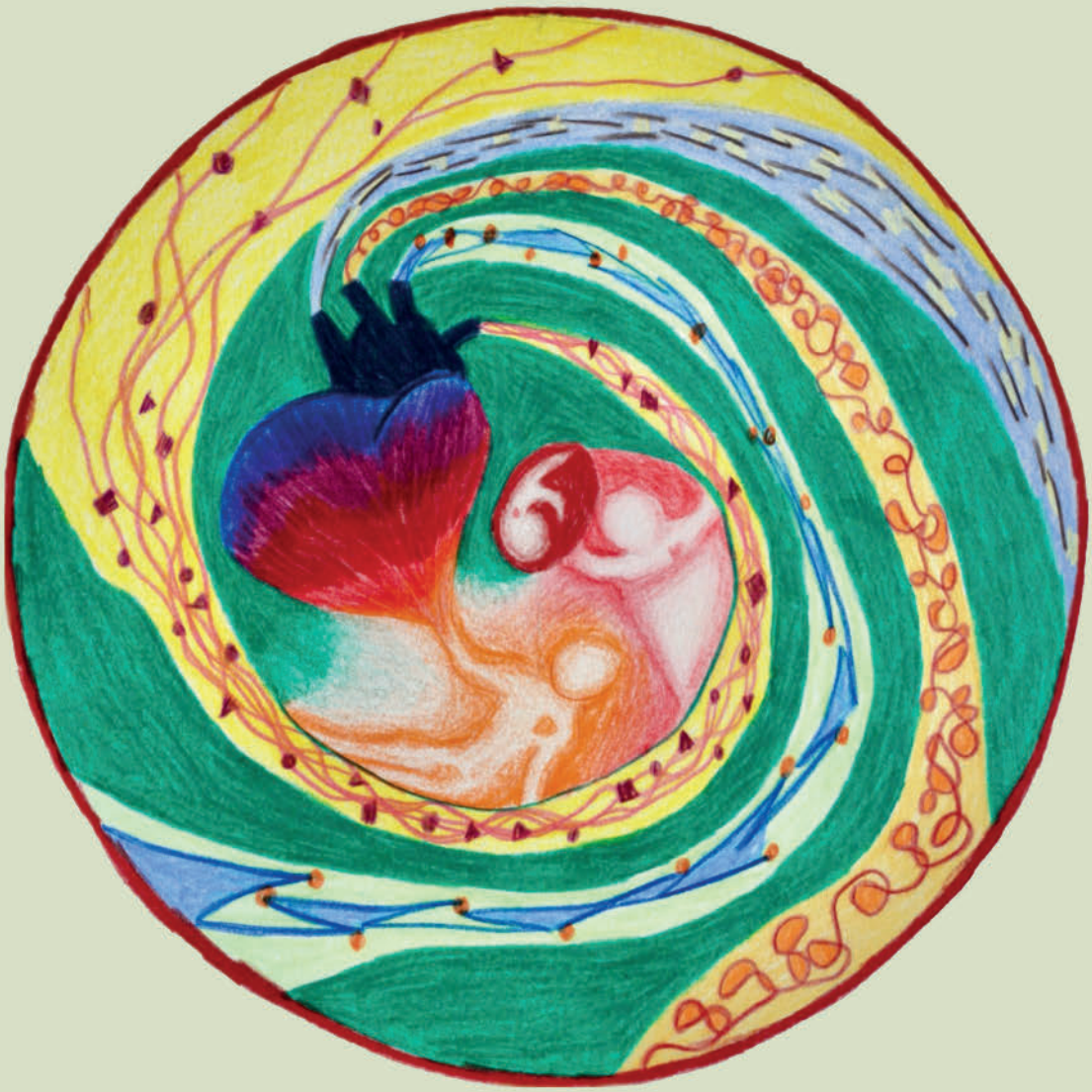
BLOOD BIOMARKERS AND CARDIAC SURVEILLANCE IN CHILDHOOD CANCER SURVIVORS



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INTRODUCTION AND THESIS OUTLINE

HEART FAILURE IN CHILDHOOD CANCER SURVIVORS

Approximately 300,000 children are diagnosed with cancer globally each year.(1) Due to improvements in the early diagnosis, treatment and supportive care of children with cancer, five-year survival has increased from 60% in the mid-1970s to more than 80% in current treatment eras.(2) This growing group of childhood cancer survivors face a high risk of treatment-related long-term adverse health effects.(3) Cardiac disease is one of the most important adverse health effects in this population and can manifest as heart failure due to left ventricular systolic dysfunction, but also as valvular disease, coronary artery disease, pericardial disease and arrhythmias.(4, 5) In this thesis, we focus on heart failure and left ventricular dysfunction in long-term childhood cancer survivors. The elevated risk of heart failure is mainly the result of treatment with anthracyclines, mitoxantrone and/or radiotherapy with the heart in the radiation field, which continue to be used in current childhood cancer treatment regimens.(6, 7, 8) Almost 11% of survivors exposed to these cardiotoxic agents will develop heart failure within 40 years from cancer diagnosis compared to 0.3% of survivors treated with other chemotherapy agents or with surgery.(7) Moreover, cardiovascular mortality is the leading non-cancer cause of death in childhood cancer survivors.(9) Part I, Chapter 2 of this thesis provides a broad overview of the literature on cardiac disease in childhood cancer survivors, including risk factors, risk prediction and prevention.

CARDIAC SURVEILLANCE IN CHILDHOOD CANCER SURVIVORS

Heart failure is typically preceded by an asymptomatic phase during which abnormalities in cardiac function are present.(10) The goal of surveillance is to detect these asymptomatic abnormalities in order to start treatment early and prevent progression to heart failure. To date, there is no direct evidence in childhood cancer survivors showing that treatment of asymptomatic abnormalities can prevent or delay the onset of heart failure. However, there is evidence that treatment with angiotensin converting enzyme inhibitors is effective to prevent heart failure in patients with asymptomatic left ventricular dysfunction after myocardial infarction(11) and in patients with asymptomatic severe left ventricular dysfunction selected from outpatient cardiology clinics. (12) Thus, early detection and treatment of asymptomatic cardiac dysfunction appears to be beneficial. The current cardiomyopathy surveillance guideline from the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) published in 2015 recommends life-long echocardiographic surveillance in all childhood cancer survivors treated with anthracyclines, mitoxantrone and/or chest-directed radiotherapy with surveillance frequency ranging from every 2 to every 5 years depending on cumulative anthracycline, mitoxantrone and chest-directed radiotherapy doses.(4)

Using cancer treatments only for risk stratification, as recommended by the IGHG guideline, assumes that the risk of heart failure is constant over time while it might be more realistic, as survivors age, to update risk stratification based on cardiac function measurements obtained during surveillance. In the Framingham Heart Study, the presence and severity of asymptomatic left ventricular dysfunction was a strong predictor for the development of heart failure among

adults in the community.(13, 14) This suggest it is meaningful to look at the extent of left ventricular dysfunction from cardiac surveillance data in childhood cancer survivors. In Chapter 3 of this thesis, we therefore studied the prognostic implications of asymptomatic left ventricular dysfunction next to anthracycline and radiotherapy doses for developing a therapeutically meaningful further decline in cardiac function.

BLOOD BIOMARKERS FOR DETECTION OF ASYMPTOMATIC CARDIAC DYSFUNCTION IN CHILDHOOD CANCER SURVIVORS

A blood biomarker with high sensitivity and high negative predictive value could be used as a triage test before conducting a surveillance echocardiogram to rule-out cardiac dysfunction in a subset of survivors in whom an echocardiogram can safely be postponed to the next surveillance time point. Also, a blood biomarker with high specificity and high positive predictive value could be used to rule-in cardiac dysfunction in survivors with a borderline abnormal result on echocardiography. At present, it is unclear what role blood biomarkers may have in the detection of asymptomatic cardiac dysfunction in childhood cancer survivors. Of the few studies performed in childhood cancer survivors, natriuretic peptides(15, 16, 17, 18, 19) and cardiac troponins(15, 18, 19) are the most frequently studied.

Natriuretic peptides

Brain natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP) are markers for increased cardiac wall tension that are widely used in the screening for heart failure in different settings, especially for rule-out.(20, 21, 22) In primary care, a NT-proBNP <125 ng/L is used to rule-out heart failure in patients suspected of having chronic heart failure on the basis of signs, symptoms and the electrocardiogram.(20) In dyspneic patients presenting at the emergency department, a NT-proBNP <300 ng/L excludes the presence of acute heart failure with high negative predictive value.(21, 22) Next to its use in the diagnosis of heart failure, NT-proBNP has also been studied for its diagnostic accuracy to detect preclinical cardiac dysfunction. In the general population, the diagnostic accuracy of NT-proBNP was better for more severe cardiac dysfunction with an area under the curve of 0.94 for detecting a left ventricular ejection fraction <40% compared to 0.78 for detecting a left ventricular ejection fraction <50%.(23) In childhood cancer survivors, the role of natriuretic peptides to detect asymptomatic cardiac dysfunction is uncertain as the performed studies were of limited sample size, used different definitions of cardiac dysfunction, did not study optimal biomarker cutoff concentrations for rule-in or rule-out and did not study the use of blood biomarkers in combination with clinical characteristics.(15, 16, 17, 18, 19)

Cardiac troponins

Cardiac troponins are markers for cardiomyocyte injury. As anthracyclines induce cardiomyocyte death, cardiac troponin elevations can be measured during or shortly after treatment and are

predictive of cardiac dysfunction in pediatric and adult cancer patients.(24, 25) Studies conducted in childhood cancer survivors >1 year after anthracycline found detectable troponins in a minority of patients.(15, 18, 19) However, most of these studies did not use a highly sensitive cardiac troponin assay which may improve diagnostic accuracy for cardiac dysfunction.

Part II of this thesis and focuses on blood biomarkers to detect cardiac dysfunction in childhood cancer survivors. Chapter 4 is a systematic review on the diagnostic accuracy of blood biomarkers for detection of cardiac dysfunction in childhood cancer survivors. Chapter 6 describes a study on the diagnostic accuracy of NT-proBNP and cardiac troponins in combination with clinical characteristics for detection of cardiac dysfunction in childhood cancer survivors utilizing data from the Dutch Childhood Cancer Survivor Study (DCCSS LATER). DCCSS LATER is a prospectively designed multicenter study in long-term childhood cancer survivors and siblings. The design of the cardiac part of DCCSS LATER is described in chapter 5. The following two chapters focus on the identification of potential novel biomarkers for anthracycline cardiomyopathy. Chapter 7 is a systematic review on extracellular matrix remodeling markers for anthracycline-induced cardiomyopathy in animal models. Chapter 8 is a case-control study in the DCCSS LATER cohort on the identification of novel candidate plasma biomarkers for anthracycline-related cardiomyopathy from a large set of plasma proteins associated with cardiovascular disease, inflammation, tissue damage and extracellular matrix remodeling.

In Part III, Chapter 9, the updated cardiomyopathy surveillance recommendations of the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) are presented. The IGHG is a collaborative effort to harmonize heterogeneity amongst surveillance recommendations from multiple guideline organizations across the globe. The guideline panel systematically searched and graded the quality of the available evidence, summarized the available evidence and formulated recommendations. Some of the evidence obtained with this thesis contributed to this guideline.

