma from DOXO-treated mice compared with sham control mice (p < 0.001) (Figures 4A and 4E).

Next, we aimed to determine if enhanced mitochondrial ROS contributed to the greater total HUVEC ROS induced by DOXO administration by assessing mitochondrial ROS bioactivity using the MitoSOX fluorescent probe (Thermo Fisher, Catalog# M36008). We observed greater mitochondrial ROS in HUVECs exposed to plasma obtained from DOXO compared with sham-treated animals (p < 0.001) (Figures 4B and 4E). To determine if this greater ROS bioactivity was linked to changes in mitochondrial volume, we incubated HUVECs with the fluorescent probe, Mito-Tracker Green (Thermo Fisher, Catalog# M7514). We found that mitochondrial volume in HUVECs was 40% lower (p < 0.001) following incubation with plasma from DOXO-treated mice compared with sham control mice (Figures 4C and 4E). By accounting for mitochondrial volume, we established that DOXO-induced stimulation of mitochondrial ROS is a result of greater mitochondrial ROS per volume of mitochondria, and not due to an increase in mitochondrial number (DOXO- vs. sham-treated mice; p = 0.002)
Collectively, these findings demonstrate that plasma from DOXO-treated mice increases total ROS bioactivity in endothelial cells ex vivo, and that greater mitochondrial ROS contributes to this effect, independent of DOXO-induced reductions in endothelial cell mitochondrial volume.

**DOXO-INDUCED CHANGES IN SYSTEMIC FACTORS RELATED TO MITOCHONDRIAL ROS BIOACTIVITY.**

**Metabolomics.** We next sought to identify changes in circulating molecular factors and pathways that may have contributed to the higher mitochondrial ROS bioactivity associated with DOXO treatment. To do so, we first performed a targeted metabolomics analysis on plasma from DOXO- and sham-treated mice, focusing on central carbon and nitrogen metabolites, which are key components of redox homeostasis (27). Partial least squares discriminant analysis showed a discrimination of the plasma metabolite profiles of the 2 groups (Supplemental Figure 1), indicating clear differences in the DOXO- and sham-treated mice. We found that selective metabolites associated with mitochondrial ROS...
differed in plasma from the DOXO versus sham animals, including: diphosphate (p = 0.048), which typically is reduced during states of mitochondrial oxidative stress (28); lactate (p = 0.031), a byproduct of glycolysis that in some physiological states may be reduced to preserve redox homeostasis in mitochondria (28); guanine (p = 0.046), a nucleotide base that could be reduced by excess mitochondrial ROS (29); ribose phosphate (p = 0.044), a product of the pentose phosphate pathway that is commonly lower during periods of oxidative stress and is associated with mitochondrial dysfunction (30); and 5-hydroxyindoleacetate (p = 0.033), a primary metabolite of serotonin that is implicated in...
oxidative stress and mitochondrial toxicity (31) (Supplemental Figure 2). To determine associations of these plasma metabolites with mitochondrial-specific aortic ROS, we performed linear regression analyses. We found that plasma diphosphate (p = 0.053) and lactate (p = 0.067) tended to be positively associated with aortic mitochondrial ROS (Supplemental Figure 3). A full report of metabolite abundance is provided in Supplemental Table 2.

**Dot blot array.** To expand on this initial analysis of potential circulating signals influenced by DOXO treatment, we next targeted inflammatory pathways, given that DOXO administration is associated with higher levels of pro-inflammatory proteins in the circulation (32). To address this aim, we assessed plasma concentrations of 20 different cytokines and chemokines via a dot-blot array. We found that all but 2 of these markers did not differ significantly in plasma from DOXO-treated mice compared with sham control mice (Table 2). The exceptions were the anti-inflammatory cytokine interleukin-4, which was only slightly (<5%), but significantly (p = 0.043) lower in the DOXO mice, and vascular endothelial growth factor-A (VEGF-A), which stood out as being 40% lower in the DOXO treated group (p < 0.001). Moreover, plasma VEGF-A was inversely associated (p = 0.001) with mitochondrial ROS (Supplemental Figure 4). These findings indicated that VEGF-A, a protein signaling molecule in endothelial cells, may be associated with DOXO-induced modulation of ROS in endothelial cells.

**RESTORING VEGF-A IN PLASMA FROM DOXO-TREATED MICE REDUCES MITOCHONDRIAL ROS BIOACTIVITY IN ENDOThelial CELLS.** We next sought to determine the possible effects of reduced circulating levels of VEGF-A in regulating DOXO-induced mitochondrial ROS bioactivity in HUVECs. To accomplish this, we supplemented plasma from

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**FIGURE 4.** Plasma From Doxorubicin-Treated Mice Increases Total and Mitochondrial Specific Reactive Oxygen Species in Vascular Endothelial Cells

Quantification of (A) whole cell reactive oxygen species (ROS) (CellROX) and (B) mitochondrial ROS (MitoSOX) in human umbilical vein endothelial cells (HUVECs) following a 24-h incubation with plasma from sham and doxorubicin (DOXO)-treated mice or with fetal cow serum (FCS) (control condition). (C) HUVEC mitochondrial number (MitoTracker Green) following a 24-h incubation with plasma from sham- and DOXO-treated mice. (D) Mitochondrial ROS expressed relative to mitochondrial number in HUVECs following 24-h incubation with plasma from sham- and DOXO-treated mice. (E) Representative images of CellROX, MitoSOX, and MitoTracker in HUVECs following 24-h incubation with plasma from sham- and DOXO-treated mice. Data are the mean ± SEM. *p < 0.05 vs. sham.
DOXO-treated mice with 90 pg/ml VEGF-A to normalize VEGF-A to concentrations observed in sham control mice, and then assessed the effect on mitochondrial ROS in HUVECs via MitoSOX fluorescence after a 24-h incubation period. We found that restoring VEGF-A in plasma from DOXO-treated mice normalized mitochondrial ROS in HUVECs compared with sham plasma (Figure 5). To demonstrate that this effect of VEGF-A supplementation of plasma was specific to DOXO-treated mice, we also supplemented sham plasma with the equivalent concentration of VEGF-A and found no change in mitochondrial ROS (sham vs. sham + VEGF-A; p = 0.937). Overall, these observations suggest that reductions in circulating VEGF-A may contribute to greater mitochondrial-derived ROS bioactivity in endothelial cells following DOXO.

**IN VIVO SUPPLEMENTATION WITH MitoQ PREVENTS DOXO-INDUCED VASCULAR ENDOTHELIAL DYSFUNCTION BY SUPPRESSING BIOACTIVITY OF MITOCHONDRIAL ROS AND PRESERVING NO SIGNALING.** Collectively, our findings strongly implicate excess mitochondrial ROS as a key mechanism mediating DOXO-induced endothelial dysfunction. As such, we postulated that targeting excess mitochondrial ROS following DOXO administration may be an effective strategy for preventing endothelial dysfunction with DOXO. To explore this possibility, we next determined if in vivo supplementation of the mitochondria-specific antioxidant MitoQ (250 μmol/l in drinking water for 4 weeks) could prevent DOXO-induced endothelial dysfunction versus administration of DOXO alone. MitoQ supplementation was initiated immediately following DOXO administration, rather than concomitantly, as the National Cancer Institute advises against adjunct antioxidant administration with chemotherapy (33). This guideline is based on concerns that antioxidant therapy may interfere with the effectiveness of these cancer-suppressing drugs and may lead to adverse clinical outcomes (34).

**Animal characteristics.** In vivo MitoQ supplementation did not influence body weight (DOXO: 26.2 ± 1.0 g vs. DOXO + MitoQ: 26.2 ± 1.4 g; p = 0.927), energy intake (DOXO: 10.7 ± 0.3 kcals/day vs. DOXO + MitoQ: 10.3 ± 0.6 kcals/day; p = 0.794), or water intake (DOXO: 2.6 ± 0.1 ml vs. DOXO + MitoQ: 2.7 ± 0.2 ml; p = 0.945) in DOXO-treated mice. MitoQ supplementation did not influence heart, skeletal muscle, or adipose tissue mass (Supplemental Table 3).

**Vascular endothelial function: NO-mediated EDD.** Carotid artery resting (DOXO: 415 ± 9 μm vs. DOXO + MitoQ: 417 ± 32 μm; p = 0.946) and maximal (DOXO: 455 ± 13 μm vs. DOXO + MitoQ: 448 ± 8 μm; p = 0.667) diameters did not differ between the groups (resting, p = 0.90; maximal, p = 0.981). Most importantly, MitoQ supplementation completely prevented DOXO-induced impairments in carotid artery EDD (peak EDD: DOXO + MitoQ vs. DOXO; p = 0.002) (Figure 6A).

Next, we determined if in vivo MitoQ supplementation prevented DOXO-induced impairments in EDD by influencing endothelium-independent dilation (i.e., by altering vascular smooth muscle sensitivity to NO). We found no difference in the dilatory responses to sodium nitroprusside between DOXO and DOXO + MitoQ mice (peak EDD; p = 0.637) or Sham and DOXO + MitoQ mice (peak EDD, p = 0.645), indicating no effects of MitoQ on smooth muscle sensitivity to NO (Figure 6B).

We then sought to determine if MitoQ supplementation prevented DOXO treatment-induced impairments in NO-mediated EDD. Administration of the NO synthase inhibitor L-NAME abolished group differences in peak EDD (DMQ vs. DOXO: p = 0.525; DMQ vs. sham: p = 0.453), indicating that MitoQ supplementation prevented the DOXO-induced impairment in NO-mediated EDD (Figure 6C).

### TABLE 2 Plasma Cytokine, Chemokine, and VEGF-A Levels (pg/ml) Following Sham (n = 4) and DOXO (n = 8) Administration

|       | Sham (n = 4) | DOXO (n = 8) | p Value |
|-------|-------------|-------------|---------|
| IL-1x | 197 ± 1     | 196 ± 2     | 0.862   |
| IL-1β | 347 ± 3     | 347 ± 1     | 0.686   |
| IL-2  | 258 ± 16    | 272 ± 6     | 0.342   |
| IL-3  | 715 ± 32    | 699 ± 16    | 0.211   |
| IL-4  | 870 ± 4     | 844 ± 5     | 0.043   |
| IL-5  | 722 ± 1     | 714 ± 2     | 0.091   |
| IL-6  | 95 ± 1      | 89 ± 7      | 0.097   |
| IL-9  | 185 ± 4     | 194 ± 4     | 0.113   |
| IL-10 | 284 ± 1     | 277 ± 5     | 0.062   |
| IL-12p70 | 162 ± 1     | 156 ± 6     | 0.216   |
| IL-13 | 155 ± 2     | 155 ± 1     | 0.303   |
| IL-17A| 150 ± 2     | 142 ± 8     | 0.053   |
| CCL5 | 251 ± 2     | 250 ± 1     | 0.607   |
| CXCL1| 227 ± 1     | 228 ± 1     | 0.764   |
| GM-CSF| 808 ± 13    | 788 ± 8     | 0.225   |
| IFN-γ| 1081 ± 2    | 1080 ± 1    | 0.644   |
| MCP-1 | 204 ± 1     | 201 ± 3     | 0.075   |
| M-CSF| 317 ± 1     | 318 ± 2     | 0.905   |
| TNF-α| 47 ± 5      | 34 ± 2      | 0.055   |
| VEGF-A| 228 ± 3     | 138 ± 2     | <0.001  |

Values are mean ± SEM and are expressed relative to an internal positive control. Values in bold indicate a statistical difference. Plasma was analyzed by immunoblot.

CCL = chemokine (C-C motif) ligand; CXCL = chemokine (C-X-C motif) ligand; GM-CSF = granulocyte-macrophage colony stimulating factor; IFN = interferon; IL = interleukin; MCP = monocyte chemotactant protein; M-CSF = monocyte-colony stimulating factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.
Last, to determine if in vivo MitoQ supplementation prevented DOXO-induced reductions in NO-mediated EDD by inhibiting mitochondrial ROS-related suppression of EDD, we incubated carotid arteries from DOXO$^+$MitoQ mice with MitoQ, prior to assessing EDD. There was no further improvement in EDD following acute MitoQ incubation (Peak EDD: DMQ vs. DMQ$^+$MitoQ; p = 0.997) (Figure 6D). Together, these observations provide experimental evidence that in vivo MitoQ supplementation might be an effective strategy for preventing DOXO treatment-associated stimulation of mitochondrial ROS, vascular endothelial dysfunction, and specifically, the NO-mediated component of EDD.

**DISCUSSION**

DOXO chemotherapy has been shown to impair endothelial function (i.e., reduce EDD) in conduit arteries of cancer survivors (18). However, the underlying mechanisms and associated therapeutic targets have not been established, because mechanism-focused investigations to date have been largely limited to endothelial cell culture models (35,36). In the present study, we first determined that DOXO treatment impaired endothelial function in conduit arteries as a result of decreased NO-mediated EDD and not due to reduced vascular smooth muscle responsiveness to NO. Next, we identified excessive ROS as a key mechanism underlying DOXO-induced endothelial dysfunction in conduit arteries, and then identified mitochondria as a key source of the excess ROS bioactivity. Subsequently, we established that systemic circulating factors from the DOXO-treated mice stimulated increased endothelial mitochondrial ROS and identified potential molecular transducers that differed in plasma obtained from the DOXO and sham groups. Because lower concentrations of VEGF-A in the DOXO mice appeared to be the strongest signal, we restored VEGF-A to sham control levels and showed that doing so normalized endothelial mitochondrial ROS. Last, to establish excessive mitochondrial ROS as a potential therapeutic target for preventing DOXO treatment-induced endothelial dysfunction in conduit arteries, we supplemented the drinking water of mice with the mitochondrial-targeted antioxidant, MitoQ, for 4 weeks following DOXO administration. Oral MitoQ supplementation prevented the impairment in EDD in DOXO-treated mice by preventing excessive mitochondrial ROS-driven oxidative stress and consequent reductions in NO-mediated EDD. Overall, our findings demonstrate that excessive mitochondrial ROS bioactivity is a key mechanism in DOXO-induced endothelial dysfunction in conduit arteries and provide initial evidence for the potential efficacy of mitochondrial ROS-lowering therapies to preserve vascular health in cancer survivors treated with such drugs.
DOXO accumulates in the mitochondria (37) and damages mitochondrial DNA (38), which can lead to an increased production of ROS from the mitochondria (39). Moreover, and consistent with our findings, in vitro experiments have shown that treatment of cultured cardiomyocytes and endothelial cells with DOXO results in elevated production of mitochondrial ROS (15) and reduced abundance of mitochondrial antioxidant enzymes (11,40). The present results extend these observations by demonstrating that DOXO treatment in vivo stimulates excess mitochondrial ROS in intact conduit arteries while suppressing SOD2 abundance, that is, rather than inducing an appropriate physiological compensatory increase in this important mitochondrial antioxidant. These findings also are in agreement with previous work reporting excess production of mitochondrial ROS and impaired vascular endothelial function in mice with genetic SOD2 insufficiency (41). It should be noted that other ROS generating pathways may also contribute, including oxido-reductases such as endothelial NO synthase or NADPH cytochrome P450 reductase (42).

Another key finding from our experiments was that changes in the composition of the circulating milieu may contribute to DOXO treatment-associated...
An increase in mitochondria-derived reactive oxygen species (ROS) with doxorubicin contributes to a state of oxidative stress and reduction in nitric oxide (NO) signaling that promotes the development of endothelial dysfunction in conduit arteries. Mitochondria-targeted antioxidant treatment with MitoQ may be a therapeutic strategy for reducing oxidative stress and preserving conduit artery endothelial function with doxorubicin to reduce cardiovascular disease risk.
stimulation of excess vascular ROS, consistent with recent observations from our laboratory in the setting of aging (22). The scope of the present investigation precluded an exhaustive interrogation of the identity of the factors involved. However, using a targeted plasma metabolomics analysis, there was a tendency to an overall lower abundance of nucleic acid bases (adenosine, thymidine, cytidine, and guanine), as well as glycolytic and pentose phosphate intermediates, all of which may be associated with states of higher oxidative stress (28,30,43). Particularly, diphosphate and lactate were positively related to vascular mitochondrial ROS. In a second targeted analysis, we assessed the abundance of circulating cytokines, chemokines, and VEGF-A in plasma from DOXO and sham mice, given that DOXO chemotherapy increases circulating abundance of pro-inflammatory cytokines (32) and inflammation can stimulate synthesis of ROS (44). This analysis revealed substantially lower circulating VEGF-A concentrations in the DOXO-treated mice. As a follow-up, we subsequently showed that restoring VEGF-A to sham levels normalized mitochondrial ROS in endothelial cells, demonstrating a role for VEGF-A in regulating endothelial cell mitochondrial ROS with DOXO. These results are in agreement with previous findings in mice in which whole-body overexpression of VEGF-B prevented DOXO-induced impairments in aortic EDD and cardiomyocyte mitochondrial respiration (17).

Chronic oral supplementation with the mitochondrial targeted antioxidant MitoQ reverses age-related impairments in NO-mediated endothelial function by mitigating excessive mitochondrial ROS-related suppression of EDD (20,45). The present study demonstrates that chronic MitoQ supplementation also prevents the increases in mitochondrial ROS and consequent reductions in NO-mediated EDD in conduit arteries induced by DOXO treatment. This is potentially clinically relevant as MitoQ is commercially available and safe for use in humans (46,47), and we have shown that 6 weeks of oral supplementation with this compound improves endothelial function in healthy older adults (45). Patients who have undergone DOXO chemotherapy also have vascular endothelial dysfunction (18), and nonspecific (48) and mitochondrial-targeted (49,50) antioxidant therapies can be effective in settings of DOXO-induced cardiomyopathy. As such, MitoQ may hold promise for anthracycline-treated patients, although the use of antioxidant treatment with chemotherapy should strictly adhere to National Cancer Institute guidelines (33).