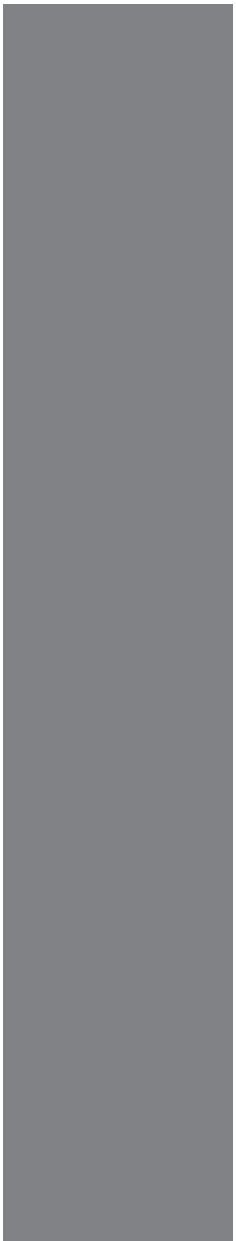
1

REFERENCES

1. Steliarova-Foucher E, Colombet M, Ries LAG, Moreno F, Dolya A, Bray F, et al. International incidence of childhood cancer, 2001–10: a population-based registry study. The Lancet Oncology. 2017;18(6):719-31.
2. Lam CG, Howard SC, Bouffet E, Pritchard-Jones K. Science and health for all children with cancer. Science. 2019;363(6432):1182-6.
3. Geenen MM, Cardous-Ubbink MC, Kremer LC, Bos vd, C., Pal vd, H. J. H., Heinen RC, et al. Medical Assessment of Adverse Health Outcomes in Long-term Survivors of Childhood Cancer. JAMA. 2007;297(24):2705-15.
4. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2015;16(3):e123-36.
5. Leerink JM, Baat ECd, Feijen EAM, Bellersen L, Dalen ECv, Grotenhuis HB, et al. Cardiac Disease in Childhood Cancer Survivors. JACC: CardioOncology. 2020;2(3):363-78.
6. van der Pal HJ, van Dalen EC, van Delden E, van Dijk IW, Kok WE, Geskus RB, et al. High risk of symptomatic cardiac events in childhood cancer survivors. J Clin Oncol. 2012;30(13):1429-37.
7. Feijen E, Font-Gonzalez A, Van der Pal HJH, Kok WEM, Geskus RB, Ronckers CM, et al. Risk and Temporal Changes of Heart Failure Among 5-Year Childhood Cancer Survivors: a DCOG-LATER Study. J Am Heart Assoc. 2019;8(1):e009122.
8. Mulrooney DA, Hyun G, Ness KK, Ehrhardt MJ, Yasui Y, Duprez D, et al. Major cardiac events for adult survivors of childhood cancer diagnosed between 1970 and 1999: report from the Childhood Cancer Survivor Study cohort. BMJ. 2020;368:l6794.
9. Armstrong GT, Chen Y, Yasui Y, Leisenring W, Gibson TM, Mertens AC, et al. Reduction in Late Mortality among 5-Year Survivors of Childhood Cancer. The New England journal of medicine. 2016;374(9):833-42.
10. Echouffo-Tcheugui JB, Erqou S, Butler J, Yancy CW, Fonarow GC. Assessing the Risk of Progression From Asymptomatic Left Ventricular Dysfunction to Overt Heart Failure: A Systematic Overview and Meta-Analysis. JACC Heart Fail. 2016;4(4):237-48.
11. Pfeffer MA, Braunwald E, Moyé LA, Basta L, Brown EJ, Jr., Cuddy TE, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. The New England journal of medicine. 1992;327(10):669-77.
12. Yusuf S, Pitt B, Davis CE, Hood WB, Jr., Cohn JN. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The New England journal of medicine. 1992;327(10):685-91.
13. Wang TJ, Evans JC, Benjamin EJ, Levy D, LeRoy EC, Vasan RS. Natural history of asymptomatic left ventricular systolic dysfunction in the community. Circulation. 2003;108(8):977-82.
14. Tsao CW, Lyass A, Larson MG, Cheng S, Lam CS, Aragam JR, et al. Prognosis of Adults With Borderline Left Ventricular Ejection Fraction. JACC Heart Fail. 2016;4(6):502-10.
15. Brouwer CA, Postma A, Vonk JM, Zwart N, van den Berg MP, Bink-Boelkens MT, et al. Systolic and diastolic dysfunction in long-term adult survivors of childhood cancer. Eur J Cancer. 2011;47(16):2453-62.
16. Mladosievicova B, Urbanova D, Radvanska E, Slavkovsky P, Simkova I. Role of NT-proBNP in detection of myocardial damage in childhood leukemia survivors treated with and without anthracyclines. Journal of experimental & clinical cancer research : CR. 2012;31:86.
17. Pourier MS, Kapusta L, van Gennip A, Bokkerink JP, Loonen J, Bellersen L, et al. Values of high sensitive troponin T in long-term survivors of childhood cancer treated with anthracyclines. Clin Chim Acta. 2015;441:29-32.
18. Ylanen K, Poutanen T, Savukoski T, Eerola A, Vettenranta K. Cardiac biomarkers indicate

a need for sensitive cardiac imaging among long-term childhood cancer survivors exposed to anthracyclines. Acta Paediatr. 2015;104(3):313-9.

19. Mavinkurve-Groothuis AM, Groot-Loonen J, Bellersen L, Pourier MS, Feuth T, Bokkerink JP, et al. Abnormal NT-pro-BNP levels in asymptomatic long-term survivors of childhood cancer treated with anthracyclines. Pediatr Blood Cancer. 2009;52(5):631-6.
20. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2021;42(36):3599-726.
21. Lee KK, Doudesis D, Anwar M, Astengo F, Chenevier-Gobeaux C, Claessens Y-E, et al. Development and validation of a decision support tool for the diagnosis of acute heart failure: systematic review, meta-analysis, and modelling study. BMJ. 2022;377:e068424.
22. Januzzi JL, van Kimmenade R, Lainchbury J, Bayes-Genis A, Ordonez-Llanos J, Santalo-Bel M, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study. Eur Heart J. 2006;27(3):330-7.
23. Costello-Boerrigter LC, Boerrigter G, Redfield MM, Rodeheffer RJ, Urban LH, Mahoney DW, et al. Amino-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide in the general community: determinants and detection of left ventricular dysfunction. J Am Coll Cardiol. 2006;47(2):345-53.
24. Lipshultz SE, Miller TL, Scully RE, Lipsitz SR, Rifai N, Silverman LB, et al. Changes in cardiac biomarkers during doxorubicin treatment of pediatric patients with high-risk acute lymphoblastic leukemia: Associations with long-term echocardiographic outcomes. Journal of Clinical Oncology. 2012;30(10):1042-9.
25. Cardinale D, Sandri MT, Colombo A, Colombo N, Boeri M, Lamantia G, et al. Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. Circulation. 2004;109(22):2749-54.



CARDIAC DISEASE AND RISK PREDICTION IN CHILDHOOD CANCER SURVIVORS

CARDIAC DISEASE IN CHILDHOOD CANCER SURVIVORS: RISK PREDICTION, PREVENTION, AND SURVEILLANCE

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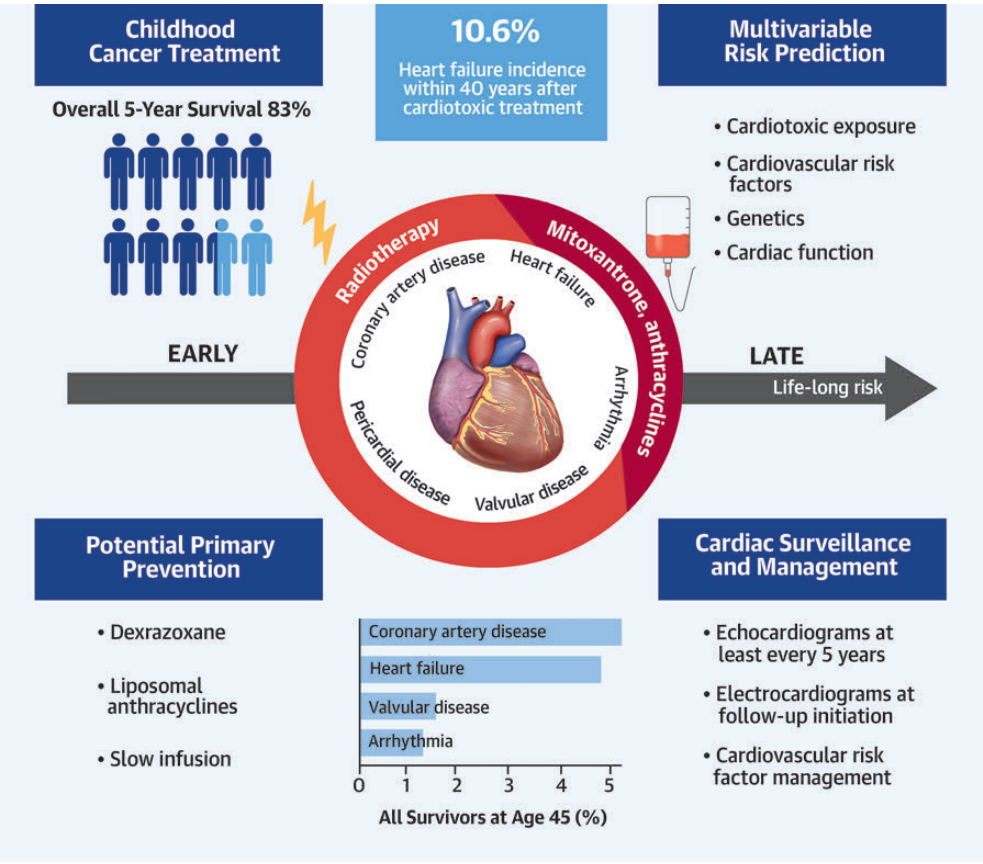
JACC CardioOncology State-of-the-Art review

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.



Central illustration. Overview of clinical practice in childhood cancer survivors at risk for cardiotoxicity. The prevalence of cardiac diseases, risk prediction models, preventive measures and surveillance recommendations are illustrated based on available evidence and promising research topics of cardiotoxicity in childhood cancer survivors. Numbers from Siegel et al., 2019(124); Feijen et al., 2019(10) and Armstrong et al., 2013(17).

Key Words

Childhood cancer survivors, cardiotoxicity, cardiovascular risk factors, risk prediction, prevention

Abbreviations and acronyms

CCS=childhood cancer survivors, chest RT=chest-directed radiotherapy, ECG=electrocardiogram, FS=fractional shortening, GLS=global longitudinal strain, IGHG=The International Late Effects of Childhood Cancer Guideline Harmonization Group, LV=left ventricle, LVEF=left ventricular ejection fraction, RCT = randomized controlled trial.

INTRODUCTION

The survival of children with cancer has considerably increased over the last decades with five-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 disease, coronary artery disease, arrhythmias and pericardial disease, depending on the exact cardiotoxic agent (3).

In this state-of-the-art review, we focus on long-term cardiac diseases after treatment for childhood cancer. We discuss the prevalence, risk factors, prevention, prediction and surveillance of cardiac disease in this population (Central Illustration). We systematically searched PubMed for studies that described cardiac adverse events in children treated with cardiotoxic cancer treatments. We limited the search to full-text articles written in English and articles published within the last 10 years. We selected articles with a study cohort of which >50% were treated for childhood cancer before the age of 21. For studies describing the prevalence or cumulative incidence of heart failure, we reviewed articles with a minimum of 500 CCS; a minimum of 100 CCS was required for the other outcomes. Studies on primary prevention strategies were identified from previous Cochrane searches (4-6). Based on these criteria, 74 studies were considered to be described in this review (Figure 1). The full search strategy is provided in the Supplemental Appendix.

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

Heart failure

Multiple studies have demonstrated 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 point to cardiotoxicity through reduction–oxidation reaction cycling and the generation of reactive oxygen species. 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 grade 3-5. Based on questionnaires in 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 demonstrated that even low-to-moderate chest RT doses increase the risk of heart failure substantially (22,23). In the Dutch LATER cohort, Feijen et al. reported a cumulative heart failure incidence of 10.6%, 40 years after childhood cancer diagnosis in CCS that received cardiotoxic cancer treatment. Interestingly, higher exposure to mitoxantrone and cyclophosphamide were suggested as novel treatment-

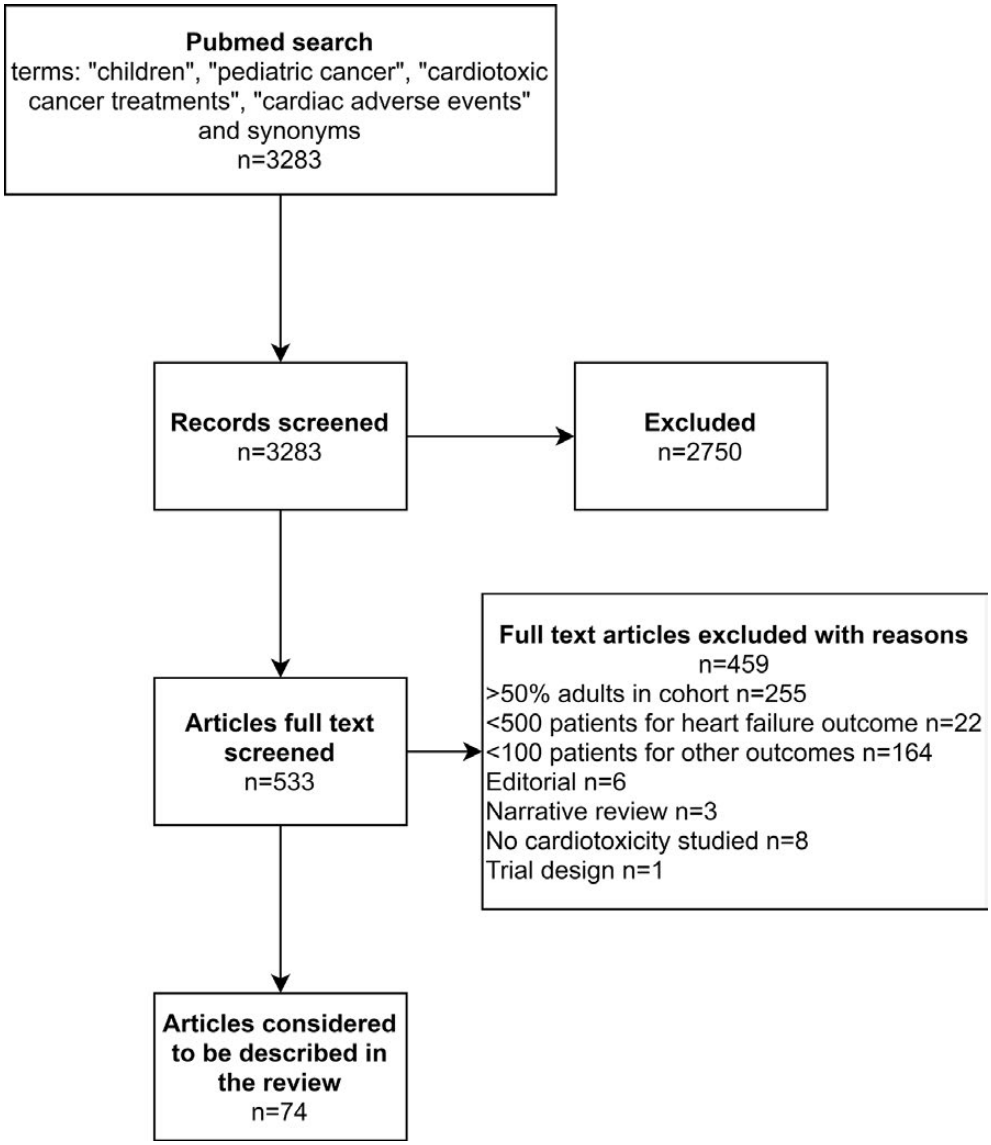


Figure 1. Flowchart of study inclusion. Flowchart describing the systematic literature search in PubMed and the inclusion of relevant studies.

related risk factors (10). While 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 non-linear 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 is substantially increased in CCS. In the Childhood Cancer Survivor Study, the cumulative incidence of coronary artery disease by age 45 years was 5.3% in survivors with and without exposure to cardiotoxic cancer treatments (31). 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 coronary artery disease at age 50 goes up to 20% in males exposed to >35Gy (18,32). The St. Jude Lifetime cohort study detected coronary artery disease, 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 aged ≤55 years (n=31) exposed to chest RT showed coronary artery lesions to be very proximal, placing large portions of the myocardium at risk (33).

Valvular heart disease

Several studies have investigated valvular abnormalities in CCS (11,17,30,34-36), with a reported prevalence of up to 31% (30,34,36). Chest RT has been identified as an important risk factor that increases at higher doses (36). Other risk factors are treatment with anthracyclines, hypertension, congenital heart disease and younger age at diagnosis, although these have not been uniformly demonstrated in all studies (11,30,34). Mild tricuspid regurgitation was most prevalent in two studies describing valvular disease, but it is important to note that this is also very common in the general population (30,34,37). 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 (36). Valvular abnormalities after chest RT are most likely caused by direct irradiation injury to the valve cusps or leaflets, causing thickening, fibrosis, and calcification (30,38). These processes progress with age and increase in prevalence over time (30,36). 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 (39). 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 (18). A single center study in CCS >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 (40).

Arrhythmias

The prevalence of symptomatic cardiac arrhythmias in long term CCS is reportedly low (11,18,31,41). In 10,724 CCS, the cumulative incidence of grade 3 to 5 arrhythmia by 45 years of age was 1.3% (31). A subsequent study (n= 23,462) demonstrated that chest RT > 35 Gy, anthracycline dose ≥250 mg/m^2, 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 (42,43). Prolonged QTc interval, which has arrhythmogenic potential, has been demonstrated in CCS that received anthracyclines or chest RT (44,45). Also, rhythm disturbances like premature ectopic beats and atrioventricular blocks have been reported in CCS (46-48). The literature on ECG abnormalities in large cohorts of long term CCS is sparse (47,48), data on the use of ambulatory ECG monitoring to define the prevalence of brady- and tachyarrhythmias induced by cardiotoxic cancer treatments are needed, but needs to be carefully weighed against the burden and clinical relevance.

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 (49). Advanced radiotherapy techniques to minimize exposure to the heart have been developed; the impact of those improvements is reflected by the decrease in coronary artery disease 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 anti-tumor efficacy or other non-cardiac adverse effects. Below we discuss three 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. It should be kept in mind that due to developmental changes and the differences in the body composition of children, data from adults cannot be reliably extrapolated to children (50).

Dexrazoxane

Dexrazoxane is one of the most widely investigated cardioprotective pharmacological 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 (51-53). These studies suggest that there are no significant differences in clinical heart failure between dexrazoxane and control patients (4,54), although dexrazoxane might have a protective effect on asymptomatic cardiotoxicity (54,55). 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 anti-tumor efficacy and the occurrence of secondary malignancies (56). 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 (51,54).

A recently published non-randomized 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 demonstrated that after a median follow-up period of 3.5 years, cardiac function was preserved with dexrazoxane without negative influence on anti-tumor efficacy or non-cardiac toxicities. Importantly, the influence of possible differences in cumulative anthracycline dose per treatment group could not be evaluated in this study (57).

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 (58). Liposomal anthracyclines have shown promising results in adults with breast cancer (5). In a meta-analysis of two 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 one 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 six hours 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 one hour was strongly recommended (59). Since data in children is 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 (60). Some CCS are at increased risk to develop metabolic syndrome due to 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 (61,62). Survivors treated with cranial radiotherapy are at risk of developing metabolic syndrome, especially obesity (63). Furthermore, abdominal radiation and nephrotoxic treatment may result in the development of cardiovascular risk factors (64,65). Hypertension is the most prevalent cardiovascular risk factor in CCS, approaching 40% in survivors aged ≥50, 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 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 pharmacological 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 (60). Although the association between lifestyle factors and cardiovascular disease has been well established in youth and aging adults (60), 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 due to 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 (62). 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 (66). Furthermore, CCS with lower exercise capacity had more emotional distress and worse attainment of social roles and health-related quality of life (67). 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 nine studies, aerobic exercise was positively related to cardiopulmonary fitness in CCS (68). A systematic review by Raber et al. identified twelve studies on physical activity interventions in CCS. Of these, five studies found that exercise training improved strength, functional mobility and flexibility and/or anthropometric fitness (69). Another systematic review on lifestyle interventions in adolescent and young adult cancer survivors targeting one or more health behaviors identified twelve studies, of which six were successful in changing health behavior (70). Three of these were focused on influencing multiple behaviors, including an individually tailored counseling program on smoking and alcohol consumption. 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 need 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 (71,72).

Development of prediction models broadly includes a development and validation phase (71). In the development phase, relevant predictors are selected based on subject knowledge and/or stepwise regression (73). 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 (73,74). Calibration refers to how well the predicted risks match the actual risks and can be assessed with a calibration plot (72). 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 (71,72). 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 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, female sex, 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 0.68-0.82 in the development and validation cohorts, respectively). The discriminatory abilities of the model were further demonstrated by a cumulative incidence of heart failure at age 40 of 0.5% in the low-risk group, while 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 authors to develop and externally validate a prediction model for ischemic heart disease before age 50 years (32). Male sex and 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 age 50 ranged from 2.3% (95% CI 1.5%-3.1%) in the low-risk group to 19.9% (95% CI 15.0%-24.7%) in the high-risk group, while this was only 1.2% (95% CI 0.4%-2.0%) in siblings. Although a clear segregation was observed between the low- and high-risk groups, the C-statistics were modest. Of note, 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 (29,32).

In a more recent study, diabetes, hypertension and dyslipidemia were used in the prediction of heart failure and ischemic heart disease in CCS aged 20, 25, 30 or 35 years at time of prediction, with relative risks comparable to moderate doses of anthracyclines (75). Cardiovascular risk factors were present in approximately 10% of the CCS at age 35 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 0.69-0.79 in the derivation cohort with successful replication in the other half of the cohort. Of importance, 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 high-risk group may benefit from more frequent surveillance and/or early interventions to modify their risk. However, low risk survivors that may be excluded from further surveillance could not be identified with these models as cumulative incidences of heart failure (~1.5-2.5%) and ischemic heart disease (~1-1.5%) were still significantly higher compared to siblings at age 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 (76). Male sex, non-white race, age at diagnosis, lymphoma history and any radiation were selected as predictors in the Cox regression model. This simple model showed modest discrimination (C-statistic 0.72-0.75) 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 inter-individual variation in the susceptibility for cardiotoxicity after anthracycline treatment (78). Genetic predisposition may explain why some children will develop cardiotoxicity at lower anthracycline doses while 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, anthracycline metabolism and transport have been described and replicated in anthracycline cardiomyopathy (Figure 2, Supplemental Table 3) (77-79). For a comprehensive overview of genetic variants implicated in anthracycline cardiomyopathy we refer the reader to an upcoming state-of-the-art review in this journal and other systematic reviews (77,78).