**STUDY LIMITATIONS.** Future preclinical studies should consider other experimental approaches to facilitate translation to patient populations, including use of tumor-bearing animals to model human cancer; administering DOXO intravenously in smaller consecutive doses over time; larger sample sizes; and mechanistic analyses to determine if the treatment has direct or off target effects. Additionally, future studies should assess cardiac function in parallel with vascular function.

Endothelial cells produce endothelin (ET)-1, a pro-vasoconstrictor and pro-atherogenic molecule that is elevated in the setting of heart failure (51) and in patients treated with DOXO chemotherapy (52,53). Moreover, ET-1 stimulates mitochondrial ROS production in endothelial cells (54). Thus, strategies aimed at preventing DOXO-induced increases in ET-1 may lower endothelial mitochondrial ROS production and improve cardiovascular function.

**CONCLUSIONS**

Our results demonstrate that DOXO-induced endothelial dysfunction in intact conduit arteries is mediated by reduced NO signaling secondary to excessive bioactivity of mitochondrial ROS in the absence of an appropriate compensatory upregulation of mitochondrial SOD (Central Illustration). Importantly, we show that chronic oral supplementation with the mitochondrial-targeted antioxidant MitoQ can prevent DOXO-induced endothelial dysfunction in conduit arteries of nontumor-bearing mice by mitigating excessive mitochondrial ROS suppression of EDD. Healthy vascular endothelial function is associated with a marked reduction in future risk of heart failure (55), the major clinical cardiovascular consequence of anthracycline treatment (56). As such, lifestyle and pharmacological strategies that preserve endothelial function hold promise for preventing heart failure and other adverse cardiovascular outcomes associated with anthracycline cancer therapy.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: This study demonstrates that excessive mitochondrial reactive oxygen species is a key mechanism underlying doxorubicin-associated vascular endothelial dysfunction. Furthermore, this study suggests that oral mitochondrial-targeted antioxidant supplementation is a potential therapeutic strategy for treating excess mitochondrial reactive oxygen species-related suppression of vascular endothelial dysfunction with doxorubicin.

TRANSLATIONAL OUTLOOK: Future studies should determine the long-term efficacy of mitochondrial-targeted antioxidant supplementation in treating doxorubicin-induced vascular endothelial dysfunction in tumor-bearing animals, so as to more closely resemble the human condition. Supplementation should occur at a time following chemotherapy that is aligned with the guidelines put forth by the National Cancer Institute. A detailed assessment of cardiac function is also necessary prior to human studies.

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KEY WORDS chemotherapy, mitochondrial antioxidant, reactive oxygen species

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this paper.
Anthracycline antibiotics have been in use for more than half a century in the treatment of cancer and the dose-limiting cardiovascular toxicity has been recognized for most of that time. Despite this and other toxicities, anthracyclines continue to be important components of chemotherapy for both solid and hematologic malignancies. There has been a great deal of effort put into understanding the mechanisms for the cardiovascular toxicity of anthracyclines and development of strategies to limit the adverse effects, while not interfering with the antitumor efficacy. Preclinical models have been used extensively, and many rodents have been sacrificed in the hopes of making anthracyclines a safer class of medications for humans. Clayton et al. (1) have added to that effort and in an elegant set of experiments advance the concept that protection of vasculature with repletion of mitochondrial coenzyme Q10 could be the answer to this age-old question.

The effects of anthracyclines on mitochondrial function with increased generation of oxidant stress are well established, including the effect on mitochondrial coenzyme Q10 synthesis (2). This has led to clinical studies examining whether coenzyme Q10 repletion can prevent cardiotoxicity (3). What is novel in the work from Clayton et al. (1) is the use of modified coenzyme Q10 that is predominantly taken up into mitochondria — “MitoQ.” Using this orally bioavailable, over-the-counter antioxidant, they show that the endothelium-dependent vasodilation of carotid arteries is protected in mice treated with a single high dose of doxorubicin. Noting a drop in the levels of vascular endothelial growth factor A (VEGF-A), the investigators advance the hypothesis that doxorubicin disruption of endothelial VEGF could be central to the effects of doxorubicin on arterial function.

Preclinical experiments and clinical studies looking to understand and prevent anthracycline-associated cardiovascular dysfunction abound, and it is worth considering how the current study jives with the rest of this literature. Anthracyclines are used clinically in multiple distinct dosing schedules, and the significance of preclinical studies should be considered in this context. In the current experimental protocol, Clayton et al. (1) use a single high-dose exposure, similar to induction chemotherapy in the treatment of some hematologic malignancies, and different from the multiple cycles of anthracycline-based chemotherapy used in solid tumors such as breast cancer. In this context, it is notable that childhood survivors of hematologic malignancies treated with anthracycline-based chemotherapy do show signs of persistent impaired vascular endothelial function (4). In contrast, these same changes do not persist in adult breast cancer survivors who received a series of lower-dose exposure to anthracyclines (5). This clinical literature suggests that the findings by Clayton et al. (1) may be most translatable to a subset of anthracycline-treated patients.

With regards to mechanistic insights suggesting disrupted VEGF-A as a mediator of the vascular...
dysfunction, some clinical data does not support that hypothesis. The effect of anthracyclines on circulating VEGF-A and other cardiovascular growth factors has been examined in samples collected from children undergoing anthracycline-based chemotherapy. Plasma levels of VEGF-A increased after anthracycline treatment in contrast to what was seen in this mouse studies of Clayton et al. (1,6). In contrast, the cardiovascular growth factors neu-reugin (NRG) and cardioprotin-1 decreased after anthracycline treatment in these same children (6). The decrease in NRG after anthracyclines has also been observed in adults undergoing anthracycline-based chemotherapy, which is interpretable as a sign of vascular endothelial cell injury (7). Endothelial cell-derived NRG acts through the ERBB receptor tyrosine kinase family to regulate the growth and survival of cardiac myocytes, and protects from anthracycline cardiac cytotoxicity (8,9). Given the interaction between VEGF-A and NRG in regulating the adaptation of the cardiovascular system to stress and the clinical data showing anthracycline-associated decline in plasma NRG, further work should be considered (10).

Perhaps the trickiest part of interpreting the work by Clayton et al. (1) is when answering questions from patients anticipating anthracycline-based treatment. MitoQ and other coenzyme Q10 preparations are available over the counter, and coenzyme Q10 supplementation has been studied not only as a way to limit injury to the cardiovascular system, but also kidney and reproductive organs (11,12). These promising preclinical results may lead people to self-medicate during their cancer treatment. As Clayton et al. (1) acknowledge, that would be a bad idea. They acknowledge that the National Cancer Institute guidelines specifically warn against taking antioxidants concurrent with chemotherapy. In fact coenzyme Q10 was among a group of dietary supplements that was associated with worse outcomes in people being treated for breast cancer (13). So, unless a definitive clinical study is conducted showing a beneficial effect of MitoQ in this clinical setting, it seems the best advice we can currently give is to leave the MitoQ on the shelf.

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**KEY WORDS** antioxidants, coenzyme Q10, doxorubicin, endothelial dysfunction, endothelium, mitochondrial function, oxidative stress, vascular endothelial growth factor
Characterization of Immune Checkpoint Inhibitor-Related Cardiotoxicity in Lung Cancer Patients From a Rural Setting

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ABSTRACT

BACKGROUND Immune checkpoint inhibitor (ICI)-related cardiotoxicity (iRC) is uncommon but can be fatal. There have been few reports of iRC from a rural cancer population and few data for iRC and inflammatory biomarkers.

OBJECTIVES The purpose of this study was to characterize major adverse cardiac events (MACE) in ICI-treated lung cancer patients based in a rural setting and to assess the utility of C-reactive protein (CRP) and neutrophil-lymphocyte ratio (NLR) in the diagnosis of iRC.

METHODS Patients with lung cancer treated with ICIs at Vidant Medical Center/East Carolina University (VMC/ECU) between 2015 and 2018 were retrospectively identified. MACE included myocarditis, non-ST-segment elevated myocardial infarction (NSTEMI), supraventricular tachycardia (SVT), and pericardial disorders. Medical history, laboratory values, pre-ICI electrocardiography (ECG), and echocardiography results were compared in patients with and without MACE.

RESULTS Among 196 ICI-treated patients, 23 patients (11%) developed MACE at a median of 46 days from the first ICI infusion (interquartile range [IQR]: 17 to 83 days). Patients who developed MACE experienced myocarditis (n = 9), NSTEMI (n = 3), SVT (n = 7), and pericardial disorders (n = 4). Ejection fraction was not significantly different at the time of MACE compared to that at baseline (p = 0.495). Compared to baseline values, NLR (10.9 ± 8.3 vs. 20.7 ± 4.2, respectively; p = 0.032) and CRP (42.1 ± 10.1 mg/l vs. 109.9 ± 15.6 mg/l, respectively; p = 0.010) were significantly elevated at the time of MACE.

CONCLUSIONS NLR and CRP were significantly elevated at the time of MACE compared to baseline values in ICI-treated patients. Larger datasets are needed to validate these findings and identify predictors of MACE that can be used in the diagnosis and management of ICI-related iRC. (J Am Coll Cardiol CardioOnc 2020;2:491-502) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The introduction of immune checkpoint inhibitors (ICIs) has significantly improved clinical outcomes in multiple cancer types, including melanoma, lung, kidney, and colorectal cancers (1). ICIs are monoclonal antibodies against programmed cell death-1 (PD-1), programmed cell death-ligand 1 (PD-L1), and cytotoxic T-cell lymphocyte antigen-4 (CTLA-4) (2). These agents function by blocking an immune checkpoint at the coreceptor and ligand interface of the T-cell and antigen-presenting cell (anti-CTLA-4) or by inhibiting the interaction between the T cell and the tumor cell (anti-PD-1 and anti-PD-L1), enabling the increased destruction of cancer cells (1,2).

A consequence of blocking the physiological immune checkpoint is overactivation of the immune system and regulatory proteins, resulting in an enhanced inflammatory response that can affect multiple organ systems, specifically termed immune-related adverse effects (irAEs) (3). The most common irAEs encountered in clinical trials and practice are colitis, pneumonitis, dermatitis, and thyroiditis (3). Immune checkpoint inhibitor-related cardiotoxicities (iRCs) are less common. Most datasets have observed iRCs in 0.1% to 0.3% of patients. Importantly, however, cases of iRC may be associated with high morbidity and mortality (4). In a recent retrospective analysis from Vigibase (World Health Organization pharmacovigilance database, Basel, Switzerland) that records drug-related adverse events, there were several cases of ICI-related myocarditis and pericarditis, with some overlap with vasculitis (5).

Studies from several groups have estimated the timing and incidence of iRCs (5-8), but potential indicators of iRC development using markers of inflammation such as the neutrophil-to-lymphocyte ratio (NLR) and C-reactive protein (CRP) have not been fully evaluated in patients with iRCs. In other studies, these markers have been used for identifying irAEs (9-11) and assessing cardiovascular disease risk. We hypothesized that patients in rural eastern North Carolina (NC), a community where cardiovascular risk factors and disease are prevalent (12,13), had a high incidence of iRCs. Furthermore, we hypothesized that the inflammatory markers NLR and CRP were associated with the development of iRCs.

Methods

Patients with a diagnosis of lung cancer who received ICI treatment between February 2015 and February 2018 were identified from the tumor registry at Vidant Medical Center/East Carolina University (VMC/ECU). The study was approved through the ECU Institutional Review Board (15-001400). Patients ≥18 years of age who completed at least 1 infusion of ICI therapy at VMC were included in the study (Figure 1). Inpatient and outpatient clinic office visits were reviewed. Demographic data including age, sex, body mass index, and ethnicity were collected. Retrospective data collected also included patient lung cancer type and stage; prior radiation therapy; prior and concomitant chemotherapy; ICI therapy and dosage; medical history; cardiac medications; baseline electrocardiograms (ECGs) and echocardiograms; cardiac biomarkers including troponin I (TnI) and brain natriuretic protein (BNP); NLR, calculated from the complete blood count profile; and CRP.

The following major adverse cardiac events (MACE) were observed after ICI: 1) non-ST-segment elevation myocardial infarction (NSTEMI) in the setting of acute coronary syndrome symptoms; 2) new onset supraventricular tachycardias (SVT); 3) myocarditis; or 4) pericardial disorders (acute pericarditis and/or nonmalignant pericardial effusion), each in the absence of sepsis, electrolyte disorders, or other confounding medical conditions. MACE in ICI-treated patients were graded based on the Common Terminology for Clinical Adverse Events version 5.0 (CTCAE v5.0, National Institutes of Health, National Cancer Institute, Bethesda, Maryland) (14). A clinical diagnosis of myocarditis was based on the European Society of Cardiology consensus statement (15) which included 2 or more of the following clinical presentations: 1) new onset (0 days up to 3 months) or subacute or chronic (>3 months) of worsening dyspnea at rest or exertion and/or fatigue with left and/or right heart failure signs and/or imaging findings of new right and/or left ventricular dysfunction or elevations of BNP or TnI; 2) sudden cardiac death or aborted cardiac death; 3) new atrioventricular block or bundle branch block, sinus arrest, ventricular tachycardia, or fibrillation and asystole; or 4) tissue characterization by cardiac magnetic resonance (CMR) imaging showing edema or late gadolinium enhancement. Post hoc review of patients based on definitions suggested by Bonaca et al. (16) as definite, probable, or possible myocarditis were further used to verify adjudication of suspected myocarditis cases. ICI-treated patients without MACE were further categorized as patients who did not experience any irAEs, or experienced noncardiac irAEs, or who had disease progression (did not complete 4 cycles of ICI due to tumor progression).
At the time of MACE, patients’ symptoms were obtained based on initial history of presenting illness documentation. Time to onset of MACE was defined as the number of days from the first ICI infusion to presentation of MACE. Presence of new pericardial effusion, new wall motion abnormalities, diastolic dysfunction, and ejection fraction (EF) at the time of MACE were compared to prior echocardiograms. Patients with evidence of malignant pericardial effusion from pathology were not considered part of the outcomes analysis. Cardiac biomarkers, obtained according to the treating clinicians’ discretion, including TnI, BNP, and inflammatory markers including NLR and CRP at the time of an irAE or MACE, were compared to respective baseline values. In addition, baseline NLR and CRP in patients with MACE were compared to baseline values of patients without MACE. TnI and BNP values of up to 6 months prior to ICI treatment were considered baseline values. In patients who did not experience any irAEs, available NLR and CRP data were also obtained at ICI cycle 6 (C6) or ICI cycle 8 (C8). Institutional normal values are as follows: TnI, <0.03 ng/ml; BNP, <200 pg/ml; and CRP, <20 mg/l. Although there is no standardized NLR range that defines normal or abnormal, a recent study suggested that, in an adult, healthy, nongeriatric population, a normal NLR is between 0.78 and 3.53 (17).

STATISTICS. Numerical data are presented as mean ± SD or median (interquartile range [IQR]). Categorical variables are presented as total number and percentage. A Fine and Gray competing risk univariable analysis was used to evaluate the associations among baseline demographics, cancer history, other irAEs, baseline laboratory values, comorbidities, and medications with MACE. To assess the cardiac presentation and diagnostic workup in patients with MACE, baseline echocardiographic and ECG parameters were compared to respective values at the time of MACE. Continuous variables (EF and PR and QTc intervals) were compared using a paired Student’s t-test, whereas categorical variables (pericardial effusion, right ventricular systolic pressure >35 mm Hg, wall motion abnormalities, diastolic dysfunction, and ECG rhythms) were compared using a McNemar test. Furthermore, baseline NLR and CRP were compared to respective values at the time of MACE, irAE, or cycle 6 by using a paired Student’s t-test. A Fine and Gray competing risk analysis was used to determine the relationship between baseline NLR or CRP and risk of MACE. A p value <0.05 was considered statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina).

RESULTS

DEMOGRAPHICS AND BASELINE CHARACTERISTICS OF ICI-TREATED PATIENTS WITH AND WITHOUT MACE. There were 196 patients who received ICIs for lung cancer at the time of this retrospective review. Patients were predominantly white and male and received ICI for non-small cell lung cancer (NSCLC) (Table 1). Patients with MACE tended to be older than patients without MACE (68.7 ± 1.8 vs. 64.3 ± 0.8 years of age, respectively; p = 0.064). Nivolumab was more frequently administered than other agents in both patients without MACE (68%) and those with MACE (83%), at a dose of 3 or 240 mg/kg. Only 25% of patients without MACE and 17% of patients with MACE received pembrolizumab. There were 11 patients without MACE who received atezolizumab (1,200 mg) and 1 patient who received a combination of nivolumab (3 mg/kg) plus ipilimumab (3 mg/kg). Patients
also received various nonimmunotherapy cancer therapy regimens, most of which were alkylating agents.