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. developed several genetic risk prediction models. Validation of the first prediction model failed in an independent cohort (80,81). 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 (AUC) of 0.79 (95% CI 0.74-0.85) in the derivation cohort and 0.76 (0.68-0.83) in the validation cohort, compared to 0.68 (0.61-0.75) for the model with clinical variables only (82). While 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, while it is likely more informative for clinician to understand the probabilities within a certain timeframe. Studies that evaluate the predictive value

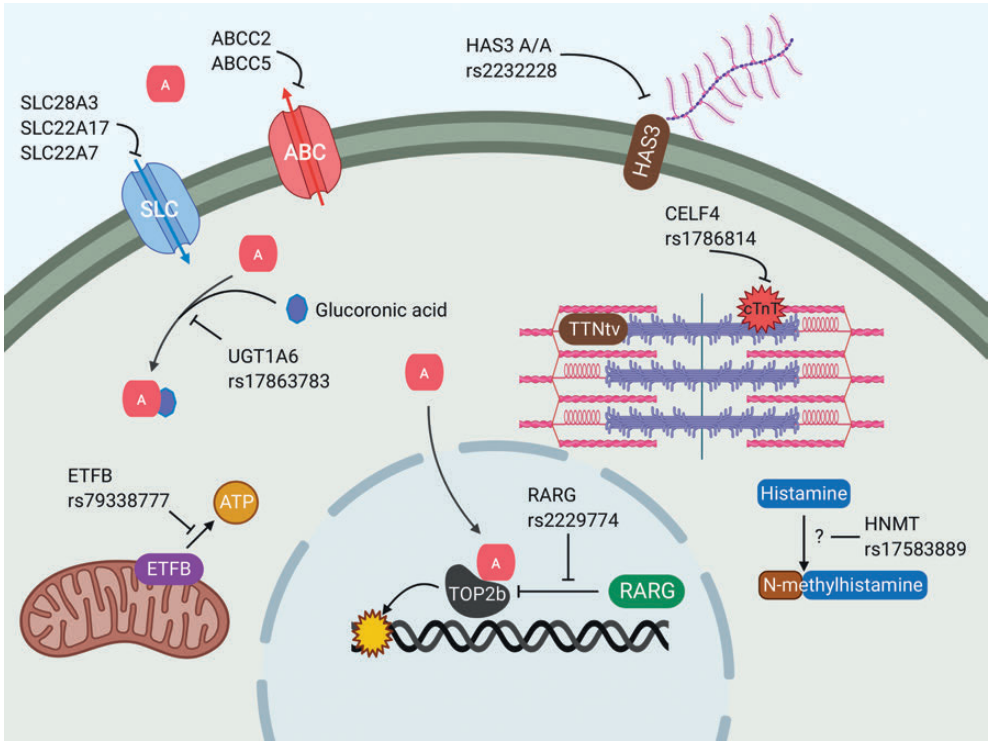


Figure 2. Replicated genetic variants associated with anthracycline-induced cardiomyopathy in childhood cancer survivors and their cellular functions. A=anthracyclines, ABC= ATP binding cassette, ATP=adenosine triphosphate, CELF4=CUGBP Elav-like family member 4, cTnT=cardiac troponin T, ETFB=electron transfer flavoprotein subunit beta, HAS3= hyaluronan synthase 3, HNMT= histamine N-methyltransferase, RARG=retinoic acid receptor gamma, SLC=solute carrier transporter, TOP2b=topoisomerase2b, TTNtv=titin truncating variant, UGT1A6=UDP glucuronosyltransferase family 1 member A6. Created with BioRender.com.

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 (7,48,83). 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 (84). Moreover, acute or early-onset cardiotoxicity is suggested as a predictor for late-onset cardiotoxicity (85).

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.

While 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 underlines 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 due to the long follow-up needed (86).

Another approach to assess clinical impact is decision modeling (86,87). Decision curves can evaluate the net benefit of a prediction model across a range of disease probability thresholds for intervention (88). 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/m^2 (87). Decision modeling provides weaker evidence on the clinical impact compared to an RCT, but 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

In order to detect anthracycline-cancer treatment induced cardiomyopathy there are different methods and techniques available. 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 shortening fraction (FS) and biplane left ventricular ejection fraction (LVEF) are widely used techniques to quantify cardiac dysfunction in survivors of childhood cancer. Fractional shortening is discouraged in patients secondary to potential

regional wall motion abnormalities (89). 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 three-dimensional echocardiography has improved inter- and intra-observer variability, which is desirable for longitudinal follow-up (90). Armstrong et al. demonstrated that the sensitivity and false-negative rate of three-dimensional echocardiography for detection of LVEF<50% measured by cardiac MRI as the gold standard, was improved compared to two-dimensional echocardiography (91).

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 (92,93). A relative GLS decrease of >15% from baseline is suggested as potentially abnormal, whereas a relative decrease of <8% seems not clinically relevant (94). Evidence on strain imaging in CCS is accumulating. Mavinkurve-Groothuis et al. showed a significant difference in GLS between asymptomatic CCS (n=111) approximately 15 years after anthracycline treatment and healthy controls (95). A large study of the St. Jude Lifetime cohort of 1,807 CCS over 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 who had a normal LVEF. Both cumulative anthracycline dose >300 mg/m^2 and any cardiac RT dose was associated with a risk of abnormal GLS (7). It is currently unknown whether an abnormal GLS is associated with a LVEF <50% or clinical heart failure in CCS.

Diastolic dysfunction after cardiotoxic cancer treatment has also been described in CCS (8,96). In the St. Jude Lifetime cohort, diastolic dysfunction grade 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 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 (97) 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 (98). A study in 114 adult survivors demonstrated 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 (91). 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 (99-102).

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 one year after cancer diagnosis was shown in a recent systematic review (103). Conflicting results on the predictive value of natriuretic peptides and troponins measured during cancer treatment for subsequent anthracycline cardiomyopathy exist in CCS (104,105). 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 timepoints (93,106).

ECG parameters may also aid in the prediction of myocardial dysfunction. A recent study in anthracycline treated CCS demonstrated that the QTc interval after chemotherapy was associated with subsequent LV dysfunction (107).

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 (108). Within the guideline development process, recommendations are formulated based on existent national follow-up guidelines and evidence summaries (109-112). 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 (113) 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/m^2, chest RT dose ≥35 Gy or a combination of anthracyclines ≥100 mg/m^2 and chest RT dose ≥15 Gy are regarded as high risk. Anthracycline doses of 100-250 mg/m^2 or chest RT doses 15-35 Gy are regarded as moderate risk, and anthracycline doses <100 mg/m^2 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 two 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 (114).

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 section 4.2.4.

Coronary artery disease surveillance guideline

The IGHG is currently finalizing a guideline for asymptomatic CAD surveillance in childhood, adolescent and young adult cancer survivors (115). Preliminary studies suggest that there is insufficient evidence to recommend a particular surveillance modality in asymptomatic childhood cancer survivors 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 twenties, clinicians should be aware of the potential atypical presentation of CAD in younger patients (17,109).

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 at risk with chest RT doses >15 Gy (113). 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 (109,111). To detect arrhythmia in an early phase, some national groups suggest performing an electrocardiogram at the initiation of long term follow-up (109,111).

Guidelines for management of cardiomyopathy in CCS

The IGHG cardiomyopathy guidelines refer to (pediatric) cardiology guidelines for further investigation and management of cardiac abnormalities (116-118). 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 40-49% should be a 'shared decision' balancing prognosis, heart failure symptoms and the individual's treatment tolerance (117,118). 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 one RCT that evaluated the initiation of ACE-inhibitors for CCS with asymptomatic cardiac dysfunction (119). This study only showed improvement in left ventricular wall stress by echocardiography. Possible reasons for failure to demonstrate an effect on clinical endpoints are the relatively short follow-up time (median 2.8 years) and liberal inclusion criteria (120).

The European Society of Cardiology published a position paper for the diagnosis and management of cancer patients and survivors in adult cardiology (121). The paper recommends prompt initiation of an ACE-inhibitor and βblocker 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 (117,118).

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 intervention studies in CCS may be due to

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 pharmacological heart failure treatments	A RCT on low dose carvedilol in high risk CCS is ongoing (125)
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	Cluster RCTs, decision curve analysis
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 cardiomyopathy	Cohort studies with uniform cardiotoxic 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, GWAS=genome wide association studies, ECG=electrocardiography, RCT=randomized controlled trial, WGS=whole genome sequencing.

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 (40). 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 (122). In addition, modeling complex interactions and non-linear 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 (123).

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. Over 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 pathophysiological mechanisms has 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 multi-disciplinary approach from researchers, epidemiologists, (pediatric) oncologists and cardiologists.

HIGHLIGHTS

- The main risk factors for cardiac disease in childhood cancer survivors are anthracyclines, mitoxantrone and chest directed radiotherapy dose.
- Primary prevention strategies may reduce the risk of anthracycline induced cardiomyopathy.
- There is an increased prevalence of traditional cardiovascular risk factors in childhood cancer survivors; screening and early management are important to modify risk.
- Multivariable risk prediction models may help to individualize prevention and surveillance strategies.

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REFERENCES

1. Gatta G, Botta L, Rossi S et al. Childhood cancer survival in Europe 1999-2007: results of EUROCARE-5--a population-based study. The Lancet Oncology 2014;15:35-47.
2. Geenen MM, Cardous-Ubbink MC, Kremer LC et al. Medical Assessment of Adverse Health Outcomes in Long-term Survivors of Childhood Cancer. JAMA 2007;297:2705-2715.
3. Armenian SH, Armstrong GT, Aune G et al. Cardiovascular Disease in Survivors of Childhood Cancer: Insights Into Epidemiology, Pathophysiology, and Prevention. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2018;36:2135-2144.
4. van Dalen EC, Caron HN, Dickinson HO, Kremer LC. Cardioprotective interventions for cancer patients receiving anthracyclines. The Cochrane database of systematic reviews 2011:Cd003917.
5. van Dalen EC, Michiels EM, Caron HN, Kremer LC. Different anthracycline derivates for reducing cardiotoxicity in cancer patients. The Cochrane database of systematic reviews 2010:CD005006.
6. van Dalen EC, van der Pal HJ, Kremer LC. Different dosage schedules for reducing cardiotoxicity in people with cancer receiving anthracycline chemotherapy. The Cochrane database of systematic reviews 2016;3:Cd005008.
7. Armstrong GT, Joshi VM, Ness KK et al. Comprehensive Echocardiographic Detection of Treatment-Related Cardiac Dysfunction in Adult Survivors of Childhood Cancer: Results From the St. Jude Lifetime Cohort Study. Journal of the American College of Cardiology 2015;65:2511-22.
8. Brouwer CA, Postma A, Vonk JM et al. Systolic and diastolic dysfunction in long-term adult survivors of childhood cancer. European journal of cancer (Oxford, England : 1990) 2011;47:2453-62.
9. Chellapandian D, Pole JD, Nathan PC, Sung L. Congestive heart failure among children with acute leukemia: a population-based matched cohort study. Leukemia & lymphoma 2018:1-10.
10. Feijen E, Font-Gonzalez A, Van der Pal HJH et al. Risk and Temporal Changes of Heart Failure Among 5-Year Childhood Cancer Survivors: a DCOG-LATER Study. Journal of the American Heart Association 2019;8:e009122.
11. Mulrooney DA, Hyun G, Ness KK et al. Major cardiac events for adult survivors of childhood cancer diagnosed between 1970 and 1999: report from the Childhood Cancer Survivor Study cohort. BMJ (Clinical research ed) 2020;368:l6794.
12. Van Der Pal HJ, Van Dalen EC, Hauptmann M et al. Cardiac function in 5-year survivors of childhood cancer: A long-term follow-up study. Archives of internal medicine 2010;170:1247-1255.
13. Armenian SH, Rinderknecht D, Au K et al. Accuracy of a Novel Handheld Wireless Platform for Detection of Cardiac Dysfunction in Anthracycline-Exposed Survivors of Childhood Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2018;24:3119-3125.
14. Christiansen JR, Kanellopoulos A, Lund MB et al. Impaired exercise capacity and left ventricular function in long-term adult survivors of childhood acute lymphoblastic leukemia. Pediatric blood & cancer 2015;62:1437-43.
15. Getz KD, Sung L, Ky B et al. Occurrence of Treatment-Related Cardiotoxicity and Its Impact on Outcomes Among Children Treated in the AAML0531 Clinical Trial: A Report From the Children's Oncology Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2019;37:12-21.
16. Zhang S, Liu X, Bawa-Khalfe T et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nature medicine 2012;18:1639-42.
17. Armstrong GT, Oeffinger KC, Chen Y et al. Modifiable risk factors and major cardiac events among adult survivors of childhood cancer. J Clin Oncol 2013;31:3673-80.
18. van der Pal HJ, van Dalen EC, van Delden E et al. High risk of symptomatic cardiac events in childhood cancer survivors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:1429-37.
19. Fidler MM, Reulen RC, Henson K et al. Population-Based Long-Term Cardiac-Specific Mortality

Among 34 489 Five-Year Survivors of Childhood Cancer in Great Britain. Circulation 2017;135:951-963.

20. Mertens AC, Liu Q, Neglia JP et al. Cause-specific late mortality among 5-year survivors of childhood cancer: The childhood cancer survivor study. Journal of the National Cancer Institute 2008;100:1368-1379.
21. Kremer LC, van Dalen EC, Offringa M, Voute PA. Frequency and risk factors of anthracycline-induced clinical heart failure in children: a systematic review. Ann Oncol 2002;13:503-12.
22. Mansouri I, Allodji RS, Hill C et al. The role of irradiated heart and left ventricular volumes in heart failure occurrence after childhood cancer. European journal of heart failure 2018.
23. Bates JE, Howell RM, Liu Q et al. Therapy-Related Cardiac Risk in Childhood Cancer Survivors: An Analysis of the Childhood Cancer Survivor Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2019;37:1090-1101.
24. Novak RF, Kharasch ED. Mitoxantrone: propensity for free radical formation and lipid peroxidation--implications for cardiotoxicity. Investigantional New Drugs 1985;3:95-99.
25. Rossato LG, Costa VM, Dallegrave E et al. Mitochondrial cumulative damage induced by mitoxantrone: late onset cardiac energetic impairment. Cardiovascular toxicology 2014;14:30-40.
26. Dalen v, E. C., Pal vd, H. J., Bakker PJ, Caron HN, Kremer LC. Cumulative incidence and risk factors of mitoxantrone-induced cardiotoxicity in children: a systematic review. European journal of cancer (Oxford, England : 1990) 2004;40:643-52.
27. Behar C, Such S, Benoit Y et al. Mitoxantrone-Containing Regimen for Treatment of Childhood acute Leukemia (AML) and Analysis of Prognostic Factors: Results of the EORTC Children Leukemia Cooperative Study 58872. Medical and pediatric oncology 1996;26:173-179.
28. Feijen EAM, Leisenring WM, Stratton KL et al. Derivation of Anthracycline and Anthraquinone Equivalence Ratios to Doxorubicin for Late-Onset Cardiotoxicity. JAMA Oncol 2019.
29. Chow EJ, Chen Y, Kremer LC et al. Individual prediction of heart failure among childhood cancer survivors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2015;33:394-402.
30. Mulrooney DA, Armstrong GT, Huang S et al. Cardiac Outcomes in Adult Survivors of Childhood Cancer Exposed to Cardiotoxic Therapy: A Cross-sectional Study. Annals of internal medicine 2016;164:93-101.
31. Armstrong GT, Oeffinger KC, Chen Y et al. Modifiable risk factors and major cardiac events among adult survivors of childhood cancer. Journal of Clinical Oncology 2013;31:3673-3680.
32. Chow EJ, Chen Y, Hudson MM et al. Prediction of Ischemic Heart Disease and Stroke in Survivors of Childhood Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2018;36:44-52.
33. Mulrooney DA, Nunnery SE, Armstrong GT et al. Coronary artery disease detected by coronary computed tomography angiography in adult survivors of childhood Hodgkin lymphoma. Cancer 2014;120:3536-44.
34. van der Pal HJ, van Dijk IW, Geskus RB et al. Valvular abnormalities detected by echocardiography in 5-year survivors of childhood cancer: a long-term follow-up study. International journal of radiation oncology, biology, physics 2015;91:213-22.
35. Schellong G, Riepenhausen M, Bruch C et al. Late valvular and other cardiac diseases after different doses of mediastinal radiotherapy for hodgkin disease in children and adolescents: Report from the longitudinal GPOH follow-up project of the German-Austrian DAL-HD studies. Pediatric Blood and Cancer 2010;55:1145-1152.
36. Christiansen JR, Hamre H, Massey R et al. Left ventricular function in long-term survivors of childhood lymphoma. The American journal of cardiology 2014;114:483-90.
37. Singh JP, Evans JC, Levy D et al. Prevalence and clinical determinants of mitral, tricuspid, and aortic regurgitation (the Framingham Heart Study). The American journal of cardiology 1999;83:897-902.
38. Desai MY, Windecker S, Lancellotti P et al. Prevention, Diagnosis, and Management of Radiation-Associated Cardiac Disease: JACC Scientific Expert Panel. Journal of the American College of Cardiology 2019;74:905-927.

39. Marinko T. Pericardial disease after breast cancer radiotherapy. Radiology and oncology 2018;53:1-5.

40. Harries I, Liang K, Williams M et al. Magnetic Resonance Imaging to Detect Cardiovascular Effects of Cancer Therapy. JACC CardioOncology State-of-the-Art Review 2020;2:270-292.

41. Hudson MM, Ness KK, Gurney JG et al. Clinical ascertainment of health outcomes among adults treated for childhood cancer. JAMA 2013;309:2371-2381.

42. Alexandre J, Moslehi JJ, Bersell KR, Funck-Brentano C, Roden DM, Salem JE. Anticancer drug-induced cardiac rhythm disorders: Current knowledge and basic underlying mechanisms. Pharmacology & therapeutics 2018;189:89-103.

43. Buza V, Rajagopalan B, Curtis AB. Cancer Treatment-Induced Arrhythmias: Focus on Chemotherapy and Targeted Therapies. Circ Arrhythm Electrophysiol 2017;10.

44. Gupta M, Thaler HT, Friedman D, Steinherz L. Presence of prolonged dispersion of qt intervals in late survivors of childhood anthracycline therapy. Pediatric hematology and oncology 2002;19:533-42.

45. Schwartz CL, Hobbie WL, Truesdell S, Constine LC, Clark EB. Corrected QT interval prolongation in anthracycline-treated survivors of childhood cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1993;11:1906-10.

46. Caru M, Corbin D, Perie D et al. Doxorubicin treatments induce significant changes on the cardiac autonomic nervous system in childhood acute lymphoblastic leukemia long-term survivors. Clin Res Cardiol 2019;108:1000-1008.

47. Pourier MS, Mavinkurve-Groothuis AMC, Loonen J et al. Is screening for abnormal ECG patterns justified in long-term follow-up of childhood cancer survivors treated with anthracyclines? Pediatric Blood and Cancer 2017;64:e26243.

48. Mulrooney DA, Soliman EZ, Ehrhardt MJ et al. Electrocardiographic abnormalities and mortality in aging survivors of childhood cancer: A report from the St Jude Lifetime Cohort Study. Am Heart J 2017;189:19-27.

49. van Dalen EC, Raphael MF, Caron HN, Kremer LCM. Treatment including anthracyclines versus treatment not including anthracyclines for childhood cancer. Cochrane Database of Systematic Reviews 2014;2014 (9) (no pagination).

50. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. The New England journal of medicine 2003;349:1157-67.

51. Chow EJ, Asselin BL, Schwartz CL et al. Late mortality after dexrazoxane treatment: A report from the Children's Oncology Group. Journal of Clinical Oncology 2015;33:2639-2645.

52. Wexler LH, Andrich MP, Venzon D et al. Randomized trial of the cardioprotective agent ICRF-187 in pediatric sarcoma patients treated with doxorubicin, 1996.

53. Lipshultz SE, Rifai N, Dalton VM et al. The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. New England Journal of Medicine 2004;351:145-153.

54. Asselin BL, Devidas M, Chen L et al. Cardioprotection and Safety of Dexrazoxane in Patients Treated for Newly Diagnosed T-Cell Acute Lymphoblastic Leukemia or Advanced-Stage Lymphoblastic Non-Hodgkin Lymphoma: A Report of the Children's Oncology Group Randomized Trial Pediatric Oncology Group 9404. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016;34:854-62.

55. Lipshultz SE, Scully RE, Lipsitz SR et al. Assessment of dexrazoxane as a cardioprotectant in doxorubicin-treated children with high-risk acute lymphoblastic leukaemia: Long-term follow-up of a prospective, randomised, multicentre trial. The Lancet Oncology 2010;11:950-961.

56. Tebbi CK, London WB, Friedman D et al. Dexrazoxane-associated risk for acute myeloid leukemia/myelodysplastic syndrome and other secondary malignancies in pediatric Hodgkin's disease. Journal of clinical oncology 2007;25:493β00.

57. Getz KD, Sung L, Alonzo TA et al. Effect of Dexrazoxane on Left Ventricular Systolic

Function and Treatment Outcomes in Patients With Acute Myeloid Leukemia: A Report From the Children's Oncology Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2020;38:2398-2406.