Among 196 patients, there were 23 patients (11%) with MACE that included possible myocarditis (n = 9 of 23; 39%), NSTEMI (n = 3 of 23; 13%), SVT (n = 7 of 23; 31%) and pericardial disorders (n = 4 of 23; 17%) (Figure 2A). Cardiotoxicity was graded based on CTCAE v5.0 criteria (14) (Supplemental Table 1). Fulfilled criteria for myocarditis was based on European
Society of Cardiology consensus statement (15) and definitions suggested by Bonaca et al. (16) in patients are detailed in Supplemental Table 2. A total of 75% had grade 3 toxicity, defined as a severe or medically significant but not immediately life-threatening adverse event that is disabling, limiting self-care of activities of daily living, and requiring hospitalization or prolonged hospitalization. Three patients (14%) experienced grade 5 toxicity (death) related to the adverse event (Figure 2B) caused by NSTEMI, possible myocarditis, and pericardial tamponade. More than 50% of the patients with MACE did not experience concomitant irAEs; however, 4 patients developed colitis (n = 2) and pneumonitis (n = 2). There were 54 patients (31%) without MACE who developed pneumonitis, 15 patients (9%) with cerebritis or encephalitis, 6 patients (3%) with colitis, 5 patients (3%) with dermatitis and other irAEs (Table 1).

In patients with and without MACE, diabetes mellitus, hypertension, hyperlipidemia, atrial fibrillation, chronic kidney disease, chronic obstructive pulmonary disease, and cerebrovascular disease were similarly present. There was a higher proportion of patients with MACE who had a history of coronary artery disease compared than patients without MACE (estimated hazard ratio [HR]: 2.79; 95% confidence interval [CI]: 1.18 to 6.59; p = 0.019). There was also a higher proportion of patients with MACE who were taking steroids than patients without MACE (n = 18 of 23; 78%; vs. n = 61 of 173; 35%, respectively; p < 0.001) (Table 2). Among the patients without MACE who were receiving steroids, 79% (n = 48 of 61) were receiving dexamethasone as palliative treatment for metastatic disease (Supplemental Table 3).

CLINICAL PRESENTATION AND CARDIAC DIAGNOSTIC WORKUP OF ICI-TREATED LUNG CANCER PATIENTS WITH MACE. More than 50% of patients without MACE presented with dyspnea and palpitations, whereas <25% of patients presented with chest pain (Figure 3C). The median time to onset of development of iRC from the first day of ICI infusion was 46 days (IQR: 17 to 83 days) (Figure 3A). The median number of doses until the onset of MACE was 3 (IQR: 2 to 4 doses) (Figure 3B).

There were only 4 patients with MACE who had a measured baseline TnI prior to ICI treatment with a mean of 0.03 ± 0.01 ng/ml. In patients without MACE with baseline measured TnI (n = 42 of 173), mean TnI was 0.02 ± 0.01 ng/ml. At the time of MACE, TnI was available in only 12 patients and was elevated to a mean of 0.98 ± 0.36 ng/ml with a peak value across 12 patients of 1.35 ± 0.49 ng/ml. There were no baseline BNP data available in patients with MACE. BNP value was available in only 18 patients at the time of MACE and was 384 ± 339 pg/ml (Table 2). Baseline BNP in 11 of the 173 patients without MACE was 158 ± 148 pg/ml. EFs were not significantly different at the time of MACE (n = 22 of 23) in comparison to baseline EFs (n = 17 of 23; 46.2 ± 16.8% vs. 50.5 ± 16.2%, respectively; p = 0.495) (Table 3). Patients were predominantly in normal sinus rhythm (63%). PR interval was
shorter (−19.6 ± 8.2 ms from baseline; p = 0.031) and QTc was more prolonged (26.8 ± 12.0 from baseline; p = 0.036) at the time of MACE (n = 17 of 23) in comparison to values at baseline (n = 12 of 173), though QTc was <500 ms.

**INFLAMMATORY MARKERS OF ICI-TREATED LUNG CANCER PATIENTS WITH AND WITHOUT MACE.**

Baseline NLR and CRP values were available in all 196 patients. Baseline NLR in patients with MACE were significantly higher than in patients without MACE (10.9 ± 8.3 vs. 8.1 ± 9.0, respectively; p = 0.022) (Table 2) and compared to patients without any irAEs (10.9 ± 8.3 vs. 7.4 ± 3.4, respectively; p = 0.029) (Central Illustration). NLR in patients who did not experience any irAEs (n = 20 of 173) at baseline and between C6 and C8 remained ≤10 at all-time points (p = 0.380). There was a significant increase in NLR observed at the time of MACE from baseline (20.7 ± 4.2 vs. 10.9 ± 8.3, respectively; p = 0.032) and in patients with other noncardiac irAEs compared to baseline values (n = 95 of 173; 7.45 ± 1.1 vs. 17.3 ± 2.4, respectively; p = 0.032) (Central Illustration). There were no statistically significant differences between baseline NLR in patients with disease progression (n = 58 of 173) and NLR values in patients with no irAEs.

CRP at the time of MACE and in patients with noncardiac irAEs was significantly elevated compared with respective baseline values (109.9 ± 15.6 vs. 42.1 ± 10.1, respectively; p = 0.010; and 107.1 ± 9.8 mg/l vs. 37.7 ± 5.4 mg/l, respectively; p = 0.031) (Central Illustration). However, baseline CRP did not differ according to MACE (HR: 1.01; 95% CI: 0.99 to 1.01; p = 0.546). Baseline CRP in patients with disease progression was similar to that in patients with MACE and with other noncardiac irAEs. CRP in patients with no irAEs was <20 mg/l at baseline and between C6 and C8 also was not significantly different (p = 0.168).

**DISCUSSION**

In this retrospective study of lung cancer patients receiving ICI, there was an 11% incidence of MACE consisting of various cardio toxicities that included possible myocarditis, NSTEMI, new onset SVT, and pericardial disorders. Importantly, a significant increase was observed in NLR and CRP at the time of MACE in comparison to patients who did not experience any irAEs (Central Illustration), highlighting its potential utility in the screening and diagnosis of MACE in ICI-treated patients.

Possible myocarditis (4.5%) and SVT (3.6%) were the predominant adverse cardiac events observed in this study. The high incidence observed for MACE may have been the result of the definition of MACE, which captured a spectrum of cardio toxicities not limited to myocarditis, inclusion of only lung cancer patients who could have higher risks to development of MACE such as chest radiation therapy (18) and the potential role of host-environmental factors unique to the rural population of eastern NC (12,13). Indeed, higher inflammatory signals and incidence of pneumonitis were also observed in this lung cancer population in comparison to those reported previously (13,19). Furthermore, in the lung cancer patients who received radiation, it has been suggested that the synergistic effect of radiotherapy and immunotherapy for priming of an endogenous antigen-specific immune response may contribute to a higher incidence of MACE by T-cell recognition of shared antigens (20).
Timing of the development of MACE with a median delay of 46 days and a median of 3 doses from the first ICI administration in this study was similar to that previously reported (5,21,22). In view of this timing of onset from earlier studies, there is a general recommendation that baseline and surveillance cardiac testing (echocardiogram, ECG, TnI and BNP) be considered during this potential MACE window period, noted as after the second and after the fourth ICI cycle administration (21). Most of the present MACE cases were not associated with a decrease in EF from baseline, which is consistent with previous studies (18,21), suggesting that relying solely on EF in ICI-treated patients with MACE may be limited for the detection of iRCs. TnI was observed to be mildly elevated at the time of MACE. However, elevations in TnI have also been observed in cancer patients receiving cancer therapy, including ICIs without any cardiotoxicities (23,24), thus suggesting that its utility may also be limited for the detection of iRC.

As demonstrated by this study, symptoms of iRC may be variable, including nonspecific symptoms of shortness of breath and palpitations that may overlap with other common cancer-related complications, which could result in underdiagnosis of iRC if unsuspected. Inflammatory markers such as CRP and NLR as diagnostic tools for identifying and monitoring irAEs have been previously documented (9-11); however, they have not been specifically investigated in iRCs. In this study, there was an elevation of CRP and NLR in patients at the time of MACE in comparison to baseline values, and this may possibly reflect similar inflammatory downstream effects such as those seen with cytokine release storm. In the setting of ICI-related pneumonitis, an upward trend of CRP has been previously documented (19) similar to that seen with cytokine release storm in chimeric antigen receptor T (CAR T) cells and tumor-infiltrating (TIL) therapy (25). In patients who did not experience any irAEs throughout the ICI treatment course, CRP remained <20 mg/l. In comparison, a significant >2-fold elevation in CRP, compared to baseline, was observed during the time of MACE and irAE. These findings are similar to a previous study conducted by

**FIGURE 3** Clinical Presentation of MACE in Lung Cancer Patients Receiving ICI

(A) Time to MACE onset from first ICI infusion. Median days to onset of MACE from the first ICI infusion was 46 days (IQR: 17 to 83 days). (B) Number of ICI doses received before MACE. Approximately 35% of patients developed MACE after 2 ICI infusion cycles with a median dose of 3 doses (IQR: 2 to 4 doses) at the time of iRC. (C) Presenting symptoms at the time of MACE. More than 50% of patients presented with palpitations and dyspnea. Less than 25% of patients presented with CP. CP = chest pain; GI = gastrointestinal (symptoms: nausea, vomiting, abdominal pain); IQR = interquartile range; iRC = immune-related cardiotoxicity; other abbreviations as in Figure 1.
our institution that observed a significant increase in CRP with nivolumab-related pneumonitis that was mitigated with use of tocilizumab (19). Similar inflammatory-mediated mechanisms such as interleukin (IL)-6 may be observed in CAR T cell-related cardiotoxicities (26) further highlighting and extending the utility of CRP in the detection of iRCs.

Another inflammatory marker that has been previously studied in noncardiac irAEs and observed to be elevated at the time of irAE is NLR (8,11). NLR is previously studied in noncardiac irAEs and observed to extend the utility of CRP in the detection of iRCs. Cardiac biomarkers n = 4 n = 12 Troponin I ng/ml 0.03 ± 0.01 0.98 ± 0.36 — — BNP, pg/ml — 384 ± 339 — — Electrocardiogram n = 12 n = 17 PR interval, ms 171.1 ± 29.9 155.9 ± 30.6 Δ−19.6 ± 8.22 −37.3 to −2.08 0.031 QTc interval, ms 442.4 ± 37.9 466.1 ± 34.8 Δ26.8 ± 12.0 1.9 to 51.7 0.036 Rhythm Normal sinus rhythm 10 (63) 7 (29) — 0.388 Sinus bradycardia 0 (0) 1 (5) — — Sinus tachycardia 1 (5) 6 (29) — 0.727 Atrial fibrillation/flutter 3 (19) 7 (33) — 0.125 Bundle branch block 2 (13) 2 (9) — 1.000 ST-segment depression/elevation 0 (0) 4 (19) — — Nonspecific T wave abnormality 5 (31) 3 (14) — 1.000

Values are mean ± SD or n (%). PR and QTc intervals at the time of MACE (n = 17) were compared to baseline (n = 12). Ejection fraction and the presence of pericardial effusion at time of MACE (n = 23) were compared to available baseline echocardiograms (n = 17).

Δ = change from baseline; BNP = brain natriuretic peptide; OR = odds ratio; RVSP = right ventricular systolic pressure; WMA = wall motion abnormalities; other abbreviations as in Tables 1 and 2.

In coronary disease, an elevation in NLR has been observed in animal models where neutrophils are recruited early after myocardial injury along with proinflammatory monocytes and lymphocytes which activate the inflammatory cascade through the neutrophil-activated CD11b/CD18, reviewed in detail elsewhere (28). The protective roles of PD-L1, PD-1, and CTLA-4 in the intricacy of the immunity-inflammatory environment against development of atherosclerotic plaque and myocardial infarction have also been previously described in animal models. Hypercholesteremic PD-L1 and PD-1 knockout mice have an exaggerated T-cell-mediated immune response and cytokine secretion that results in a larger atherosclerotic lesion size composed of numerous CD8− T cells and apoptotic core (29,30). Similarly, CTLA-4 inhibition results in accelerated atherosclerosis that can be mitigated by CTLA-4-Ig (31). Thus, in the setting of ICI use, this may result in a proatherosclerotic inflammatory signal leading to an increased risk for the development of iRC in patients with underlying coronary heart disease. In cases of ICI-related myocarditis, an increase in inflammatory cells, particularly abundant T cells and macrophages were observed in the postmortem assessment of the myocardium (4). Although the mechanisms of ICI-related myocarditis remain to be fully elucidated, mistaken recognition of cardiac self-antigen for foreign antigen is one plausible pathway.
(A) NLR in ICI-treated patients with and without MACE. In patients who did not experience any irAEs, NLR at baseline and C6 to C8 were <10. NLR at baseline was >10 in patients with MACE and patients who had disease progression. NLR increased significantly at the time of MACE and noncardiac irAEs (red bars). (B) CRP in ICI-treated patients with and without MACEs. In patients who did not experience any irAEs, CRP was <20 mg/l at baseline and C6 to C8. CRP was elevated in patients with MACE and in patients with disease progression and who had noncardiac irAEs. At the time of irAE or MACE, CRP was significantly elevated in comparison to baseline CRP. *$p<0.05$ in comparison to respective baseline values; †$p<0.05$ in comparison to baseline values in patients with no irAEs. C6-C8 — cycle 6 to cycle 8; CRP — C-reactive protein; ICI — immune checkpoint inhibitor; irAE — immune-related adverse events; MACE — major adverse cardiac events; NLR — neutrophil-lymphocyte ratio.
Interestingly, a significant proportion of patients with MACE were receiving concomitant corticosteroids predominantly for palliative treatment. The effect of baseline steroids concurrent with ICI treatment on outcomes is complex, whereby chronic prednisone usage of >10 mg per day in NSCLC has been shown to be associated with decreased clinical benefit and shorter overall survival (32,33) in comparison to transient use (33); however, other retrospective studies have debated this (34,35). ICI therapy efficacy may not be adversely affected by steroids due to the dose-dependent, cell cycle-dependent, and time-dependent effects of steroids on T-cell survival (36). Therefore, it is possible that, after ICI-mediated T cell activation or upregulation, T cells may be protected from chronic corticosteroid-induced T-cell cytolysis, resulting in increased risk for irAEs or iRCs.

NLR in cancer patients receiving ICI may also predict the response and development of irAEs. In solid cancers and melanoma, an elevated NLR prior to and during ICI treatment has demonstrated poor response to chemoradiation therapy (37,38) as well as worse outcomes in overall and progression free survival (39,40). This may be explained on the basis of ICI therapy, which recruits lymphocytes for tumor destruction such that an elevated NLR may represent an impaired immune tumor response. Although there is no standardized NLR range that defines normal, in an adult, healthy, nongeriatric population, a normal NLR is between 0.78 and 3.53 (17). NLR at baseline in patients with MACE and patients who experienced noncardiac irAEs was also significantly elevated in comparison to baseline NLR values of patients who did not develop any irAEs. Baseline NLR in patients who had significant tumor disease progression was almost equal to the baseline NLR of patients with MACE, which may be explained by activation of a proinflammatory status with rapid release of poorly differentiated neutrophils that has been seen in patients with significant tumor progression (40). Although the current findings may seem to conflict with some prior studies which observed an association with low baseline NLR and development of irAEs (38,39), increased NLR and a higher proportion of steroid use suggests an inflammatory state quite consistent with the cardiotoxic events experienced by the present patients who were receiving ICIs.

**STUDY LIMITATIONS.** There are several limitations to this study stemming from the bias inherent in any single-institution retrospective analysis. The present study population was also small with the potential for uncontrolled confounding and missing data. The definitions of MACE encompassed various cardiotoxicities that were from International Classification of Diseased codes and electronic health record documentation. At the time of data acquisition, iRCs were not always well recognized, and CMR imaging was not routinely performed at our institution, thus resulting in myocarditis diagnosis based on clinical and diagnostic criteria without myocardial biopsy and cardiac CMR. Baseline values of cardiac biomarkers were missing in a number of patients, as it was not considered standard of practice to obtain in the outpatient setting or in asymptomatic patients. As such, trends and utility of these cardiac biomarkers could not be fully evaluated. As such, these results are hypothesis-generating. Earlier studies have demonstrated that combination ICI is associated with higher mortality (5) in comparison to either anti-PD-1 or anti-PD-L1 monotherapy. The different effects of monotherapy versus ICI combination therapy on MACE and changes in inflammatory markers could not be assessed because most patients received nivolumab at our institution.

**CONCLUSIONS**

The introduction of ICIs has significantly changed treatment strategies for many cancer patients. Although ICI therapy is highly effective for some cancer patients, these agents may adversely affect other organs, including the heart. iRCs are uncommon but, as shown, myocarditis and SVT were the major MACE noted in our patient population. The most striking finding from this study was the rise in the inflammatory biomarkers CRP and NLR at the time of MACE. Studies are needed to understand whether CRP and NLR can be used as data to inform multidisciplinary decisions among the patient, oncologist, and cardiologist regarding diagnosis and optimal therapeutic management of ICI therapy. Further prospective studies are needed to identify risk factors of iRC, chronic effects of ICIs, and the utility of NLR and CRP in the prognostication of MACE.

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KEY WORDS immune checkpoint inhibitors, inflammatory markers, myocarditis, neutrophil-to-lymphocyte ratio

APPENDIX For supplemental tables, please see the online version of this paper.
Inflammatory Biomarkers to Detect Immune Checkpoint Inhibitor-Associated Cardiotoxicity in Lung Cancer Patients Ready for Prime Time?*

Mandar A. Aras, MD, PhD,a John R. Power, MD,b Javid J. Moslehi, MDb

Lung cancer is the leading cause of cancer-related death in the United States and worldwide. Non-small cell lung cancer (NSCLC) represents nearly 80% of all lung cancer cases, with most of these cases diagnosed at an advanced stage (1). Although targeted therapies have redefined treatment options for patients with molecularly defined NSCLC (e.g., epidermal growth factor receptor [EGFR] mutant and anaplastic lymphoma kinase [ALK]-rearranged NSCLC), these therapies are ineffective in those whose tumors lack such genetic alterations. Immune checkpoint inhibitors (ICI) harness the immune system and have emerged as novel treatment options for patients with locally advanced or metastatic lung cancer (2). This approach has resulted in improved survival and a more favorable toxicity profile than conventional chemotherapy.