58. Lao J, Madani J, Puertolas T et al. Liposomal Doxorubicin in the treatment of breast cancer patients: a review. J Drug Deliv 2013;2013:456409.
59. Loeffen EAH, van Dalen EC, Mulder RL, van de Wetering MD, Kremer LCM, Tissing WJE. The duration of anthracycline infusion should be at least one hour in children with cancer: A clinical practice guideline. Pediatric blood & cancer 2018;65.
60. Benjamin EJ, Blaha MJ, Chiuve SE et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation 2017;135:e146-e603.
61. Oudin C, Simeoni MC, Sirvent N et al. Prevalence and risk factors of the metabolic syndrome in adult survivors of childhood leukemia. Blood 2011;117:4442-8.
62. Smith WA, Li C, Nottage KA et al. Lifestyle and metabolic syndrome in adult survivors of childhood cancer: a report from the St. Jude Lifetime Cohort Study. Cancer 2014;120:2742-50.
63. van Waas M, Neggers SJ, Pieters R, van den Heuvel-Eibrink MM. Components of the metabolic syndrome in 500 adult long-term survivors of childhood cancer. Ann Oncol 2010;21:1121-6.
64. Dekkers IA, Blijdorp K, Cransberg K et al. Long-term nephrotoxicity in adult survivors of childhood cancer. Clinical journal of the American Society of Nephrology : CJASN 2013;8:922-9.
65. Meacham LR, Dilley KJ. Radiotherapy: Diabetes risk after radiation--not out of the woods. Nature reviews Clinical oncology 2012;9:618-9.
66. Ness KK, Plana JC, Joshi VM et al. Exercise Intolerance, Mortality, and Organ System Impairment in Adult Survivors of Childhood Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2020;38:29-42.
67. Hayek S, Brinkman TM, Plana JC et al. Association of Exercise Intolerance With Emotional Distress, Attainment of Social Roles, and Health-Related Quality of Life Among Adult Survivors of Childhood Cancer. JAMA Oncol 2020.
68. Bourdon A, Grandy SA, Keats MR. Aerobic exercise and cardiopulmonary fitness in childhood cancer survivors treated with a cardiotoxic agent: a meta-analysis. Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer 2018;26:2113-2123.
69. Raber M, Swartz MC, Santa Maria D et al. Parental involvement in exercise and diet interventions for childhood cancer survivors: a systematic review. Pediatr Res 2016;80:338-46.
70. Pugh G, Gravestock HL, Hough RE, King WM, Wardle J, Fisher A. Health Behavior Change Interventions for Teenage and Young Adult Cancer Survivors: A Systematic Review. J Adolesc Young Adult Oncol 2016;5:91-105.
71. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. BMJ (Clinical research ed) 2015;350:g7594.
72. Steyerberg E, Vergouwe Y. Towards better clinical prediction models. 2014:1925-1931.
73. Harrell F. REGRESSION MODELING STRATEGIES : with applications to linear models, logistic and ordinal ... regression, and survival analysis. [Place of publication not identified]: SPRINGER, 2016.
74. Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Statistics in medicine 2011;30:11-21.
75. Chen Y, Chow EJ, Oeffinger KC et al. Traditional Cardiovascular Risk Factors and Individual Prediction of Cardiovascular Events in Childhood Cancer Survivors. Journal of the National Cancer Institute 2020;112:256-265.
76. Oikonomou EK, Athanasopoulou SG, Kampaktsis PN et al. Development and Validation of a Clinical Score for Cardiovascular Risk Stratification of Long-Term Childhood Cancer Survivors. The oncologist 2018;23:965-973.
77. Aminkeng F, Ross CJ, Rassekh SR et al. Recommendations for genetic testing to reduce the incidence of anthracycline-induced

cardiotoxicity. British journal of clinical pharmacology 2016;82:683-95.

78. Leong SL, Chaiyakunapruk N, Lee SWH. Candidate Gene Association Studies of Anthracycline-induced Cardiotoxicity: A Systematic Review and Meta-analysis. Scientific reports 2017;7:39.

79. Tripaydonis A, Conyers R, Elliott DA. Pediatric Anthracycline-Induced Cardiotoxicity: Mechanisms, Pharmacogenomics, and Pluripotent Stem-Cell Modeling. Clinical pharmacology and therapeutics 2019;105:614-624.

80. Visscher H, Ross CJ, Rassekh SR et al. Pharmacogenomic prediction of anthracycline-induced cardiotoxicity in children. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:1422-8.

81. Visscher H, Ross CJ, Rassekh SR et al. Validation of variants in SLC28A3 and UGT1A6 as genetic markers predictive of anthracycline-induced cardiotoxicity in children. Pediatric blood & cancer 2013;60:1375-81.

82. Visscher H, Rassekh SR, Sandor GS et al. Genetic variants in SLC22A17 and SLC22A7 are associated with anthracycline-induced cardiotoxicity in children. Pharmacogenomics 2015;16:1065-1076.

83. Leerink JMvdP, H.J.H.; Kremer, L.C.M.; Feijen, E.A.M.; Meregalli,P.G.; Pourier, M.S.; Merkx, R.; Bellersen, L.; van Dalen, E.C.; Loonen, J.; Pinto, Y.M.; Kapusta, L.; Mavinkurve-Groothuis, A.M.C.; Kok, W.E.M. The added value of echocardiography for the 10-year prediction of left ventricular systolic dysfunction in long-term survivors of childhood cancer. submitted, 2020.

84. Tomer A, Rizopoulos D, Nieboer D, Drost F-J, Roobol MJ, Steyerberg EW. Personalized Decision Making for Biopsies in Prostate Cancer Active Surveillance Programs. Medical Decision Making 2019;39:499-508.

85. Temming P, Qureshi A, Hardt J et al. Prevalence and predictors of anthracycline cardiotoxicity in children treated for acute myeloid leukaemia: retrospective cohort study in a single centre in the United Kingdom. Pediatric blood & cancer 2011;56:625-30.

86. Hendriksen JMT, Geersing GJ, Moons KGM, de Groot JAH. Diagnostic and prognostic prediction models. Journal of Thrombosis and Haemostasis 2013;11:129-141.

87. Yeh JM, Nohria A, Diller L. Routine echocardiography screening for asymptomatic left ventricular dysfunction in childhood cancer survivors: a model-based estimation of the clinical and economic effects. Annals of internal medicine 2014;160:661-671.

88. Vickers AJ, van Calster B, Steyerberg EW. A simple, step-by-step guide to interpreting decision curve analysis. Diagnostic and Prognostic Research 2019;3:18.

89. Lang RM, Badano LP, Mor-Avi V et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. European Heart Journal - Cardiovascular Imaging 2015;16:233-271.

90. Thavendiranathan P, Grant AD, Negishi T, Plana JC, Popovic ZB, Marwick TH. Reproducibility of echocardiographic techniques for sequential assessment of left ventricular ejection fraction and volumes: application to patients undergoing cancer chemotherapy. Journal of the American College of Cardiology 2013;61:77-84.

91. Armstrong GT, Plana JC, Zhang N et al. Screening adult survivors of childhood cancer for cardiomyopathy: comparison of echocardiography and cardiac magnetic resonance imaging. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:2876-84.

92. Negishi K, Negishi T, Hare JL, Haluska BA, Plana JC, Marwick TH. Independent and incremental value of deformation indices for prediction of trastuzumab-induced cardiotoxicity. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography 2013;26:493-8.

93. Sawaya H, Sebag IA, Plana JC et al. Assessment of echocardiography and biomarkers for the extended prediction of cardiotoxicity in patients treated with anthracyclines, taxanes, and trastuzumab. Circ Cardiovasc Imaging 2012;5:596-603.

94. Plana JC, Galderisi M, Barac A et al. Expert consensus for multimodality imaging evaluation

of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. European heart journal cardiovascular Imaging 2014;15:1063-93.

95. Mavinkurve-Groothuis AM, Groot-Loonen J, Marcus KA et al. Myocardial strain and strain rate in monitoring subclinical heart failure in asymptomatic long-term survivors of childhood cancer. Ultrasound in medicine & biology 2010;36:1783-91.

96. Dorup I, Levitt G, Sullivan I, Sorensen K. Prospective longitudinal assessment of late anthracycline cardiotoxicity after childhood cancer: the role of diastolic function. Heart (British Cardiac Society) 2004;90:1214-6.

97. Nagueh SF, Smiseth OA, Appleton CP et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography 2016;29:277-314.

98. Jordan JH, Hundley WG. MRI of Cardiotoxicity. Cardiol Clin 2019;37:429-439.

99. Tham EB, Haykowsky MJ, Chow K et al. Diffuse myocardial fibrosis by T1-mapping in children with subclinical anthracycline cardiotoxicity: relationship to exercise capacity, cumulative dose and remodeling. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 2013;15:48.

100. Toro-Salazar OH, Gillan E, O'Loughlin MT et al. Occult cardiotoxicity in childhood cancer survivors exposed to anthracycline therapy. Circ Cardiovasc Imaging 2013;6:873-80.

101. Tong X, Li VW, Liu AP et al. Cardiac Magnetic Resonance T1 Mapping in Adolescent and Young Adult Survivors of Childhood Cancers. Circ Cardiovasc Imaging 2019;12:e008453.

102. Mawad W, Mertens L, Pagano JJ et al. Effect of anthracycline therapy on myocardial function and markers of fibrotic remodelling in childhood cancer survivors. European heart journal cardiovascular Imaging 2020.

103. Leerink JM, Verkleij SJ, Feijen EAM et al. Biomarkers to diagnose ventricular dysfunction in childhood cancer survivors: a systematic review. Heart (British Cardiac Society) 2019;105:210-216.

104. Moazeni S, Cadeiras M, Yang EH, Deng MC, Nguyen K-L. Anthracycline induced cardiotoxicity: biomarkers and "Omics" technology in the era of patient specific care. Clin Transl Med 2017;6:17-17.

105. Kremer LC, Bastiaansen BA, Offringa M et al. Troponin T in the first 24 hours after the administration of chemotherapy and the detection of myocardial damage in children. European journal of cancer (Oxford, England : 1990) 2002;38:686-9.

106. Cardinale D, Sandri MT, Colombo A et al. Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. Circulation 2004;109:2749-54.

107. Markman TM, Ruble K, Loeb D et al. Electrophysiological effects of anthracyclines in adult survivors of pediatric malignancy. Pediatric blood & cancer 2017;64.

108. Kremer LC, Mulder RL, Oeffinger KC et al. A worldwide collaboration to harmonize guidelines for the long-term follow-up of childhood and young adult cancer survivors: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. Pediatric blood & cancer 2013;60:543-9.

109. Children's Oncology Group - Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancers. Version 5.0 - October 2018.

110. Scottish Intercollegiate Guidelines Network (SIGN). Long term follow up of survivors of childhood cancer. Edinburgh: SIGN; 2013. [March 2013].

111. Dutch Childhood Oncology Group. Guidelines for follow-up in survivors of childhood cancer 5 years after diagnosis. English translation 2014.

112. United Kingdom Children's Cancer Study Group. Therapy Based Long Term Follow Up. Practice Statement. 2nd edition, April 2005.

113. Armenian SH, Hudson MM, Mulder RL et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer:

a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology 2015;16:e123-e136.

114. Hudson MM, Leisenring W, Stratton KK et al. Increasing cardiomyopathy screening in at-risk adult survivors of pediatric malignancies: a randomized controlled trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:3974-81.

115. Mulrooney D, Van Dalen E, Bardi E et al. Coronary Artery Disease Surveillance for Childhood, Adolescent and Young Adult Cancer Survivors – Recommendations from the International Guideline Harmonization Group. Abstracts from the 51st Congress of the International Society of Paediatric Oncology (SIOP) Lyon, France, October 23–26, 2019: Wiley, 2019.

116. Kirk R, Dipchand AI, Rosenthal DN et al. The International Society for Heart and Lung Transplantation Guidelines for the management of pediatric heart failure: Executive summary. [Corrected]. The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation 2014;33:888-909.

117. Ponikowski P, Voors AA, Anker SD et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European heart journal 2016;37:2129-2200.

118. Yancy CW, Jessup M, Bozkurt B et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology 2013;62:e147-239.

119. Cheuk DKL, Sieswerda E, van Dalen EC, Postma A, Kremer LCM. Medical interventions for treating anthracycline‐induced symptomatic and asymptomatic cardiotoxicity during and after treatment for childhood cancer. Cochrane Database of Systematic Reviews 2016.

120. Silber JH, Cnaan A, Clark BJ et al. Enalapril to prevent cardiac function decline in long-term survivors of pediatric cancer exposed to anthracyclines. J Clin Oncol 2004;22:820-8.

121. Zamorano JL, Lancellotti P, Rodriguez Munoz D et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). European heart journal 2016;37:2768-2801.