ICI target immune “brakes,” systematically activating of immune cells, particularly T lymphocytes, and providing antitumor responses. ICI include antibodies against programmed cell death receptor (PD-1), programmed cell death ligand (PD-L1), and cytotoxic T-cell lymphocyte antigen 4 (CTLA-4). However, ICI treatment can result in immune-related adverse events (irAEs) targeting any organ. Cardiovascular irAEs, particularly myocarditis, have received considerable attention due to their potentially fatal outcome (3,4). ICI were initially tested and approved as single therapy in patients with metastatic melanoma, a cancer type that historically had few treatment options and poor survival (5). Later, these therapies were tested in patients with lung cancer and renal cell carcinoma (6,7). In the latter studies, ICI were tested either in combination with or following exposure to classic chemotherapies or targeted therapies. As a result, there was a growing need to define the cardiovascular sequelae in lung cancer patients treated with ICI, where the cardiovascular risk was complicated by exposure to cardiotoxic non-immune-based cancer therapies and a high prevalence of conventional cardiovascular risk factors (8).

Based on this rationale, in this issue of JACC: CardioOncology, Moey et al. (10) performed a retrospective analysis of lung cancer patients who received ICI therapy at a single medical center from 2015 to 2018. The authors defined major adverse
cardiac events (MACE) following ICI therapy as: 1) non-ST-segment elevation myocardial infarction (NSTEMI); 2) new onset supraventricular tachycardia (SVT); 3) myocarditis; and 4) pericardial disorders. Among their overall cohort of 196 patients, 23 patients (11%) developed MACE, including 9 patients with “possible” myocarditis, 3 with NSTEMI, 7 with SVT, and 4 with pericardial disorders. The investigators hypothesized that baseline inflammatory state, as measured by a neutrophil-to-lymphocyte ratio (NLR) and C-reactive protein (CRP), would be higher in patients with MACE than those without. Indeed, baseline NLR was elevated in patients who developed MACE compared to those without MACE, as well as those without other irAEs. There were no differences between baseline CRP concentrations among the groups. Both NLR and CRP at the time of MACE were significantly increased from baseline values. Based on those findings, the authors concluded that NLR and CRP may be useful in the screening and diagnosis of MACE in patients with lung cancer treated with ICI.

Cardiovascular toxicities after ICI therapy in lung cancer patients have not been well described, so the authors should be congratulated on completing this study. However, there are a number of limitations with phenotyping this cohort (11). First, the authors used a broad and nonstandard definition of MACE, which conventionally has been defined as cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, and hospitalization for heart failure (12). In this case, the authors defined MACE as myocarditis, NSTEMI, pericardial disease, or SVT and assumed those endpoints all represented immune-related cardiotoxicities. Although myocarditis has an immune-related pathophysiology and an established and biologically plausible complication of ICI treatment, that may not be the case for isolated myocardial infarction or SVT. It was also unclear if the same underlying biomarkers would predict these inherently different disease processes. Also concerning was the lack of application of standard diagnostic modalities to diagnose myocarditis. Of the 9 patients who developed myocarditis, none underwent tissue characterization using cardiac magnetic resonance imaging or biopsy to confirm the presence of myocardial inflammation (13). Whether the patients truly had myocarditis remains unclear. It is worth noting that, although all 9 myocarditis cases in this study only met “possible” criteria, suspected cases could still meet “probable” or “definite” myocarditis criteria without advanced imaging through a robust standard cardiac workup (13). Similarly, pericardial diseases may represent pericarditis, which may be immune-related, or pericardial effusion, which is related to the cancer itself. Radiation therapy prior to immunotherapy has been postulated to prime an endogenous antigen-specific immune response; however, this “double hit” may make lung cancer patients more susceptible to pericardial disorders (14). Prior analysis of VigiBase (World Health Organization, Basel, Switzerland), the WHO’s global Individual-Case-Safety-Report database, suggested that pericardial disorders were overrepresented in patients receiving anti-PD-1/PD-L1 therapy for lung cancer (15). In the present study, the incidence of pericardial disorders in an exclusive lung cancer cohort was lower than expected (2%). Whether the pericardial disorders reported were driven by immune toxicity or complications from malignancy needs further clarification. The lack of standardization in methods and quality assurance in evaluating cardiovascular irAEs represents a major need for the field of cardio-oncology.

The second issue is the lack of adjustments for confounders and the need for a multivariable analysis. The authors were interested in evaluating commonly obtained inflammatory markers as diagnostic tools for identifying and monitoring irAEs. In the present study, NLR and CRP levels were retrospectively evaluated at baseline and at the time of MACE. Although these assays are routinely available and inexpensive, elevations in NLR and CRP were not demonstrated to be specific to cardiovascular irAEs. Univariable analysis showed that baseline NLR was indeed statistically different among patients with MACE compared to patients without MACE. However, most baseline laboratory values, including hemoglobin, white blood cell counts, platelets, and creatinine levels were also significantly different between the 2 groups. By not adjusting for potential confounders, including any hematological markers, the specificity of baseline NLR in predicting ICI-associated irAE is unclear. Indeed, for NLR or CRP to emerge as important biomarkers, a multivariable analysis would be a more appropriate approach. This is especially relevant in this cohort where the use of higher doses of steroid in the MACE group (78% vs. 35%, respectively; p < 0.001) could be the primary driver of neutrophil demargination and elevated NLR. The authors admit that CRP has been previously shown to be elevated in their population presenting with ICI-related pneumonitis, and NLR was elevated in patients that developed noncardiac irAEs. Thus, the search for a specific biomarker indicative of cardiac irAEs continues and likely requires a prospective clinical trial.

In summary, the characterization of cardiovascular toxicity among lung cancer patients receiving...
immunotherapy represents an important contribution to the field of cardio-oncology. As the indications for immunotherapy continue to expand, the need to understand the risk factors for the development of potentially life-threatening cardiovascular toxicities arising from immunotherapy is imperative. Although inflammatory biomarkers may be associated with the development of MACE, it remains to be determined whether these markers are specific enough to change life-prolonging immunotherapy treatment strategies.

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KEY WORDS immune checkpoint inhibitors, inflammatory markers, myocarditis, neutrophil-to-lymphocyte ratio
Outcomes of Cancer Patients Undergoing Transcatheter Aortic Valve Replacement

Transcatheter aortic valve replacement (TAVR) has evolved as a first-line treatment modality for patients with symptomatic aortic stenosis (AS). The proportion of patients with cancer who require TAVR has gradually increased, with a prevalence close to 4% (1). Although surgical aortic valve replacement (SAVR) has been a long-standing treatment option for AS patients, cardiac surgery in cancer patients carries an increased risk of infection, conduction abnormalities, bleeding, and post-procedural intensive care requirement (2). As a less-invasive option, TAVR is therefore promising. Limited data have assessed the short- and long-term outcomes of cancer patients, and there are no data regarding readmissions in this population. We investigated a large, representative, nationwide cohort to evaluate the feasibility and short-term outcomes of TAVR in this group.

The Nationwide Readmission Database (NRD) is a database created by the Agency for Healthcare Research and Quality for the Healthcare Cost and Utilization Project that encompasses weighted estimates of one-half of the total hospitalizations in the United States (3). We used this registry to retrospectively select patients who were admitted between January 2012 and September 2015 and underwent TAVR using the appropriate International Classification of Diseases-9th Revision procedure codes (35.05 and 35.06). Among included patients, we assessed for the presence of a malignancy using the International Classification of Diseases-9th Revision diagnoses codes (140.X to 209.X). We performed chi-square tests for categorical variables and Mann-Whitney U tests for continuous variables to evaluate comorbidities and outcomes, as well as multivariable logistic regression analyses to assess mortality predictors after adjusting for age, sex, and all comorbidities (Table 1). All regression models were multivariable, and results were presented as odds ratio (OR) with 95% confidence interval (CI). All statistical analyses were performed using SPSS version 26 (IBM, Armonk, New York) for the weighted values of observations as provided by the NRD to measure national estimates. A 2-sided value of p < 0.05 was set for statistical significance. NRD data are anonymized and considered nonhuman subject research; thus, institutional review board approval was not required.

A total of 63,352 patients underwent TAVR and were included, of which 2,850 (4.5%) had a malignancy. Cancer patients were more likely to have an underlying cardiomyopathy (10.8% vs. 8.8%; p < 0.001), and heart failure (11.2% vs. 8.9%; p < 0.001), but less likely to have hypertension, atrial fibrillation, diabetes mellitus, dyslipidemia, chronic lung disease, and other comorbidities (Table 1). Post-procedural outcomes, including all-cause in-hospital mortality, stroke, bleeding, and permanent pacemaker implantation, did not differ in patients with and without cancer (Table 1). However, cancer patients were more likely to develop acute kidney injury (17.9% vs. 16.2%; p = 0.023), and to be readmitted within 30 days of discharge (20.2% vs. 17.4%; p < 0.001). After adjusting for age, sex, and all comorbidities mentioned in Table 1, there remained no difference in all-cause in-hospital mortality (OR: 0.873 [95% CI: 0.715 to 1.066]; p = 0.183), but there was a higher likelihood of 30-day readmission (OR: 1.21 [95% CI: 1.09 to 1.34]; p < 0.001). Mortality rates were similar irrespective of stage or site of cancer. When analyzed specifically by site, only patients with colorectal (OR: 3.66 [95% CI: 2.30 to 5.82]; p < 0.001), urinary/bladder (OR: 1.87 [95% CI: 1.17 to 2.98]; p = 0.009), and uterine (OR: 5.03 [95% CI: 2.33 to 10.89]; p < 0.001) cancers were associated with the increased risk of 30-day readmission, when compared with patients without cancer. The most common cause for readmission in both groups was heart failure, followed by infections and sepsis.

Current guidelines recommend TAVR to be performed in patients with a life expectancy >12 months (4). However, it is seldom possible to predict the life expectancy of cancer patients, and successful treatment of AS may allow for more intensive cancer treatment modalities, which in turn could affect survival. In many cases, symptomatic AS may be the...
rate-limiting step in cancer management. Thus, a multidisciplinary decision-making team of interventionalists and oncologists is warranted. A recent multicenter study comparing 222 cancer patients with 2,522 “no-cancer” patients undergoing TAVR showed that the 2 groups had similar 30-day outcomes, and 1-year mortality was higher in individuals with advanced cancer (5). The novelty of our study lies in the reporting of higher readmission rates in such patients, as well as the observation that certain types of cancers were more commonly associated with early readmission, most notably from heart failure and infection. These summative findings support that TAVR in cancer patients is appropriate on a case-by-case basis, and that optimal post-procedural cardiovascular rehabilitation as well as careful observation for post-procedural infections may result in overall better outcomes.

Our study is not without limitations. There is a paucity of information with respect to patient-level data regarding cancer treatments, as well as other unaccounted comorbidities and causes of death. Being an administrative database, it relies on physician/hospital reporting of outcomes. In addition, because of the retrospective nature of the analysis, it is not possible to differentiate active malignancies from history of malignancy. Information on dates of in-hospital outcomes and post-discharge out-of-hospital mortality are not recorded in NRD, which prohibits conducting a competing risk analysis for in-hospital mortality are not recorded in NRD, which prohibits conducting a competing risk analysis for in-hospital outcomes or readmission. It is noteworthy that our comparator arm represents a high-surgical risk population, as TAVR in intermediate- and low-risk patients obtained approval in 2016 and 2019, respectively. It would be interesting to see how this affects the findings of future trials, especially those that also address the question of quality of life, which is an important consideration in decision-making in advanced cancer patients undergoing palliative therapy.

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| Cancer (n = 2,849) | No Cancer (n = 60,503) | p Value |
|-------------------|-----------------------|---------|
| **Age, yrs**      | 83 (76-87)            | 83 (77-88) | <0.001 |
| **Sex**           |                       |          |        |
| Male              | 1,748 (61.4)          | 31,549 (52.1) |      |
| Female            | 1,101 (38.6)          | 28,954 (47.9) |      |
| **Cancer stage**  |                       |          |        |
| Localized/regional| 2,675 (93.9)          |          |        |
| Metastatic        | 175 (6.1)             |          |        |
| **Cancer site**   |                       |          |        |
| Prostate cancer   | 388 (13.6)            |          |        |
| Breast cancer     | 147 (5.1)             |          |        |
| Leukemia/lymphoma | 1,476 (51.8)          |          |        |
| Lung cancer       | 206 (7.2)             |          |        |
| Colorectal cancer | 92 (3.2)              |          |        |
| Urinary bladder cancer | 102 (3.6) |          |        |
| Uterine corpus cancer | 27 (0.9) |          |        |
| Other cancers     | 413 (14.5)            |          |        |
| **Comorbidities** |                       |          |        |
| Hypertension      | 2,098 (73.6)          | 48,489 (80.1) | <0.001 |
| Atrial fibrillation| 1,139 (40)           | 26,706 (44.1) | <0.001 |
| Cardiomyopathy    | 309 (10.8)            | 5,317 (8.8)  | <0.001 |
| Diabetes mellitus | 849 (29.8)            | 21,324 (35.2) | <0.001 |
| Heart failure     | 320 (11.2)            | 5,413 (8.9)  | <0.001 |
| Previous MI       | 326 (11.4)            | 6,830 (11.3) | 0.811 |
| Carotid artery disease | 157 (5.5) | 4,150 (6.9)  | 0.005 |
| Dyslipidemia      | 1,472 (51.7)          | 35,906 (59.3) | <0.001 |
| Chronic lung disease | 868 (30.5) | 20,261 (33.5) | 0.001 |
| Renal failure     | 1,010 (35.5)          | 21,758 (36)  | 0.589 |
| Obesity           | 308 (10.8)            | 9,720 (16.1) | <0.001 |
| Smoking           | 760 (26.7)            | 16,239 (26.8) | 0.845 |
| Alcohol abuse     | 47 (1.6)              | 638 (1.1)   | 0.004 |
| **Outcomes**      |                       |          |        |
| Length of stay, days | 6 (4-11)            | 6 (3-9)   | <0.001 |
| In-hospital mortality | 107 (3.8)           | 2,300 (3.8) | 0.954 |
| In-hospital stroke | 58 (2)               | 1,438 (2.4) | 0.257 |
| Post-procedural blood transfusion | 709 (24.9) | 12,830 (21.2) | <0.001 |
| In-hospital acute kidney injury | 509 (17.9) | 9,826 (16.2) | 0.023 |
| Permanent pacemaker implantation | 291 (10.2) | 6,380 (10.5) | 0.594 |
| 30-day readmission | 494 (20.2)          | 9,018 (17.4) | <0.001 |
| 30-day in-hospital mortality | 27 (1.1)         | 585 (1.1)   | 0.988 |

Values are median (interquartile range) or n (%). *For 30-day readmission rates, we excluded patients who died within index hospitalization and patients who were discharged in December each year (and September 2015) to allow for at least 30 days of follow-up for all patients.

ML = myocardial infarction; TAVR = transcatheter aortic valve replacement.

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Assessment of Cardiac Function in Chemotherapy Naive Women With Breast Cancer Undergoing Contemporary Radiation Therapy

Radiation therapy (RT) is an established treatment for breast cancer and is associated with a reduction in disease recurrence and death. Thoracic RT is associated with incidental cardiac exposure, which can lead to a spectrum of radiation-induced heart disease (RIHD) (1). The mechanisms underlying RIHD are multifactorial, including direct cellular damage, endothelial dysfunction, inflammation, and fibrosis (1). Identification of surrogate endpoints that predict the development of latent cardiac RIHD could help to guide primary prevention (2). Previous research has included heterogeneous populations with variability in RT dose and use of combination chemoradiation (2,3), resulting in difficulty in differentiating the adverse effects of each treatment modality. Accordingly, we sought to isolate a population of breast cancer patients undergoing RT without adjuvant chemotherapy.

Chemotherapy-naive women with left-sided breast cancer were consecutively enrolled. The protocol was approved by the institution’s Human Research Ethics Committee (HREC/17/Austin/533). Whole-breast radiation without regional nodal radiation was delivered with tangential, 3-dimensional conformal methods. Prescription dose was 50 Gy in 25 fractions or 42.4 Gy in 16 fractions administered over 6 weeks. Treatment planning included deep inspiration breath-hold scans. Mean heart dose (MHD) was calculated.

Patients underwent serial echocardiography with global longitudinal strain (GLS) at 3 time points: baseline, at completion of RT, and 12 months. Left ventricular ejection fraction (LVEF) and GLS were calculated according to the American Society of Echocardiography guidelines. A significant decline in left ventricular function was defined as a decrease in LVEF of more than 10% to below the lower limit of normal (ejection fraction 53%) or a decrease of >15% in GLS compared with baseline, independent of symptoms. The reproducibility of echocardiographic parameters including GLS in our laboratory has previously been reported (4).

High-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro-hormone brain natriuretic peptide (NT-proBNP) were measured daily during the first week of RT, and weekly thereafter. hs-cTnT assays have a limit of detection of 5 ng/l and a coefficient of variation of <10% at the 99th percentile upper reference limit of 14 ng/l. The reference range for NT-proBNP was <125 pg/ml for patients age 0 to 74 years and <450 pg/ml for patients age 75 to 99 years.

Continuous variables are expressed as mean ± SD or median with 25th and 75th percentiles (Q1 to Q3) and compared using the Student’s paired t-test; change in biomarker concentrations was evaluated by 1-way repeated analysis of variance with Bonferroni correction. Tests of normality were performed using the Shapiro-Wilk test. The data analysis was carried out using Stata 13.0 (StataCorp LP, College Station, Texas). A p value <0.05 was considered statistically significant.

In total, 20 patients were recruited and underwent baseline assessment. One patient was lost to follow-up. Of the 19 patients included in the final analysis, the mean age was 64 ± 12 years. Patient and echocardiographic characteristics are presented in Table 1. The mean heart dose was 1.3 ± 0.7 Gy and the maximum heart dose was 3.9 Gy. Baseline LVEF was 62.8 ± 3.9% and GLS was -20.1 ± 2.5%. No significant change was recorded at 6 weeks post-RT in LVEF (63.1 ± 4.7%; p = 0.77) or GLS (-20.2 ± 2.8%; p = 0.95), or at 12 months post-RT (LVEF 64.1 ± 4.2%; p = 0.36 and GLS -20.0 ± 2.8; p = 0.91). Serum hs-cTnT and NT-proBNP were unchanged throughout RT (median [Q1 to Q3] NT-proBNP 59 pg/ml [39 to 115 pg/ml] at baseline vs. 69 pg/ml [48 to 120 pg/ml] at conclusion of the study; p = 0.99; hs-cTnT 5 ng/l [5 to 7 ng/l] at baseline vs. 6 ng/l [5 to 7 ng/l] at the conclusion of the study; p = 0.42).

Although contemporary RT techniques limit the MHD, it is not yet known whether this translates to reduced cardiotoxicity. There is growing enthusiasm in the detection of surrogate endpoints for latent RIHD that could identify patients at risk and guide preventative therapies. Although the use of biomarkers and GLS has shown potential for detecting early signs of chemotherapy-induced cardiotoxicity, we did not determine significant changes in these markers with RT. Unlike previous studies, our patient
In this single-center study, we found no evidence of subclinical myocardial dysfunction or injury during and up to 12 months post-RT. This suggests that RT delivered with contemporary techniques is not associated with early cardiac sequelae. Larger studies are needed to further verify this hypothesis and establish the long-term risks of RIHD.

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Late-Onset Giant Cell Myocarditis Due to Enterovirus During Treatment With Immune Checkpoint Inhibitors

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Immune checkpoint inhibitor (ICI) therapy has significantly improved the prognosis of many advanced cancers. ICIs target cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed cell death-1 (PD-1), or programmed cell death-ligand 1 (PD-L1) and increase the antitumor immune response. Several immune-related side effects, including colitis, pneumonitis, hepatitis, and thyroiditis, have been reported since the U.S. Food and Drug Administration approval of the first ICI, ipilimumab, in 2011 (1). Cardiovascular adverse effects such as myocarditis are uncommon, but bear high mortality rates of nearly 50% (2). ICI myocarditis typically occurs early, and potential factors associated with ICI myocarditis are combination therapy (e.g., nivolumab [anti-PD-1] and ipilimumab [anti-CTLA-4] therapy), presence of a thymoma, and diabetes (3,4). Acute myocarditis after a first course of combination ICI therapy was assessed through a detailed study of 2 clinical cases (5). In both patients, myocardial lymphocytic CD8 infiltrates were identified on autopsy with no clear evidence of acute viral infection. As far as the pathophysiology, the theory of a common antigen or high frequency of T-cell receptor sequences amongst the myocardium, skeletal muscle, and tumors has been suggested.

However, the clinical presentation of ICI myocarditis is heterogeneous, with preserved left ventricular ejection fraction in one-half of the cases, and high rates of conduction abnormalities and ventricular arrhythmias (3). ICI myocarditis can also occur late, although notably, there is limited information on late-onset myocarditis with long-term ICI therapy (2). We herein describe a case of late myocarditis in a patient on long-term ICI therapy.

CASE PRESENTATION

A 57-year-old male patient presented with myalgias and dyspnea after the 27th course of 2nd-line ICI monotherapy (Nivolumab [anti-PD-1] 3 mg/kg every 3 weeks) for metastatic renal cell carcinoma. The electrocardiogram showed sinus tachycardia, low QRS voltage, and T-wave inversion in the anterior leads with no ST-segment changes. Cardiac biomarkers were elevated (troponin I 4,700 ng/l, B-type natriuretic peptide 466 ng/l). Left heart catheterization revealed normal coronary arteries. Cardiac magnetic resonance
demonstrated severe and diffuse myocardial edema (mean myocardial T2 = 74 ms, normal values <55 ms [Figure 1], high T1, values of 1,163 ms, high extravascular volume of 35.5%), and severe biventricular dysfunction (left ventricular ejection fraction [LVEF] 32%). A right ventricular endomyocardial biopsy (EMB) was performed and intravenous steroids (methylprednisolone 1 g/day) were started within 24 h of intensive care unit admission and continued for 3 days, then tapered to prednisone 2 mg/kg/day. Peak high-sensitivity troponin I was 5,800 ng/l on day 3 of his hospitalization.

Histopathological analysis of the EMB specimen showed intense myocardial inflammation comprised of numerous giant cells associated with many mononuclear cells, and prominent myocyte necrosis (Figure 1). Immunohistochemistry showed that the inflammatory cells were mainly CD68-positive giant cells and macrophages and CD8-positive T-lymphocytes, many of them exhibiting granzyme B, perforin, and TIA1 cytotoxicity markers. PD-L1 was expressed only by inflammatory cells and not by cardiomyocytes. CD4 and CD20 lymphocytes as well as Nkp46-positive NK cells were poorly represented (not shown).

The patient’s hemodynamics remained stable, without cardiogenic shock, and the LVEF improved gradually over 8 days in the intensive care unit. No sustained ventricular arrhythmias or high-degree conduction abnormalities were observed. The LVEF improved to 50% before discharge, although there was right ventricular impairment. The patient was discharged on oral heart failure medications (angiotensin-converting enzyme inhibitors and beta-blockers) and prednisone 1 mg/kg/day on day 13. Rechallenge with ICI therapy was deemed too high-risk at the time and was withheld after a multidisciplinary discussion. The patient had close follow-up in the cardio-oncology clinic, heart failure medications were titrated to maximum-tolerated doses, and steroids were subsequently tapered. An ambulatory 24-h Holter monitor showed no significant rhythm or conduction disorders.

To investigate the presence of viral markers of cardiac infection, frozen EMBs were sampled and analyzed at a quaternary university hospital laboratory (EA-4684, Cardiovir, Reims, France). Molecular assays for the detection of viral genomes were performed for enteroviruses (EVs), human herpesviruses (HHV1 to HHV8), and human parvovirus B19 (HPVB19). Only enterovirus RNA genomes were detected, and the RNA genome copy number was then estimated by a standardized RT-quantitative polymerase chain reaction assay. The mean EV-B viral load was 6000 genome copies/μg of total nucleic acid extracted, indicative of a moderate viral genomic replication load in the cardiac tissue. To differentiate between acute and persistent EV-B infection, we investigated the proportions and 5’ uncoding region sizes of EV-B populations using referenced molecular assays. Three EV-B populations were identified with a major proportion (45.6%) of full-length viral forms associated with 2 minor viral RNA populations, characterized by deletions ranging respectively from 8 to 36 (34.1%) and 37 to 50 nucleotides (20.3%) in the 5’ uncoding region. Sequencing of the viral protein 1 gene was performed and resulted in the genotypic identification of an original echovirus-19 strain (GenBank accession no. MN596948); nucleotide and amino acid sequences percent identities were 78.51% and 93.42%, respectively, with the echovirus-19 strain Burke (GenBank accession no. AY302544.1). Sequence identities between the EMB virus isolate and the prototype echovirus-19 strain Burke demonstrated a homologous serotype. Immunohistochemistry for viral protein 1 detection was performed on heart biopsies and showed foci of positive cardiomyocytes surrounded by inflammatory infiltrates, indicating endomyocardial viral protein synthesis activities.

Steroids were gradually tapered and discontinued at 3 months in light of the viral analysis. Cardiac magnetic resonance imaging showed at 6 weeks: 1) resolution of myocardial edema (average myocardial T2 = 50 ms (Figure 1), T1 values of 917 ms, and extravascular volume decreased to 29%); 2) recovery of LVEF to 68%; and 3) improvement of right ventricular function with an ejection fraction of 44% at 6 weeks to 51% at 6 months follow-up. The cancer had not progressed at 6 months follow-up, and the patient reported normal functional status. ICI therapy was not restarted at the time of last follow-up.

**DISCUSSION**

This case highlights the heterogeneity of pathogenesis of acute myocarditis on ICI therapy. We describe here acute myocarditis with long-term ICI therapy, secondary to giant cell myocarditis due to enterovirus.

Giant cell myocarditis is uncommon; its pathogenesis is poorly understood, and prognosis is poor. Recent data from an international registry including 220 patients with histologically proven severe myocarditis demonstrated that giant cell myocarditis bears the worst prognosis (6). The favorable outcomes in our patient,
in terms of biventricular systolic recovery and the absence of further relapse, are unexpected outcomes in this case of giant cell myocarditis. To the best of our knowledge, only 1 other case of giant cell myocarditis on ICI therapy has thus far been published. The context differed from our patient’s given that the tumor was melanoma, the therapy was ipilimumab (targeting CTLA-4), and cardiovascular death was the outcome (7).

The moderate EV-B RNA load associated with major 5’ full-length viral populations supported the hypothesis of an opportunistic acute viral infection as the cause of the late-onset fulminant myocarditis in our patient. Endomyocardial inflammatory damage could result from EV-induced cytopathic effects and cellular autoimmune mechanisms. The presence of endomyocardial giant cells may have been related both to viral and ICI-induced autoimmune cellular dysfunctions, acting synergistically and resulting in myocarditis. As far as the pathophysiology, the PD-1/PD-L1 pathway plays a critical role in suppressing inflammation induced by viral infection, allograft transplantation, as well as autoimmune mechanism, protecting tissues from acute histopathological damage. Viruses, specifically EV-B, have developed various strategies to escape from innate immune responses including blockade of the PD-1/PD-L1 pathway. Indeed, cleavage activities of EV-B proteinases can down-regulate PD-L1 expression in cardiac cells in vivo and in vitro inducing severe inflammatory damage (8). Here, on EMB, PD-L1 was only expressed by inflammatory cells and not by cardiomyocytes. Total disruption of the PD-1/PD-L1 pathway both by EV-B infection and ICI in cardiac tissue might explain the clinical and histopathological severity of our case. We further hypothesized that ICI therapy cessation may have reestablished T-cell immunity, resulting in viral clearance and complete clinical and imaging recovery.

Although the number of patients eligible to ICI treatment is increasing dramatically, the pathogenesis of myocarditis needs to be further investigated. Based on only 2 extensively studied cases (5) and 2 retrospective cohorts limited to approximately 30 participants each (3,4), societies have released case definitions for these
emerging clinical syndromes (9). The American Heart Association (AHA) has recently proposed diagnostic criteria for ICI myocarditis diagnosis based mainly on clinical characteristics, abnormal biomarkers, and/or cardiac imaging abnormalities (9). The recent publication of ICI myocarditis definitions by the AHA has the value of standardizing criteria to improve reporting. Steroids are the first-line treatment of ICI myocarditis, despite poor evidence to support this, followed by other immune modulators, plasma exchange, or even CTLA-4 agonist (abatacept) infusions. In the present study case, steroids were introduced and then discontinued after the confirmation of active viral markers on EMB. Indeed, the prognosis of T-cell ICI myocarditis is partly driven by early initiation of steroid treatment (10), which should not be held when awaiting historical or viral results.

Endomyocardial biopsy is recommended whenever possible in fulminant myocarditis (9). It is, however, likely to be overlooked in cancer patients. This case report emphasizes the risk for opportunistic viral infection during long-term ICI therapy and utility of biopsy. The first mechanism to be considered in the setting of ICI myocarditis is T-cell mediated, prompting steroids as a first-line therapy early after hospital admission. Endomyocardial biopsy should be performed whenever possible to rule out infectious causes of myocarditis to guide therapy and further our understanding of the potential varying presentations of ICI myocarditis.

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KEY WORDS enterovirus, giant cell myocarditis, imaging, immune checkpoint inhibitors, immunotherapy, myocarditis
CardioMEMS-Guided CAR T Cell Therapy for Lymphoma in a Patient With Anthracycline-Induced Cardiomyopathy

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CASE REPORT

A 66-year-old woman with advanced diffuse large B-cell lymphoma (DLBCL) was referred for chimeric antigen receptor (CAR) T cell therapy. She was diagnosed with DLBCL 6 years prior to presentation and had no significant past medical history. She received a total of 10 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), with a lifetime exposure of doxorubicin >500 mg/m². She underwent an autologous hematopoietic stem cell transplant after 4 cycles of R-CHOP. Four years later, her DLBCL recurred, requiring 6 more cycles of R-CHOP. One month after she completed the last cycle, her transthoracic echocardiogram (TTE) demonstrated a left ventricular ejection fraction (LVEF) of 55% to 60% and global longitudinal strain of −12% (normal: −19% to −22%). Two months following therapy, she was admitted to the hospital with acute decompensated heart failure (HF). Repeat TTE demonstrated an LVEF of 25% with global left ventricular (LV) dysfunction.

Positron emission tomography scan imaging demonstrated progression of her DLBCL with new pulmonary nodules. She was referred to our center for consideration of CAR T cell therapy. Coronary computed tomography demonstrated mild coronary artery disease, and cardiac magnetic resonance imaging showed no evidence of infiltrative or inflammatory disease. She was presumed to have anthracycline-induced cardiomyopathy and was initiated on furosemide and guideline-directed medical therapy (GDMT), including lisinopril 2.5 mg and metoprolol succinate 12.5 mg daily. Up-titration of GDMT was limited, and spironolactone or eplerenone could not be added, secondary to hypotension.

She was referred to a cardio-oncologist for risk stratification prior to CAR T cell therapy. She had good exercise tolerance, riding a stationary bike for 10 to 15 min at a time. A right heart catheterization (RHC) demonstrated mildly elevated biventricular filling pressures with normal cardiac output (right atrium 10 mm Hg, pulmonary artery 36/20/27 mm Hg, pulmonary capillary wedge pressure 16 mm Hg, Fick cardiac output 5.1 l/min, Fick cardiac index 2.9 l/min/m², and a mixed venous oxygen saturation 57.4%).

It was felt that CAR T cell therapy was her only therapeutic option. Multidisciplinary discussions between her hematologist/oncologist, cardio-oncologist, and advanced HF team were held to determine feasibility. Given the recent onset of her cardiomyopathy, the decision was made to give her a 3-month trial of GDMT for HF optimization. Of particular concern was how she would be able to withstand cytokine release syndrome (CRS), a common complication of CAR T cell, in which a rapid release of cytokines occurs from the CAR T cells when they engage with the target antigen expressed on malignant cells. Manifestations of CRS include fevers and derangements in blood pressure and oxygenation, ranging from mild hypotension and hypoxemia to
vasodilatory shock requiring vaspressors and severe hypoxemia necessitating intubation. The hypoxemia typically results from capillary leak syndrome, a low-pressure pulmonary edema. Other possible cardiac complications include atrial or ventricular arrhythmias and ventricular dysfunction. Invasive pulmonary artery catheter monitoring was considered as an option to facilitate appropriate volume management. However, the risk of infection was considered prohibitive given that lymphodepleting chemotherapy is administered prior to CAR T cell therapy. Considering these circumstances, the decision was made to implant a CardioMEMS (Abbott, Abbott Park, Illinois) device that was approved under compassionate use criteria.

After 3 months of GDMT, repeat RHC demonstrated normal filling pressures with preserved cardiac output (right atrium 2 mm Hg, pulmonary artery 25/12/18 mm Hg, pulmonary capillary wedge pressure 15 mm Hg, Fick cardiac output 4.7 l/min, Fick cardiac index 2.8 l/min/m², and mixed venous oxygen saturation 61.5%). During the RHC, she had successful placement of the CardioMEMS device. Repeat TTE demonstrated no improvement in her LVEF (24.6%), but did show that her right ventricular performance improved from mildly to moderately reduced to normal.

The following week, she was admitted for pre-treatment with a lymphodepleting chemotherapy regimen consisting of fludarabine and cyclophosphamide. During pre-treatment, she developed neutropenic fever and hypotension, and was started on intravenous antibiotics. GDMT was held. On day 1, 2 days later, she received the infusion of axicabtagene ciloleucel (Yescarta, Kite Pharma/Gilead, Los Angeles, California) CAR T cells. On day 2, she developed mild CRS with fevers, sinus tachycardia to the 150s, and systolic blood pressures in the 90s. The CardioMEMS demonstrated an elevated pulmonary artery diastolic pressure (PAD) of 20 mm Hg (Figure 1). She was given intravenous furosemide 20 mg and a dose of tocilizumab, an interleukin-6 receptor antagonist used in the treatment of CRS. By day 3, she had worsening tachypnea and a new oxygen requirement of 2 l nasal cannula, requiring a second dose of tocilizumab. Chest x-ray revealed diffuse pulmonary opacities (Figure 2), with a PA_{L} of 10 mm Hg on CardioMEMS, suggestive of noncardiogenic pulmonary edema. Given the normal PA_{L}, the treatment team held diuretic agents to prevent hypotension. Monitoring the patient’s volume status required significant coordination between the oncology and cardiology services. The oncology nurses performed the CardioMEMS measurements 3 to 4 times/day. The advanced HF consult service rounded on the patient daily, and made recommendations based on the CardioMEMS readings.