122. Leerink JM, Feijen ELAM, van der Pal HJH et al. Diagnostic tools for early detection of cardiac dysfunction in childhood cancer survivors: Methodological aspects of the Dutch late effects after childhood cancer (LATER) cardiology study. American Heart Journal 2020;219:89-98.

123. Gunturkun F, Davis RL, Armstrong GT et al. Deep learning for improved prediction of late-onset cardiomyopathy among childhood cancer survivors: A report from the St. Jude Lifetime Cohort (SJLIFE). Journal of Clinical Oncology 2020;38:10545-10545.

124. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7-34.

SUPPLEMENTAL MATERIAL

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3

REFINING THE 10-YEAR PREDICTION OF LEFT VENTRICULAR SYSTOLIC DYSFUNCTION IN LONG-TERM SURVIVORS OF CHILDHOOD CANCER

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ABSTRACT

Background

In childhood cancer survivors (CCS) at risk for heart failure, echocardiographic surveillance recommendations are currently based on anthracycline- and chest-directed radiotherapy dose. Whether the ejection fraction (EF) measured at an initial surveillance echocardiogram can refine these recommendations is unknown.

Objective

To assess the added predictive value of EF at >5 years after cancer diagnosis to anthracycline and chest-directed radiotherapy dose in CCS, for the development of left ventricular dysfunction with an EF<40% (LVD40) .

Methods

Echocardiographic surveillance was performed in 299 CCS from the Emma Children's Hospital in the Netherlands. Cox regression models were built including cardiotoxic cancer treatment exposures with and without EF to estimate the probability of LVD40 at 10 years follow-up. Calibration, discrimination and reclassification were assessed. Results were externally validated in 218 CCS.

Results

Cumulative incidences of LVD40 at 10 years follow-up were 3.7% and 3.6% in the derivation and validation cohort, respectively. Addition of EF resulted in an integrated area under the curve increase from 0.74 to 0.87 in the derivation cohort and from 0.72 to 0.86 in the validation cohort (likelihood-ratio p<0.001). Reclassification of CCS without LVD40 improved significantly (non-case cNRI 0.50, 95% CI [0.40-0.60]). A predicted LVD40 probability ≤3%, representing 75% of the CCS, had a negative predictive value of 99% (95% CI 98-100%) for LVD40 within 10 years. However, patients with mid-range EF (40-49%) at initial screening had an incidence of LVD40 of 11% and a 7.81 (95% CI 2.07-29.50) fold increased risk of LV40 at follow-up.

Conclusions

In CCS, an initial surveillance EF, in addition to anthracycline- and chest-directed radiotherapy dose, improves the 10-year prediction for LVD40. Through this strategy, both the identification of low-risk survivors in whom the surveillance frequency may be reduced and a group of survivors at increased risk of LVD40 could be identified.

INTRODUCTION

The survival of childhood cancer has increased considerably over the last decades, with 80% of children with cancer becoming long-term (≥5 year) survivors.(1) However, the same treatment that successfully cured their childhood cancer places them at an increased risk of adverse events up to 40 years after childhood cancer diagnosis.(2) Cardiotoxicity in childhood cancer survivors (CCS) is a well-known late effect after treatment with anthracyclines, mitoxantrone or chest-directed radiotherapy.(3, 4, 5) The cumulative incidence of symptomatic heart failure at 40 years past cancer diagnosis is 10.6% in CCS treated with cardiotoxic cancer therapies.(5) In addition, asymptomatic left ventricular (LV) dysfunction is frequently present in CCS and is associated with an increased risk of developing symptomatic heart failure in the general population.(6) When defined as an ejection fraction (EF) <50%, or as a fractional shortening <28%, asymptomatic LV dysfunction has been reported in 6-8 % of CCS at a median of 9 to 23 years after cancer diagnosis.(7, 8, 9, 10)

Currently, to detect and treat asymptomatic LV dysfunction early, the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) recommends to perform an echocardiogram once every five years in all CCS treated with cardiotoxic cancer therapies. (11, 12) More frequent surveillance is thought reasonable in high risk CCS treated with cumulative doses of anthracyclines ≥250mg/m2, chest-directed radiotherapy ≥35 Gray or a combination of the two (anthracycline ≥100mg/m2 and chest-directed radiotherapy ≥15 Gray).(11) Referral to a cardiologist is recommended once asymptomatic LV dysfunction (EF<50%) is identified. However, the recommendation for pharmacological treatment is, due to a lack of evidence in CCS(13, 14), based on guidelines for adults with asymptomatic LV dysfunction from other causes.(11),(15, 16) These guidelines recommend treatment for symptomatic patients and for patients with asymptomatic LV systolic dysfunction, although their direct relevance to CCS is unknown.(17, 18)

The current IGHG surveillance guidelines do not include measurements of LV function in the risk stratification for cardiomyopathy.(11) We hypothesized that EF measured at the first long-term follow-up echocardiogram may improve cardiomyopathy risk stratification and may further serve to personalize surveillance frequency recommendations. With the knowledge that an EF<40% is a strong and widely accepted indication to start heart failure medications(15), an optimal surveillance strategy should be directed to timely identify CCS with an EF<40%, regardless of the presence of heart failure symptoms.

In this study, we assessed and externally validated the added predictive value of EF at first long-term follow-up echocardiogram in asymptomatic CCS treated with cardiotoxic cancer therapies for the development of left ventricular dysfunction with EF<40% (LVD40).

METHODS

Study population

The derivation cohort consisted of CCS from the Emma Children's Hospital in Amsterdam, The Netherlands. This cohort included CCS with a primary childhood malignancy between 1966 and 1997, treated with anthracyclines, mitoxantrone and/or chest-directed radiotherapy who were at least 5 years past cancer diagnosis.(8)

The validation cohort consisted of CCS from the Radboud University Medical Center in Nijmegen, the Netherlands.(19, 20, 21) CCS treated with anthracyclines, who were at least 5 years past cancer diagnosis and who visited the survivorship outpatient clinic between 2006-2012 were included in this cohort.

From both cohorts, we selected CCS >18 years of age at the first follow-up echocardiogram who were treated with anthracyclines, mitoxantrone and/or chest-directed radiotherapy. CCS with a history of heart failure before the first available follow-up echocardiogram ≥5 year after cancer diagnosis were excluded. Asymptomatic CCS with an EF<40% before or at the first echocardiogram were also excluded. For the longitudinal analysis, CCS with ≥2 follow-up echocardiograms were included when the time interval between each echocardiogram was ≤5 years and the total follow-up was ≥1 year. We chose the time interval of ≤5 years between each echocardiogram because this is the time interval recommended by the cardiomyopathy surveillance guidelines.(11) Informed consent for participation in the late effects study cohort was previously obtained from all participants and the study was approved by the medical ethics boards of the Emma Children's Hospital/Academic Medical Center and the Radboud University Medical Center.(8, 19, 20)

Data collection

Data were retrospectively collected from medical records, digitally archived echocardiograms and the database of prior studies within these cohorts(8, 19, 20). Variables of interest included: sex, cancer diagnosis, age at cancer diagnosis, age at first echocardiogram (see below, 'Echocardiograms'), time since cancer diagnosis at first echocardiogram, cardiovascular risk factors (hypertension, dyslipidemia and/or diabetes reported in questionnaires or diagnosed by a physician), heart failure medication prescriptions, cumulative doses of anthracycline (summed according to doxorubicin-equivalent ratios(22)), mitoxantrone and chest-directed radiotherapy.

Chest-directed radiotherapy was defined as radiotherapy involving the heart region and included total body irradiation, left or whole abdominal irradiation, spinal irradiation, thoracic irradiation, and inverted Y field irradiation. For the chest-directed radiotherapy dose we used the maximum prescribed dose to the smallest field and added the total body irradiation dose.(23)

Echocardiograms

The first available echocardiogram ≥5 years after cancer diagnosis was used to measure the initial EF. All subsequent echocardiograms after the first echocardiogram were systematically collected. All echocardiograms were performed by trained sonographers and supervised by an imaging cardiologist. Fractional shortening was measured in the parasternal long axis and calculated from the LV internal diameter at end-diastole and end-systole at the base of the LV by M-mode echocardiography. Biplane EF was measured in the apical chamber views with the modified Simpson's method.(24) In cases where biplane EF could not be measured, EF was calculated using the Teichholz formula that has been shown to accurately estimate EF in the absence of dyssynchrony and wall motion abnormalities.(25) We assessed the agreement between Teichholz and biplane-derived EF in 323 echocardiograms where both metrics were available (Table S1,

Figure S1). The overall agreement on the endpoint of an EF<40% or EF≥40% was 97%.(26) In 30 randomly selected echocardiograms the intraclass correlation coefficient for the intraobserver variability of biplane EF was 0.83 (95% confidence interval (CI) 0.67-0.92) and the intraclass correlation coefficient for interobserver variability was 0.79 (95% CI 0.61-0.90), which is comparable to values reported in the literature.(27)

Statistical analyses

Continuous variables are presented as mean ± standard deviation (SD) when normally distributed and as median (25th-75th percentile) when asymmetrically distributed. Categorical variables are presented as number with percentages. Patient characteristics were compared between groups with the Student's t-test for normally distributed continuous variables, the Kruskal-Wallis test for asymmetrically distributed continuous variables and the Chi-square or Fisher's exact test for categorical variables.

The primary endpoint was the onset of left ventricular dysfunction with EF<40% (LVD40) after the first follow-up echocardiogram. Time was considered from the point at which the initial EF was obtained. The cumulative incidence of LVD40 was estimated with death as a competing risk and compared CCS with an EF 40-49% to CCS with an EF≥50% at first echocardiogram using the Fine and Gray's test.(28)

Hazard ratios (HRs) with 95% CIs were estimated with multivariable Cox regression models. Anthracycline and chest-directed radiotherapy dose that are currently used for risk stratification in the IGHG surveillance guideline(11) were entered in the model with and without the addition of initial EF. EF was categorized to estimate the risk associated with an EF 40-49% (mid-range) compared to an EF≥50% (preserved). Continuous EF was used in the prediction model development because continuous covariates have superior predictive power.

The proportionality assumption was tested with the Schoenfeld residual test and by inspecting the Schoenfeld residuals over time.(29) Non-linearity of the covariates was tested for with restricted cubic splines (results in supplementary material).(30)

We estimated the baseline hazard with the Breslow estimator at 10 years follow-up (H0(t)) in both cohorts and calculated the linear predictor (LP) with the coefficients derived from the model fitted in the derivation cohort. Subsequently, individual 10-year probabilities for LVD40 (LVD40prob) were estimated with the formula(31): $LVD40prob(t=10) = 1-[H0(t=10)exp(LP)]$

Calibration was evaluated by plotting the observed versus the predicted 10-year probabilities for LVD40 in 5 groups. In the derivation cohort, improvement in model performance with the addition of initial EF was tested using the likelihood ratio test.(32) Discrimination was quantified with the integrated area under the receiver operating characteristic curve (iAUC), which represents a weighted average of time-dependent AUC measures.(33, 34) Bias and 95% CIs of the iAUCs were assessed using 2000 bootstrap samples. The continuous net reclassification improvement (cNRI) of the model with addition of the initial EF value as compared to the model without EF was calculated. The cNRI indicates the proportion of patients that accurately change in their predicted risk with the addition of EF to the model and can be calculated for cases and non-cases (Table S4).(35) Time-

dependent accuracy measures (sensitivity, specificity, negative and positive predictive values) of the model with EF were calculated with the 'timeROC' package, which accounts for censoring.(36)

To adjust for selection bias that might have resulted from the exclusion of CCS, we performed a sensitivity analysis in the derivation cohort where we weighted the HR estimates with the inverse of the sampling probability.(37) To estimate the sampling probability we used a logistic regression model with selection for this study (yes/no) as the outcome, and sex, age at cancer diagnosis (as a spline), cumulative anthracycline dose (as a spline), chest-directed radiotherapy dose, cumulative mitoxantrone dose, cancer diagnosis year and LVD40 or heart failure (yes/no) as covariates. Additional sensitivity analyses wereperformed with heart failure medication use and cardiovascular risk factors (hypertension, dyslipidemia and diabetes).