On day 6, her course was complicated by atrial flutter with rapid ventricular rates in the 140s. She developed acute hypoxic respiratory failure secondary to cardiogenic pulmonary edema, as evidenced by a PA_{L} of 23 mm Hg. She required intubation and initiation of low-dose norepinephrine. She was started on amiodarone for the atrial flutter, converted, and remained in sinus rhythm thereafter.

**Figure 1** CardioMEMS Pulmonary Artery Pressure Monitoring

CardioMEMS guided diuretic administration and dosing (e.g., intravenous furosemide 20 mg [black triangles] and 40 mg [black squares]) throughout hospitalization. Red line – pulmonary artery (PA) systolic; blue line – PA mean; green line – PA diastolic; CAR – chimeric antigen receptor; MICU – medical intensive care unit.
With continued guidance from the CardioMEMS device she received intermittent doses of intravenous furosemide 20 to 40 mg to keep her PA\textsubscript{d} between the range of 10 to 20 mm Hg. She was weaned off vasopressors and extubated on day 12. Daily discussions amongst the many teams involved throughout her hospital course were paramount in preventing discordant care, especially when her clinical status had many rapid changes while she was in the intensive care unit. She completed CAR T cell therapy and was discharged to acute rehabilitation after a one-month hospitalization. She was unable to be restarted on GDMT due to hypotension. Her PA\textsubscript{d} was 6 mm Hg at discharge. The plan was to obtain a positron emission tomography scan after acute rehabilitation to reevaluate her cancer.

Her initial course at the facility was uncomplicated, and she was able to participate in physical therapy. She had weekly appointments in the oncology clinic and an appointment with the advanced HF team. The providers at rehabilitation continued to transmit CardioMEMS readings to the advanced HF team. Furosemide 20 mg daily by mouth was restarted when her PA\textsubscript{d} was 18 mm Hg, which occurred 3 weeks after hospital discharge. At that time, the physician at the facility reported that she continued to do well. Fifteen days later (34 days after hospital discharge) the patient woke up with acute shortness of breath, and was found to have an oxygen saturation of 88%. Prior to this episode she had not reported any dyspnea, but had noted nausea for the past 3 days. She was taken by ambulance to an outside hospital. Initial chest x-ray was interpreted as pulmonary edema and a possible left lower lobe infiltrate. PA\textsubscript{d} at the time of hospitalization was 22 mm Hg. She was intubated for progressive hypoxic respiratory failure. Per outside hospital records, a bedside TTE showed severe LV dysfunction but normal right ventricular function. She was treated with multiple vasopressors for presumed mixed septic and cardiogenic shock. She experienced a cardiac arrest due to ventricular tachycardia and expired later that day.

**DISCUSSION**

CAR T cell therapy is a newer treatment for hematological malignancies and a form of immune therapy that uses a patient’s immune system to detect and kill tumor cells (1). T cells are collected from the patient via apheresis and are then genetically engineered in a laboratory to produce CAR proteins on their surface, which allow them to recognize antigens and kill targeted tumor cells when reintroduced into the patient. Importantly, CAR T cell therapy can be effective in chemotherapy-refractory lymphoma, inducing durable complete remissions lasting >2 years in some patients with DLBCL (1).

The side effects of CAR T cell therapy, such as CRS and arrhythmias, can be challenging to manage in patients with HF. CRS, which is predominantly mediated by interleukin-6, can occur in up to 70% to 90% of
patients (2). Hypotension is typically treated with intravenous fluids to maintain systolic blood pressure >90 mm Hg. Third-spacing of fluids with capillary leak is common, and this can lead to noncardiogenic pulmonary edema. A single-center retrospective study demonstrated that 12% of patients developed the composite outcome of arrhythmias, decompensated HF, or cardiovascular mortality (3).

The cardiac toxicities associated with CAR T cell therapy have led many centers to view significant cardiovascular disease as a relative contraindication (4). In 2 of the main clinical trials for CAR T cell therapy in patients with refractory DLBCL, patients were excluded if their LVEF was ≤45% to 50% (4). Our team decided to proceed with CAR T cell therapy in the patient described in this report because of her lack of other comorbidities, preserved performance status, and normal invasive hemodynamics. It was also her only treatment option for refractory DLBCL.

The CardioMEMS device—which is typically used to manage outpatients with HF—was an essential component of our patient’s medical treatment (5). For example, at 2 periods in the patient’s hospitalization she was found to have diffuse pulmonary infiltrates, hypoxemia, and hypotension. Her PA_{a} was 10 and 23 mm Hg during these respective episodes. Hemodynamic monitoring by means of CardioMEMS allowed proper utilization of diuretic therapy, i.e., avoidance in the setting of hypovolemia and administration in the setting of volume overload. Diuretic management on the basis of physical examination and chest x-rays alone would have been challenging due to marked fluctuations in volume status with third spacing of fluids both peripherally and in the pulmonary vasculature. To our knowledge, this is the first study documenting the use of CardioMEMS to monitor the hemodynamic variation with CAR T cell therapy. Investigators are exploring other novel applications for CardioMEMS, especially in patients with rapid changes in hemodynamics. For example, HEMO-VAD (Design and rationale of haemodynamic guidance with CardioMEMS in patients with a left ventricular assist device: the HEMO-VAD pilot study) is a single-center prospective study evaluating the safety and feasibility of CardioMEMS for optimization of LV assist device pump settings (6).

This case demonstrates that CAR T cell therapy is feasible in patients with symptomatic heart failure, when there is close collaboration between oncologists and cardiologists, and there is reliable hemodynamic data to guide treatment decisions. However, the fact that our patient died 1 month after discharge highlights how vulnerable these patients are, and that long-term success may be difficult to achieve in patients with severe LV dysfunction. It is not clear what precipitated the patient’s acute decompensation with possible mixed septic and cardiogenic shock. Some have questioned whether there could be a latent period with continued circulation of CAR T cells after treatment, leading to delayed cardiac toxicities such as arrhythmias and decompensated HF; this warrants further study (3). Also, risk assessment using cardiopulmonary exercise testing in addition to RHC may help to identify suitable patients better. The use of hemodynamic monitoring with CardioMEMS may enable oncologists and cardiologists to offer potentially life-saving therapy to a broader population of patients, but more research is warranted.

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KEY WORDS: anthracycline, cardiomyopathy, heart failure, immunotherapy, lymphoma
Multimodality Treatment for Advanced Cervical Cancer With Isolated Metastasis to Interventricular Septum of the Heart

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Cervical cancer is one of the most common malignancies in women worldwide (1). Approximately 90% are squamous cell carcinomas (SCC), whereas the rest are adenocarcinomas. Due to increased awareness and global early detection programs, most patients are diagnosed at an early stage. Lymphatic and hematogenous spread of the disease is uncommon and associated with a dismal prognosis, with one-half the patients dying within 6 months (1). Involvement of the heart is extremely rare (2–4). Only 38 cases were reported during the last 50 years, of whom only a handful had attempted complete (R0) resection (2–4). We report a patient with advanced stage SCC of the cervix and a single metastasis to the interventricular septum (IVS) discovered incidentally during comprehensive clinical staging. Multimodality treatment including chemotherapy, antivascular endothelial growth factor therapy, radiotherapy, and radical cardiac surgery resulted in exceptional survival.

CLINICAL CASE

A 40-year-old healthy White female presented with irregular vaginal bleeding 6 months after delivering her third child. Physical examination, pelvic ultrasound, magnetic resonance imaging (MRI), and computed tomography (CT) revealed a 6.4 × 4.3-cm invasive uterine cervical tumor and a lytic bone lesion in the right ileum. Total body 18F-fluorodeoxyglucose positron emission tomography (FDG-PET)/CT imaging (Figure 1) showed intense uptake of the tracer in the pelvic mass, the lytic bone lesion, and the IVS (standardized uptake values [SUV] = 7.0) corresponding to a single 2.4 × 1.6-cm tumor mass observed in the contrast-enhanced CT. Transthoracic echocardiography showed normal biventricular function (left ventricular ejection fraction [LVEF] of 55%), no valvular abnormalities, and an ill-defined solid mass. CT angiography of the heart showed a heterogenous mass involving the mid-distal septum with delayed contrast enhancement. Cardiac MRI (CMR) characteristics of the mass included rapid gadolinium uptake in first-pass perfusion, delayed contrast enhancement, as well as isointense and hyperintense signals in T1-weighted and T2-weighted sequences, respectively. A biopsy of the cervical mass and the lytic bone lesion confirmed the diagnosis of metastatic poorly differentiated SCC. Biopsy of the cardiac mass was deemed unnecessary at this stage, given the bone metastasis and multimodality imaging of the heart findings that were highly suggestive of malignancy. The multidisciplinary cardiac tumor team comprising medical and radiation oncology, cardiology, radiology, and cardiothoracic surgery specialists recommended systemic cancer therapy. The patient received 18 courses of carboplatin and paclitaxel in combination with bevacizumab (Avastin, Genetech, South San Francisco, California) with complete resolution of the cervical tumor and the bone metastasis after 4 months of therapy. However, the cardiac metastasis decreased only slightly in size, and 18F-FDG-PET/CT imaging showed an...
increase in tracer uptake intensity from 7.0 to 13.1 SUV. Given the patient’s young age, excellent response to systemic therapy (excluding the heart), and lack of alternative effective therapy, we proceeded with surgical resection of the metastasis.

Surgery (Figure 2) was performed using normothermic cardiopulmonary bypass and cardioplegic arrest. The tumor was exposed via a longitudinal incision in the right ventricle parallel to the IVS, carefully preserving the left anterior descending coronary artery. A 6 × 5-cm elliptical portion of the septum including the tumor mass was excised, preserving the septal papillary muscle of the tricuspid valve. It was difficult to ascertain a clean margin in the posterior-basal septum area. The septum was reconstructed using a bovine pericardial patch sewn into the left ventricular aspect of the septum. The ventriculotomy was then closed and the patient weaned off cardiopulmonary bypass. Transesophageal echocardiography after separation from cardiopulmonary bypass showed mildly reduced LVEF (40%) secondary to asynchronous contraction and loss of septal contribution to global left ventricular function, as well as trace mitral regurgitation and mild tricuspid regurgitation.

Gross pathology clearly demonstrated the tumor. However, tumor histology revealed areas of interstitial fibrosis, necrosis, and areas of inflammation, and there was no evidence of a viable tumor in the entire specimen. Because the pathological report was consistent with no active disease in the heart and a follow-up 18F-FDG PET/CT after surgery confirmed complete response of all tumor sites, the multidisciplinary team recommendation was to proceed with therapy with curative intent. Shortly after surgery, the patient received 50 Gy of external beam irradiation to the pelvis boosted by 27.5 Gy of vaginal brachytherapy.

The patient recovered uneventfully, returned to New York Heart Association functional class I, and continued clinical and imaging follow-up at 3- to 4-month intervals. Repeat 18F-FDG PET/CT showed complete resolution of the malignancy. Two years after surgery, CMR and 18F-FDG PET/CT were highly suggestive of local cardiac recurrence showing a 1.6-cm mass in the posterior-basal IVS. The 18F-FDG PET/CT tracer avidity increased from 0 to 4.7 and up to 13 SUV within a few months. Echocardiographic assessment of the septum was suboptimal, possibly due to post-operative changes and the presence of patch. Transesophageal echocardiography-guided endomyocardial biopsy was negative for tumor. Nevertheless, systemic treatment using the same combination of carboplatin, paclitaxel, and bevacizumab (Avastin, Genentech, South San Francisco, California) was resumed. Forty-five months after initial diagnosis, the patient was in New York Heart Association functional class I to II. CMR showed a slight decrease in the IVS mass size to 1.4 cm. The pathological avidity in FDG-PET decreased to 5 SUV. There were no other systemic or pelvic new findings.
DISCUSSION

The incidence of secondary metastatic tumors to the heart is 22 to 132 times more common than primary malignant tumors, and estimated to be in the range of 0.7% to 3.5% at autopsy series in the general population, and up to 14.0% in patients with known malignancies (5,6). The incidence of cardiac metastases has increased over the past decades, likely due to improved life expectancy of oncological patients and advances in diagnosis (5,6). The most common primary malignancies metastasizing to the heart include lung cancer, breast cancer, malignant melanoma, germ cell tumors, and hematological malignancies (5–7).

The most frequent mode of metastatic spread of SCC of the cervix is via the lymphatics to the para-iliac and para-aortic lymph nodes (1). Hematogenous spread is unusual and most commonly involves the lungs, bone, liver, and brain (1). Cardiac metastases from cervical cancer are exceedingly rare (2–4). The vast majority of cases have involved the right heart, presumably due to hematogenous spread of cervical cancer to the inferior vena cava via the uterine veins, coupled with filtration of tumor cells by the lungs.

Patients with cardiac metastases from cervical cancer present with a myriad of symptoms that depend on tumor location and size, leading to heart failure, arrhythmias, or tamponade (2–4). Many cases were diagnosed postmortem. Antemortem diagnosis was usually achieved by multimodality imaging including transthoracic and transesophageal echocardiography, CT angiography of the heart, CMR, and 18F-FDG PET/CT (2–7). Tissue diagnosis should be pursued for prognostic implications and optimizing treatment, but may not be feasible. Our patient had no symptoms related to the cardiac metastasis. The tumor was discovered by 18F-FDG PET/CT during the process of initial clinical staging of the disease. 18F-FDG PET/CT is a particularly useful imaging modality in this setting. Together with CMR for detailed tumor characterization, these imaging modalities provide a comprehensive assessment of disease status that is essential for optimal shared decision making (6,7).

The median survival of patients diagnosed with cardiac metastasis from cervical cancer is 4 months (0 to 13 months) (2–4). The management of these patients should be individualized and discussed within a dedicated multidisciplinary tumor heart team (5–7). A multimodality treatment strategy including chemoradiation, biological therapy, and surgery is necessary to prolong survival and maintain quality of life. In this regard, in a prospective randomized trial, bevacizumab has been shown to improve survival when added to the commonly used platinum–paclitaxel and topotecan–paclitaxel chemotherapy regimens (8). Of note, treatment with bevacizumab has been shown to be associated with increased incidence of adverse cardiovascular side-effects such as uncontrolled hypertension, thromboembolism, cardiac ischemia, and heart failure (8). The decrease in LVEF in our patient occurred immediately after resection and remained stable, and our patient did not have severe cardiac limitations during follow-up. Her low LVEF was readily managed with cardiac medications.
Surgical resection should be considered for isolated cardiac metastasis in patients with favorable response to initial therapy and in whom complete resection is deemed feasible (2–7). Our patient had excellent response of the disease in the cervix and of the single pelvic bone metastasis with no evidence of new disease, with the exception of the cardiac metastasis, which remained metabolically active. This is not an infrequent clinical phenomenon in patients with primary or secondary malignant tumors of the heart (7), presumably due to differential response of the tumor to cancer therapy in different tissue environments (9). Given the patient’s young age, good functional status, imaging suggesting feasibility of complete resection, as well as lack of an alternative effective treatment, we proceeded with surgery followed by intensive chemoradiation and bev-acizumab with curative intent. Although the pathological examination in our case did not reveal residual tumor in the tissue specimen, surgical resection had an important impact on the design of her concurrent therapy and prognosis. The local recurrence supports the presence of tumor and findings of the pre-operative 18F-FDG PET/CT, highlighting the potential for false-negative tumor histological examinations following neoadjuvant chemotherapy (10). The radiological evidence of local recurrence in the area of slim resection margins—the basal-posterior septum—underscores the technical challenge of achieving complete R0 resection with clean margins when treating primary or secondary malignant tumors of the heart (6,7).

In summary, cardiac metastases from cervical cancer should be included in the differential diagnosis of secondary malignant tumors of the heart. We believe that the patient-specific, multimodality management with curative intent strategized and delivered by an experienced and dedicated multidisciplinary team was key to achieving exceptional survival in our patient.

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KEY WORDS cardiac mass, cervical cancer, heart metastasis, multidisciplinary team, multimodality treatment
A 68-year-old Caucasian man with a history of Waldenstrom macroglobulinemia (WM) on treatment with ibrutinib for 2 years presented with syncope. He had no prior cardiovascular history or risk factors. On the day of presentation, he had an episode of dizziness followed by a sudden loss of consciousness while watching television. Two minutes later, he spontaneously regained consciousness. Upon initial evaluation by emergency medical services, he was found to be in atrial fibrillation (AF). In transit to the hospital, he had 4 episodes of polymorphic ventricular tachycardia (VT) that required multiple debrillations (Figure 1). In the emergency department (ED), he was hemodynamically stable. Physical examination revealed normal S1 and S2 with no murmurs, equal peripheral pulses with warm and well-perfused extremities, and unremarkable neurological and pulmonary findings. His home medication list included only ibrutinib and no over-the-counter medications. In the ED, he was started on amiodarone for recurrent episodes of VT and admitted to the cardiac intensive care unit.

DIFFERENTIAL DIAGNOSIS

Myocardial ischemia: Leading the differential diagnosis of polymorphic VT in a 68-year-old man was acute coronary syndrome.
Heart failure (HF): He did not have symptoms or signs of hyperviscosity syndrome or HF. Systemic amyloidosis associated with WM leading to amyloid cardiomyopathy was also considered.
Drug toxicity: A high index of suspicion was maintained about the potential association between ibrutinib and ventricular arrhythmia (VA).

CLINICAL COURSE

Electrocardiogram at the time of evaluation by emergency medical services showed AF with a ventricular rate of 120 beats/min and nonspecific ST/T-wave abnormalities. Complete blood count, serum electrolytes, and renal function were within normal limits at the time of admission, and serial serum troponin levels were undetectable. A transthoracic echocardiogram showed normal biventricular size and function with no regional wall motion abnormalities. Furthermore, coronary angiography demonstrated very mild luminal irregularities without obstructive disease. In the cardiac intensive care unit, he was noted to have multiple premature ventricular complexes and nonsustained VT with a left bundle branch block morphology and right superior axis, indicating an origin at the right ventricular apex (Figure 2). He underwent cardiac magnetic resonance imaging, which demonstrated a small area of mid-myocardial delayed enhancement in a nonvascular...
distribution in the basal inferolateral and anterolateral free wall of the left ventricle. The etiology of this scar was unclear because the patient had no prior cardiac history, and to our knowledge, ibrutinib had not been known to be associated with myocardial scar. No abnormality was noted in the right ventricular apical region, which was the likely origin of his VA.

**MANAGEMENT**

Initially, the patient was managed with intravenous amiodarone for recurrent VT. After extensive multidisciplinary discussions among cardiology, electrophysiology and oncology teams, ibrutinib was discontinued due to its potential association with VT. The patient was started on metoprolol, and an implantable cardioverter-defibrillator (ICD) was placed for secondary prevention. In terms of AF, his CHA2DS2-VASc score was 1, and anticoagulation was deferred, particularly given the potential bleeding risks with ibrutinib (1).

Although it remained uncertain if ibrutinib played an integral role in the pathophysiology of VT, the medication was discontinued with close outpatient cardiology and oncology follow-up. Because he had been in remission for several years, he was not started on alternative therapy for WM. On follow-up visits with close surveillance every 3 months with CBC, comprehensive metabolic panel, and IgM levels, he continued to be without evidence of symptomatic cytopenias, hyperviscosity, hepatosplenomegaly, lymphadenopathy, neuropathy, amyloidosis, cryoglobulinemia, or cold agglutinemia and did not meet criteria for reinitiation of therapy. On ICD interrogation at 3 months after cessation of ibrutinib, there were no further arrhythmias.

**DISCUSSION**

Ibrutinib is an oral, irreversible Bruton’s tyrosine kinase inhibitor used to treat a broad spectrum of B-cell proliferative disorders, including chronic lymphocytic leukemia (CLL) as first-line therapy, mantle cell lymphoma, marginal zone lymphoma, and WM (2). Cardiovascular toxicities associated with ibrutinib are

![Rhythm Strips at the Time of Presentation](image-url)
supraventricular tachyarrhythmias, VAs (VT and ventricular fibrillation) sudden cardiac death, conduction disorders, HF, hypertension, CNS hemorrhagic events, and CNS ischemic events (3). Its association with AF has been well-described. In a meta-analysis of 4 RCTs, the pooled incidence rate of AF in patients treated with ibrutinib was 3.3 per 100 person-years compared with 0.84 per 100 person-years in those who received non-ibrutinib therapy (4).

The association of ibrutinib with VAs is becoming increasingly recognized (3,5,6). In randomized controlled trials, the incidence of all-grade VAs in patients treated with ibrutinib (n = 1157) compared with patients in the control arm (n = 958) was 1.0% versus 0.4%, and for grade 3 or greater VAs was 0.3% versus 0% (7). In analyses from a U.S.-based comprehensive cancer registry cohort, male sex, previous AF, HF, coronary artery disease, diabetes, widened QRS, and valvular disease were associated with the development of any arrhythmias (VA and supraventricular tachyarrhythmias) (6). Among those without baseline HF or coronary artery disease, the estimated 100,000 person-year incidence rate for VAs was 596 compared with 48.1 among similar nonibrutinib-treated subjects, which suggested an observed versus expected relative risk of 12.4 (p < 0.001). Regarding drug dosage, in 1 study in which approximately 80% of the patients experienced VAs, 91% were taking at least 420 mg of ibrutinib per day, and only 9% were taking 280 mg or lower per day (3). Among those with ibrutinib-associated VAs, the median time-to-event was 16 months (range 0.7 to 57.6 months) (6).

Ibrutinib has been reported to cause polymorphic VT without QTc prolongation as well as in the absence of structural heart disease (8,9). Tomcsányi et al. (9) reported the case of a 74-year-old woman on ibrutinib for CLL with underlying left bundle branch block and AF who experienced ibrutinib-induced polymorphic VT in the absence of other causes (9). The initiation of VT was not characterized by short-long-short cycles as is seen in torsade de pointes. In polymorphic VT not related to prolonged QT, an alteration in the cardiac calcium homeostasis associated with ryanodine receptor-calmodulin-dependent protein kinase pathways is suspected. As such, it has been hypothesized that an interaction between these and PI3K-Akt pathways could potentially lead to polymorphic VT with ibrutinib (3).

Although the awareness of ibrutinib-associated VA is increasing, there are no published management guidelines. Our patient was initially treated with amiodarone, then started on a beta-blocker. In the case described by Tomcsányi et al. (9), the patient was treated with amiodarone and remained arrhythmia-free for 4 months. Amiodarone was thereafter discontinued due to pulmonary toxicity, and discontinuation led to the recurrence of VT. Sotalol was found to be ineffective, as were Class I antiarrhythmic agents (9). As ibrutinib is
Ibrutinib is a Bruton's tyrosine kinase inhibitor associated with a well-known side effect of AF. Reported cases of VAs associated with ibrutinib are rare. We present a case of VT storm in a patient receiving ibrutinib for WM.

In general, ICD implantation is recommended for the secondary prevention of sudden cardiac death due to life-threatening VT/ventricular fibrillation in patients in whom a completely reversible cause cannot be identified (10). Our patient had hemodynamically unstable VT episodes requiring resuscitation, and although ibrutinib was felt to be the culprit, the presence of delayed enhancement on cardiac magnetic resonance imaging made it difficult to rule out underlying structural heart disease, such as an infiltrative cardiomyopathy. Additionally, an ICD is indicated if it is expected to improve overall mortality. Because our patient’s long-term prognosis from WM was favorable, it was felt that he would benefit from an ICD, particularly because ibrutinib or another therapy could be indicated in the future. In patients with cancer whose overall prognosis is poor (i.e., low expectation of survival with an acceptable functional status beyond 1 year), ICD therapy is not recommended. As ibrutinib is often used in patients with CLL whose prognosis is generally favorable, ICDs for secondary prevention have a potential for long-term benefit. Another additional advantage of an ICD is providing prognostic parameters, such as the burden of atrial arrhythmia, nonsustained VT, and treated episodes of VT/ventricular fibrillation, which could help to risk-stratify patients in the future prior to reintroduction of potentially cardiotoxic drugs.

CONCLUSIONS

Ibrutinib is a Bruton’s tyrosine kinase inhibitor associated with a well-known side effect of AF. Reported cases of VAs associated with ibrutinib are rare. We present a case of VT storm in a patient receiving ibrutinib for WM. As the recognition of ibrutinib-associated VAs is increasing, more data are needed to guide best management strategies for VAs triggered by ibrutinib.

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KEY WORDS ibrutinib, ventricular tachycardia, Waldenstrom macroglobulinemia
Despite impressive advances in diagnosis and treatment, cancer remains a serious disease with a significant risk of recurrence and death. Although personalized and less toxic therapies have decreased the public health burden associated with many cancer types, the ideal cancer-mitigating intervention focuses on prevention through adoption of healthy lifestyle behaviors and avoidance of carcinogens. Cancer prevention strategies also include risk-modifying interventions (e.g., prophylactic mastectomy, prophylactic oophorectomy), some of which may be associated with an increased risk of atherosclerotic cardiovascular disease (ASCVD).

The following clinical scenarios focused on cancer prevention highlight the value of systematic cardiovascular risk assessment in individuals at high risk of cancer who are treated with cancer risk-reducing therapies. Recommendations regarding the evaluation of lipid abnormalities and the role of coronary artery calcium (CAC) scan are provided, balancing existing evidence with the need for rigorous data that specifically applies to cancer populations.

**CASE 1: PATIENTS AT HIGH RISK FOR OVARIAN CANCER UNDERGOING PROPHYLACTIC OOPHORECTOMY**

A 45-year-old Caucasian woman with a known pathogenic BRCA1 mutation underwent bilateral prophylactic oophorectomy 1 year ago. She had not had menstrual periods since then, and is considered menopausal. She is treated with amlodipine for hypertension. She is a heavy smoker and follows a sedentary lifestyle. She does not have diabetes mellitus (hemoglobin A1C, 5.4%). Her office blood pressure is 145/95 mm Hg, weight is 187 pounds (85 kg), and body mass index is 29.3 kg/m². Her lipid profile is as follows: low-density lipoprotein cholesterol 105 mg/dl, total cholesterol 177 mg/dl, high-density lipoprotein cholesterol 45 mg/dl, and triglyceride level 135 mg/dl. Her 10-year ASCVD risk using the pooled cohort equation is 5.3%. She is reluctant to consider statin therapy as she is already taking multiple medications.

In terms of additional potential cardiovascular risk factors, BRCA1 has been implicated in the repair of DNA double-stranded breaks, and its loss-of-function is associated with reduced cardiac performance and...
ACCELSRATED CARDIOMYOCYTE DEATH IN MURINE MODELS. HUMAN STUDIES INVESTIGATING THE ASSOCIATION BETWEEN BRCA1/2 MUTATIONS AND INCIDENT CARDIOVASCULAR DISEASE (CVD) HAVE SHOWN conflicting results, potentially due to variable sample sizes and ethnic-specific differences in the pathogenesis of CVD (1). Bilateral prophylactic oophorectomy is associated with a 96% reduction in the risk of epithelial ovarian cancer in BRCA1/2 mutation carriers. In women younger than 45 years of age, the risk of cardiovascular death after surgical oophorectomy is 44% higher than that of healthy control subjects (2). Premature menopause was shown to predict future coronary heart disease and stroke in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort and represents an important risk-enhancing factor (3).

PRACTICAL STRATEGIES

The patient has a borderline-range (5% to 7.4%) estimated ASCVD risk in the presence of a risk-enhancing factor, namely premature menopause. Given this, we would advocate for a discussion regarding initiation of moderate-intensity statin therapy. However, the patient has expressed a preference to avoid taking additional medications. In this case, a CAC scan could help refine ASCVD risk assessment (4). An Agatston score of ≥100 and/or a CAC score ≥75th percentile for the patient’s age, sex, and race would suggest a benefit to statin initiation, although there are no randomized clinical trial data supporting the use of CAC in treatment decisions. A CAC score of 300 would increase this patient’s 10-year coronary heart disease risk to 10% using the MESA risk score calculator, and would further support the use of lipid-lowering therapy. Nicotine cessation should be a primary strategy to reduce CVD risk in this patient, while the importance of healthy lifestyle habits, normal-range blood pressure and lipid profile, weight loss, and glycemic control should also be emphasized.

CASE 2: PATIENTS AT HIGH RISK FOR COLORECTAL CANCER RECEIVING CELECOXIB

A 53-year-old Caucasian man with familial adenomatous polyposis (FAP) underwent prophylactic total colectomy 3 months prior and was subsequently started on sulindac to delay the development of adenomas in the upper gastrointestinal tract and in the retained rectum. The patient has a history of hypertension controlled with hydrochlorothiazide and diet-controlled diabetes mellitus. He is a nonsmoker and maintains a physically active lifestyle. His family history is significant for a brother who experienced ST-segment elevation myocardial infarction at the age of 49 years. His office blood pressure is 125/75 mm Hg, body weight 160 pounds (68 kg), and body mass index 22 kg/m². His lipid profile is as follows: low-density lipoprotein cholesterol 145 mg/dl, total cholesterol 198 mg/dl, high-density lipoprotein cholesterol 37 mg/dl, and triglyceride level 80 mg/dl. His 10-year ASCVD risk using the pooled cohort equation is 13%.

Aspirin and sulindac are commonly used, contemporary pharmacoprophylactic therapies in patients who are at high risk of colorectal cancer, with the former being the preferred drug for individuals with sporadic adenomas and the latter for those with FAP. In the late 1990s, celecoxib was approved for use in patients with FAP. The risk-reduction benefit appeared to be significant with a 31% lower colorectal polyp burden rate in FAP patients treated with celecoxib and a 15% lower incidence of duodenal polyps. However, in a large study of colorectal adenoma prevention, long-term use of celecoxib (200 or 400 mg twice daily) was associated with a 3.4-fold increased risk of death from CVD, myocardial infarction, stroke, or heart failure (5).

In addition, a meta-analysis of randomized controlled trials showed that celecoxib use was associated with a 3-fold increased risk of myocardial infarction. A subsequent prospective investigation, assessing the safety of a lower dose of celecoxib (200 mg daily), did not demonstrate a statistically significant hazard; as such, the cardiovascular risk mediated by celecoxib remains incompletely understood (6). In 2004, due to high cardiovascular event rates, rofecoxib was removed from the market, and a black-box warning was issued for celecoxib the following year.

PRACTICAL STRATEGIES

This patient is in an intermediate-risk ASCVD category, but has 2 additional factors that increase his likelihood of future cardiovascular events: chronic nonsteroidal anti-inflammatory drug (NSAID) use and a strong family history of premature ASCVD. Importantly, the presence of diabetes mellitus in an individual 40 to 75 years of age is an indication for the use of moderate-intensity statins regardless of ASCVD score, and in an individual...
with multiple ASCVD risk factors, high-intensity statin therapy should be considered. NSAID use and family history are not incorporated into the pooled cohort equation, but represent established potentiators of CVD.

It is important to understand that patients with FAP who are treated with preventive total colectomy have favorable long-term survival outcomes. The notion that CVD represents an important competing risk to cancer mortality should be conveyed and serve as a motivating factor to introduce lifestyle changes and consideration of pharmacological therapies. Consideration of chronic NSAID initiation in this patient, especially in the context of conventional cardiovascular risk factors, should prompt referral to an internist or cardiologist.
Importantly, decisions regarding long-term NSAID therapy should balance the reduced polyp and potential colorectal cancer rate with the increased CVD risk.

Attainment of optimal blood pressure and glycemic control should be targeted. In addition, a CAC scan could be considered. If highly abnormal, it could be used to counsel and motivate this patient to further modify his lifestyle (4). It would also be reasonable to check a lipoprotein(a) level given the strong family history of premature atherosclerosis. Due to the expected long-term use of sulindac, statin therapy and improved lifestyle habits would be appropriate risk-reduction interventions in this case.

**CONCLUSIONS**

Cardiovascular risk assessment in individuals who are deemed to be at high risk for cancer is distinct from that in patients with an established cancer. Specific therapies aimed at reducing the risk of cancer may augment cardiovascular risk and deserve careful consideration. Two important tenets of cardiovascular risk assessment include evaluation of lipid abnormalities and understanding the role of CAC imaging in refining ASCVD risk stratification in this unique population.

Although more research is needed regarding the value of CAC burden in patients with cancer, available evidence suggests that elevated CAC scores are predictive of both future cardiovascular events and cancer. In a recent study, CAC >300, when compared with a score of 0, was associated with a 3.7-fold increase in the risk of CV death and a 30% increase in the risk of cancer death (7). The most common cause of death among individuals with 0 CAC was cancer (50%), whereas patients with CAC >300 experienced most of the mortality due to CVD.

In a study of 464 patients with locally-advanced nonsmall-cell lung cancer treated with thoracic radiation therapy, an increased CAC score, measured from planning radiation therapy computed tomography, was associated with an elevated risk of all-cause mortality (hazard ratio: 1.29; confidence interval: 1.0 to 1.6; p = 0.027) (8). In a separate analysis of breast cancer patients who had received radiation therapy, higher pre-RT CAC scores were associated with a higher likelihood of acute coronary syndrome at 9 years of follow-up (hazard ratio: 1.42; confidence interval: 0.49 to 4.17; p = 0.519) (9). Coronary calcification is therefore a predictor of CVD in the cancer population and further research is needed to better characterize the contribution of cause-specific mortality and cardiovascular events in cancer patients according to CAC levels (10). Last, breast arterial calcifications detected on screening mammogram are an independent marker for the presence of coronary artery disease and may help identify women at higher risk of ASCVD.

Lipid-lowering therapies should be considered in patients with cardiovascular risk factors or disease, as per the standard ASCVD risk score. Particular medical interventions to decrease the risk of cancer, such as prophylactic oophorectomy or long-term nonsteroidal anti-inflammatory drugs, may increase the risk of ASCVD and should be regarded as deleterious factors.

In the coming decade, cardio-oncologists may be asked to assess and manage cardiovascular toxicities in individuals who receive cancer risk-reducing interventions but who are free of cancer. Given the increasing data supporting an overlap between the molecular and clinical underpinnings of cancer and heart disease, oncologists should consider involving their cardiovascular medicine colleagues for risk assessment and preemptive management. In addition, longitudinal cardiovascular safety evaluations of specific patient subgroups in whom prophylactic anticancer therapies are considered should be performed to provide an evidence-based understanding of the risks and benefits of such therapies.

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KEY WORDS BRCA, cardio-oncology, cardiovascular risk assessment, prevention
Reflections on Our Inaugural Year of JACC: CardioOncology, With Gratitude and Tireless Devotion

Bonnie Ky, MD, MSCE, Editor-in-Chief, JACC: CardioOncology

One year ago, we launched the first issue of JACC: CardioOncology, wherein I outlined my vision for this journal: to become the essential resource for the field of cardio-oncology; to play a vital and transformative role in advancing the field and care of our patients by innovating science and positively impacting clinical care; to be driven by the highest standards of excellence; and to educate, engage, and strengthen the international community (1).

Excellence, rigor, and community. These are the principles to which we at JACC: CardioOncology strive for and use to guide us in our decision-making. Each journal issue has been built on a strong foundation of original basic, translational, and clinical science; seminal reviews and primers; educational case challenges; impactful patient perspectives; and global viewpoints from key leaders in cardiology and oncology. We have held international sessions at the China International Heart Failure Congress and the Global Cardio-Oncology Society Meeting in Brazil (2), and have hosted live, interactive Journal clubs (3); virtual case presentations (4); and podcast interviews highlighting the patient’s perspective (5).

As I reflect on the past year and the wonderful advances we have made together as a community, I feel a tremendous sense of gratitude. I am thankful to the communities of cardiology and oncology, and to our enthusiastic and engaged readers who turn to JACC: CardioOncology to learn the most current knowledge in our field. We are committed to providing highly accessible content, and we launched original research podcast summaries in March 2020, recorded by our Associate Editors to provide their unique context to the papers. Our podcasts have had >1,500 downloads since April 2020.

I am grateful to the authors who have entrusted us with their work. Manuscript submissions have continued to grow, and we have a strong, healthy submission volume. The depth and breadth of topics published in JACC: CardioOncology within our first year have been remarkable, with some of our most downloaded pieces covering topics including coronavirus disease-2019 (COVID-19) (6,7); amyloidosis (8); cardioprotective strategies in both primary and secondary prevention (9-12); cancer therapies, including osimertinib, CAR T cell, androgen deprivation therapy, tyrosine kinase inhibitors, and their cardiotoxic risk (13-16); and the intersection between cardiovascular disease and cancer in our patients (17-19). In the second quarter of 2020 alone, we had nearly 130,000 article usage sessions, and each quarter, this number has grown. The work of our authors is being read for utilization in clinical practice and research, which is always the most important metric for a JACC journal.

As a physician scientist, I recognize that the choice of “which journal” is not an easy one and requires careful consideration. We remain committed to serving our authors and working tirelessly to ensure that the peer review process is respectful, fair, constructive, and as seamless as possible. Our average time to first decision in second quarter 2020 was 15 days, and we will strive to maintain this standard. We seek to partner and work collaboratively with our authors to ensure the highest quality contribution to our community. I am grateful to the reviewers, who provide timely, incisive insight and lend their valued expertise to help ensure the quality of our journal. We have had nearly 300 peer reviewers contribute their evaluations over the past year. We know this takes...
dedicated time and effort, amid busy professional and personal lives and amid the painful challenges of COVID-19 and racial injustice that continue to afflict our world today.

Of course, I am grateful to my many mentors, including Dr. Fuster and the JACC family Editors-in-Chief: Drs. Doug Mann, Dave Moliterno, Julia Grapsa, Chris O’Connor, Chandra Shekhar, and Shiv Kumar; the leadership team at the American College of Cardiology; and the entire American College of Cardiology publishing team, led by Justine Varieur Turco, Divisional Senior Director. Eileen Cavanagh, Nandhini Kuntipuram, Colleen Whipple-Enro, Tamika Eadair at the ACC, and the entire team at Elsevier have been wonderful collaborative partners in ensuring our success. I have to especially acknowledge the remarkable efforts of Michelle McMullen, our Managing Editor. Launching JACC: CardioOncology has been a community effort, and I am thankful to our excellent multidisciplinary Editorial Board, including our International and Senior Advisors, Social Media Editors, Editorial Consultants, Assistant Editors, and Guest Editor, Dr. Anju Nohria; our highly committed Associate Editor team, comprised of our Deputy Editors Drs. Saro Armenian and Dan Lenihan; and our Associate Editors Drs. Greg Armstrong, Ana Barac, Anne Blaes, Paul Burridge, Katie Rudy, and Ron Witteles. Each has worked tirelessly and selflessly as we together cohesively advance our mission.

As I look forward to the year ahead, I am hopeful. I am proud to announce we are now indexed on Scopus, and much of our U.S. National Institutes of Health-funded science and COVID-19 papers are already indexed on PubMed. Our goal is that we continue to stimulate, innovate, and inspire rigorous peer-reviewed science and advance clinical care. Over the next year, we will improve access to JACC: CardioOncology through additional platform changes to improve searchability and integration across the JACC Journals.

Clinical, translational, and basic science original research manuscripts will continue to serve as our foundation. As we evaluate each manuscript, we will continue to ask ourselves the following key questions: Are the findings valid? Is the methodology rigorous? Is the topic of clinical importance? Are the findings incremental to our current understanding of the topic and do they fulfill an evidence gap? What is the potential clinical impact and the potential for advancing the field? We will continue to publish State-of-the-Art Reviews and Primers, and similarly ask ourselves: is this an authoritative, critical appraisal of the literature? Is it comprehensive, yet focused? Is this data-driven and accurately reflective of the current evidence? We will look to Clinical Case Challenges to provide evidence-based descriptions of unique cases that thoughtfully illustrate the diagnostic and therapeutic dilemmas that we as clinicians face as we care for our patients (20). Our Viewpoints will continue to express opinion pieces on important and timely topics, and present thought-provoking, community-building, evidence-based perspectives. We will continue to grow our international engagement events, podcasts, live Journal clubs, and dynamic case presentations, each occurring at least once per quarter. We also will launch a new “How To” series that will offer practical, evidence-based education on common clinical questions that are relevant to the everyday cardiovascular care of our cancer patients.

I am grateful. I serve a wonderful community, one made of patients, physicians, scientists, and care providers who inspire and motivate me daily. I look forward to continuing to work with a tireless devotion to “never feeling satisfied”—in our mission to educate our global community and positively impact the care of our patients.

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Florida Inter-Specialty Collaborative Project to Improve Cardio-Oncology Awareness and Identify Existing Knowledge Gaps*

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Cancer and cardiovascular disease are 2 primary causes of morbidity and mortality in the United States (1). In addition to the physical burden of disease, there is an emotional and financial toll on patients, family members, and the health care system at large (2). The success of novel cancer therapies has resulted in significantly improved disease-free and progression-free survival for many cancers; however, many conventional and newer cancer treatments, including radiation therapy, chemotherapeutic agents, targeted therapies, and immunotherapies, are also associated with an increased risk of a number of adverse effects, including cardiovascular toxicity (3).

By 2040, it is estimated that there will be approximately 26.1 million cancer survivors in the United States alone. Many of these patients also may be at risk for or experience cardiovascular risk factors or disease (4). Traditionally, cardio-oncology programs that specialize in the monitoring, prevention, detection, and treatment of cardiotoxicity in patients with cancer have been located in large academic quaternary institutions. However, an increasing number of programs are currently being developed in smaller community practices. In 2014, the American College of Cardiology (ACC) National Cardio-Oncology Survey identified multiple factors that have traditionally acted as barriers to developing cardio-oncology programs nationally, including lack of funding, limited interest, lack of infrastructure, and lack of educational opportunities (5). Such limitations directly impact the number of practicing cardiologists who have expertise in cardio-oncology, which may result in significant public health implications, given the rapidly increasing number of cancer survivors.

FLORIDA ACC AND AMERICAN SOCIETY OF CLINICAL ONCOLOGY PROGRAM DEVELOPMENT

To comprehensively evaluate current cardio-oncology awareness among clinicians in the State of Florida, we developed a collaborative program between the Florida Chapter of the American College of Cardiology (FCACC) and the Florida American Society of Clinical Oncology (FLASCO) and administered a survey to its members. Our goal was to extrapolate the educational needs of both cardiologists and oncologists who actively treat patients with cancer and develop educational materials to help bridge the identified knowledge gaps. In 2019, the FCACC was awarded a grant by the ACC Board of Governors to

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the JACC: CardioOncology author instructions page.

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survey the knowledge of and resources available concerning cardio-oncology among cardiologists and oncologists in Florida. The investigators established cardio-oncology subcommittees for both FCACC and FLASCO to optimally promote participation among specialists from both organizations.

A 16-question Web-based survey was sent to all members of FCACC and FLASCO via electronic platforms. Both surveys were available at the following links:

FCACC: https://www.surveymonkey.com/r/FPQPNQ

FLASCO: https://www.surveymonkey.com/r/FLASCO-CardioOncology

The FLASCO survey included several additional questions directed specifically to the oncology community. The surveys were designed to enable participants to complete answering all questions in <5 min to enhance response rate. Clinicians received reminders 4 times over a 3-week period. The results of the surveys were analyzed to determine current levels of awareness and to identify opportunities to enhance and expand education and address knowledge gaps for both specialist groups. Data were directly and securely exported to Microsoft Excel (2010), within which frequency tables were tabulated using Visual Basic for Applications-coded commands. Data are reported in frequency and proportion format.

After identifying specific knowledge gaps from these surveys, the cardio-oncology subcommittees of FCACC and FLASCO developed brief educational materials focusing on 11 fundamental topics in cardio-oncology. These documents were designed to be delivered electronically to FCACC and FLASCO memberships in early 2020. The topics were not intended to be in-depth reviews, but instead brief outlines in crucial areas of cardio-oncology for clinicians. Each topic also included linked references for those interested in obtaining further information (6).

A total of 303 physicians completed the surveys: 165 members (5.8%) from FLASCO and 138 members (5.5%) from the FCACC. Only 23 (14%) of 163 oncologists and 22 (16%) of 134 cardiologists reported feeling “very comfortable” treating patients with cardiovascular complications secondary to cancer treatment (Figure 1). As detailed in the figure, cardio-oncology services were available in less than one-half of each of the respondent groups. When cardio-oncology services were available, oncologists more commonly referred patients to cardio-oncology compared with the cardiologists. However, it is important to note that there were a small number of responses to this question from oncologists (n = 44), and the absolute number of providers (n = 41) referring to cardio-oncology were the same for both specialties. Interestingly, fewer than one-half of respondents felt that there was optimal cooperation between their specialties concerning the management of these complex patients. More than one-half of the oncologists rated their knowledge of cardio-oncology above average, whereas more than one-half of the cardiologists rated their knowledge of cardio-oncology average or below average (Figure 1). Many of the cardiologists (40%) and more than one-half of the oncologists (56%) had not previously attended any cardio-oncology sessions.

ONCOLOGY-SPECIFIC QUESTIONS

Oncologists primarily consulted general cardiology (58%), as compared with cardio-oncology (38%) for evaluation of treatment-related cardiotoxicity. Overall, a lack of awareness (63%) and lack of funding (40%) were there primary barriers toward establishing a cardio-oncology program. Additional reasons included lack of mentoring (27%), lack of interest (26%), and inadequate reimbursement (16%). Lack of importance was noted by 16%. Whereas 29% (47 of 161) of oncologists treated patients with potential cardiotoxities more than once per week, 36% cared for patients with cardiotoxities less than once a week and 35% cared for these patients less than once per month. The most common cardiotoxities reported by participating oncologists included heart failure or reduced left ventricular ejection fraction (84%), arrhythmias and atrial fibrillation (43%), arterial or venous thromboembolism (42%), and QT prolongation (36%). Myocardial infarction/ischemia/vasospasm was reported by only 10% of respondents.

The survey results indicated that oncologists most commonly initiated cardiac evaluations or consultations for patients treated with the following cancer treatments: anthracyclines (95%), trastuzumab (88%), vascular endothelial growth factor inhibitors (55%), multitargeted tyrosine kinase inhibitors (TKIs) (47%), and immune checkpoint inhibitors (39%). Many oncologists reported being unfamiliar with cardiotoxities related to proteasome inhibitors (45%), targeted TKIs (33%), 5-flourouracil (30%), and immune checkpoint inhibitors (23%).

FLORIDA EDUCATIONAL COLLABORATIVE PROGRAM

The survey presented herein represents the first step of a statewide, collaborative initiative dedicated to enhancing cardio-oncology knowledge and education. With the results collected from our surveys, we
have developed a program of basic education in cardio-oncology, which is available via the electronic platforms of the FCACC and FLASCO (6). The society platforms act as a permanent repository of information and include monthly e-mail blasts that highlight cardio-oncology topics. Based on the limited number of cardiologists and oncologists with expertise in cardio-oncology, we hope that involvement at the local and state levels will encourage an increased focus on cardio-oncology in more provider groups. This may improve access to care for these complex patients, because most patients are treated in community practices rather than in large academic centers. For institutions without dedicated cardio-oncology programs, our hope is that such efforts to enhance education and increase awareness may improve prevention, early detection, and coordination of care of patients who suffer from both cancer and cardiovascular disease.

We developed focused educational documents for Florida practitioners with brief discussions regarding 11 fundamental cardio-oncology topics (6). The goal of this program is to provide basic information regarding the most common cardiotoxic cancer treatments, including the following: anthracyclines, anti-HER-2 therapies, 5-fluorouracil/cape-citabine, TKIs, proteasome inhibitors, immune checkpoint inhibitors, and radiation therapy. These educational materials also focus on specific cardiac topics for patients with cancer, including thromboembolism, arrhythmias, cardiac imaging, and survivorship. The documents can be found at: https://accfl.org/Cardio-Oncology.

This project is unique in that it sought to address current cardio-oncology knowledge gaps specific to the cardiovascular care of patients with cancer among both oncologists and cardiologists in Florida; however, the proportion of respondents was very small, and we recognize this as an important limitation (7). The study design did not account for differences in responses by age, sex, or practice setting demographics. Of the respondents, 49% reported that they practiced in an academic/hospital setting, and 43% indicated that they were in private practice, with the latter group most likely being underrepresented relative to the Florida physicians’ workforce. Thus, the data provided by the respondents may not be generalizable to all members of the FCACC and FLASCO. This survey is not a representative sample of Florida’s physician population at large and may have sampling bias, reflecting the opinion of physicians who are more actively engaged with medical professional societies than other physicians. However, it highlights the need for further awareness, advocacy,

![FIGURE 1 Main Survey Results](image)

Survey Results
303 physician respondents:
165 of 2,800 oncologists (FLASCO) and 138 of 2,500 cardiologists (FCACC)

| Question in the Survey                                      | Oncologists N=165 | Cardiologists N=138 |
|-------------------------------------------------------------|-------------------|---------------------|
| Very comfortable treating cardio-oncology patients          | 23/163 (14%)      | 22/134 (16%)        |
| Cardio-oncology services in their communities               | 74/161 (46%)      | 58/137 (42%)        |
| Refer to cardio-oncology services if available              | 41/44 (93%)       | 41/120 (34%)        |
| Excellent cooperation between cardiology and oncology       | 61/160 (38%)      | 46/135 (34%)        |
| Lack of local cardio-oncology educational resources         | 103/160 (64%)     | 23/115 (20%)        |
| Attended none or 1 educational session in cardio-oncology (past 3 years) | 134/160 (84%)     | 81/124 (65%)        |

How would you rate your knowledge in cardio-oncology?

![How many educational programs/sessions regarding cardio-oncology have you attended in the previous 3-5 years?](image)

This figure shows the main findings from oncologists and cardiologists responses to the FCACC FLASCO Survey and Collaborative Project. ACC = American College of Cardiology; AHA = American Heart Association; GCOS = Global Cardio Oncology Summit; MSKCC = Memorial Sloan Kettering Cancer Center.
and education in the growing field of cardio-oncology.

Considering these respondents likely represented a more engaged group of practitioners, there was a lack of general cardio-oncology knowledge, uncertainty of local available resources, and low reported rate of cooperation between cardiologists and oncologists. Although we were not able to assess the reasoning for these observed practice patterns, as it was beyond the scope of this study, enhanced collaboration between disciplines will be important and necessary to deliver optimal cardiovascular and oncologic care to this patient group.

Previous studies indicate that approaches vary between cardiologists and oncologists when using cardio-oncology services for the treatment of patients with cancer. Peng et al. (7) reported that most cardiologists (55%) felt that they should monitor for cardiotoxicity even in the absence of symptoms. However, the same study indicated that only 12.5% of oncologists shared this view. Furthermore, 50% of oncologists felt that cardiologists should be involved only when patients developed cardiotoxicities, but only 6.5% of cardiologists agreed with that opinion. Most cardiologists believed that access to cardio-oncology services would improve prognosis (88.3%), whereas only 45.8% of oncologists shared this view. Our study did not directly address this same question, but found that only 38% of oncologists and 34% of cardiologists indicated that they felt “very comfortable” interacting with their colleagues for co-management of cancer and heart disease.

One study surveyed 303 oncologists about knowledge of cardiotoxicities in France. The results showed that only 35% of oncologists actively followed guidelines from oncological societies, and no oncologists were aware of recommendations from cardiac societies (8). However, 88% of respondents did support the development and implementation of cardio-oncology programs. These findings strongly suggest that lack of cooperation between cardiologists and oncologists is an international phenomenon. Fortunately, there also appears to be increasing support for creating new programs to address this need.

The next step of our statewide advocacy, survey, and educational program is the establishment of a large multistate network with involvement of the ACC and the American Society of Clinical Oncology (ASCO) state chapters. This will allow different states to use a similar platform to assess the needs of their own ACC and ASCO members and to develop and share methods to close knowledge gaps. Thus far, this burgeoning network includes members from 19 ACC state chapters: Florida, Missouri, Indiana, Tennessee, Michigan, North Carolina, Texas, Maryland, Virginia, Illinois, Georgia, Ohio, California, Connecticut, Pennsylvania, New York, Minnesota, Colorado, and Milwaukee. It also includes 6 ASCO chapters from both academic and private practice settings (9) and has now also incorporated members from 9 countries with International Cardio-Oncology Society-affiliated chapters (ic-os.org), including Canada, Mexico, Brazil, Argentina, England, France, Poland, Japan, and India. This network will become a platform for multiple future collaborations. We look forward to expanding our network to meet the needs of our colleagues and provide enhanced care for cardio-oncology patients.

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