All analyses were performed in R (version 3.5.1, R Foundation, Vienna, Austria), a two-sided p-value <0.05 was considered statistically significant. No missing data was present in the predictor variables.

RESULTS

Characteristics of CCS in the derivation and validation cohort

In the derivation cohort, 690 CCS received cardiotoxic cancer treatment and survived ≥5 years after diagnosis (Figure 1). Eighty-four CCS died before available echocardiographic follow-up (four of heart failure). Other reasons for exclusion included: heart failure or an EF<40% before the first follow-up echocardiogram (n=16), <2 follow-up echocardiograms performed (n=200), or ≥5 years between the follow-up echocardiograms (n=91). In total, 299 CCS were eligible for this study. Compared to the CCS that were excluded, those included were more often female (56.2% versus 33.7%, p<0.001) and were treated with higher anthracycline doses (median 280 mg/m2 [25th-75th percentile 180-400] versus 200 mg/m2 [150-360], p=0.013) (Table S2).

In the validation cohort, 400 CCS were treated with cardiotoxic cancer treatments and survived ≥5 years after diagnosis and 218 of them were eligible for inclusion (Figure 1). Reasons for exclusion were an age <18 years during echocardiographic follow-up (n=70), >5 years between the follow-up echocardiograms (n=60), <2 follow-up echocardiograms performed (n=49) and heart failure before echocardiographic follow-up (n=3).

Patient characteristics of both cohorts are presented in Table 1. Compared to the derivation cohort, CCS in the validation cohort were more often treated with anthracyclines at lower doses (derivation cohort 280 mg/m2 [180-400]; validation cohort 180 mg/m2 [150-301]) and had a higher initial EF (derivation cohort mean 61.6%±7.1 versus validation cohort mean 57.1%±6.9). A mid-range initial EF (EF 40-49%) was present at baseline in 13.7% CCS in the derivation cohort and in 5.5% of the patients in the validation cohort. CCS with a mid-range EF were exposed to higher anthracycline doses compared to CCS with a preserved EF (EF≥50%). Follow-up after the first echocardiogram was longer in the derivation cohort (median 10.9 years [8.2-13.1]) as compared to the validation cohort (median 8.9 years [5.2-10.9]) (Table 1).

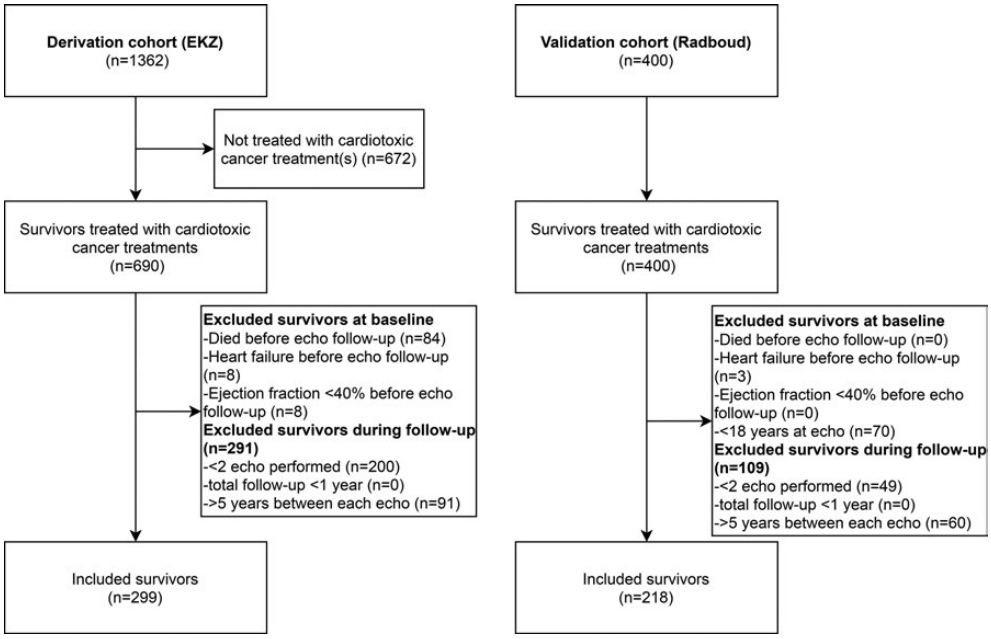


Figure 1. Flowchart of Patient Inclusion. Flowchart describing the inclusion of childhood cancer survivors (CCS) in the derivation and validation cohort. Adult survivors who were previously treated with cardiotoxic cancer treatments with at least 2 surveillance echocardiograms performed at more than 5 years from cancer diagnosis and with <5 years between each echocardiogram were selected. Survivors with heart failure or an ejection fraction <40% before or at the first surveillance echocardiogram were excluded. echo = echocardiogram; EKZ = Emma Children's Hospital; Radboud = Radboud University Medical Center.

Incidence of LVD40 and characteristics of CCS with LVD40 in the derivation cohort

In the derivation cohort, the cumulative incidence of LVD40 at 10-years follow-up after the first echocardiogram was 3.7% (11 events, 95% CI 1.4%-5.9%). In six patients, LVD40 was accompanied by symptomatic heart failure and 10 patients were treated with heart failure medications. At a median follow-up of 7.2 years (25th-75th percentile 6.2-9.7) after the initial EF, 12 CCS died, with 10 due to cancer, one due to nervous system disease in a patient with a cerebral drain, and one had unexplained sudden death without a known cardiomyopathy diagnosis. The cumulative LVD40 incidence 10 years after the initial EF was significantly higher in CCS with an initial mid-range EF (11.0%) compared to CCS with an initial preserved EF (2.6%; Gray's test p=0.012). In CCS with LVD40, the median time from first echocardiogram to LVD40 onset was 7.2 years (25th-75th percentile 3.8-8.4; range 1.2-12.2) and was not significantly different between CCS with a mid-range EF and those with a preserved EF (median 7.2 years [3.3-8.9] versus 6.6 years [4.7-7.7], p=0.085). In multivariable analysis adjusted for anthracycline and chest-directed radiotherapy, CCS with an initial mid-range EF had a higher risk of LVD40 compared to CCS with a preserved EF (HR 7.8, 95% CI 2.1-29.5) (Table 2). All CCS who developed LVD40 were treated with cumulative anthracycline doses ≥100

mg/m2 or chest-directed radiotherapy doses ≥15 Gray, corresponding to a moderate or high risk according to the cardiomyopathy surveillance guideline.(11)

Table 1. Characteristics of the CCS in the derivation and validation cohort

	Derivation cohort: Amsterdam	Validation cohort: Nijmegen	P-value
n	299	218	
Sex, female	168 (56.2)	109 (50.0)	0.192
Age at cancer diagnosis, y	7.22 [4.01, 11.71]	7.02 [4.00, 12.46]	0.625
Time since cancer diagnosis at first follow-up echo, y	16.74 [11.83, 23.15]	16.95 [12.99, 21.70]	0.512
Age at first follow-up echo, y	24.06 [19.60, 30.71]	22.63 [20.05, 28.06]	0.399
Tumor			<0.001
ALL	55 (18.4)	71 (32.6)	
AML	14 (4.7)	15 (6.9)	
Hodgkin lymphoma	23 (7.7)	30 (13.8)	
Non-Hodgkin lymphoma	61 (20.4)	37 (17.0)	
Nephroblastoma	46 (15.4)	14 (6.4)	
Soft-tissue sarcoma	28 (9.4)	7 (3.2)	
Ewing sarcoma	18 (6.0)	14 (6.4)	
Osteosarcoma	24 (8.0)	13 (6.0)	
CNS tumour	17 (5.7)	4 (1.8)	
Germ cell tumour	4 (1.3)	1 (0.5)	
Neuroblastoma	2 (0.7)	9 (4.1)	
Other	7 (2.3)	2 (0.9)	
Anthracyclines	239 (79.9)	214 (98.2)	<0.001
Cumulative anthracycline dose, mg/m2	280.00 [180.00, 400.00]	180.00 [150.00, 301.38]	<0.001
Chest RT	105 (35.1)	59 (27.1)	0.065
Chest RT dose, Gy	25.00 [18.00, 33.25]	20.00 [18.00, 30.00]	0.406
Anthracyclines and chest RT	45 (15.1)	56 (25.7)	0.004
Mitoxantrone	12 (4.0)	7 (4.2)	1.000
Cumulative mitoxantrone dose, mg/m2	12.00 [12.00, 16.00]	40.00 [20.00, 40.00]	0.003
EF at first follow-up echo	57.10 ± 6.94	61.62 ± 7.11	<0.001
EF 40-49% at first follow-up echo	41 (13.7)	12 (5.5)	0.004
Hypertension	15 (5.0)	-	
Dyslipidemia	4 (1.34)	-	
Diabetes Mellitus	2 (0.7)	-	
Heart failure medication(s) use at first echo	4 (1.3)	3 (1.4)	1.000
Follow-up after the first follow-up echo, y	10.90 [8.19, 13.05]	8.86 [5.22, 10.86]	<0.001
Number of follow-up echoes per patient	5.00 [3.00, 6.00]	3.00 [2.00, 4.00]	<0.001
Echocardiographic surveillance rate, per 5 y	2.26 [1.96, 2.67]	1.93 [1.57, 2.52]	<0.001
Left ventricular dysfunction with EF<40% during follow-up	11 (3.7)	7 (3.2)	0.965

Values are n (%), mean ± SD, or median [25th and 75th percentile]. Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; Chest RT, chest-directed radiotherapy; CNS, central nervous system; Gy, gray; n, number; y, year.

Table 2. Multivariable Cox regression of potential risk factors for LVD40 during follow-up.

Model without first EF	HR (95% CI)	P-value
Anthracycline dose (per 100 mg/m^2 increment)	1.71 (1.21-2.40)	0.002
Chest-directed radiotherapy dose (per 10 Gy increment)	1.65 (1.20-2.26)	0.002
Model with continuous first EF		
EF at first echocardiogram (per 10 points decrease)	9.62 (2.84-32.57)	<0.001
Anthracycline dose (per 100 mg/m^2 increment)	1.43 (1.04-1.98)	0.026
Chest-directed radiotherapy dose (per 10 Gy increment)	1.67 (1.21-2.30)	0.002
Model with categorized first EF		
Mid-range versus preserved EF at first echocardiogram	7.81 (2.07-29.50)	0.002
Anthracycline dose (per 100 mg/m^2 increment)	1.70 (1.22-2.36)	0.002
Chest-directed radiotherapy dose (per 10 Gy increment)	1.91 (1.34-2.72)	<0.001

Abbreviations: CI, confidence interval; EF, ejection fraction; LVD40, left ventricular dysfunction with EF<40%; HR, Hazard ratio; Gy, Gray.

Prediction model development

Lower initial EF increased the risk of LVD40 during follow-up (HR 9.6 per 10 points EF decrease, 95% CI 2.8-32.6)(Table 2). Addition of initial EF to the prediction model with anthracycline and chest-directed radiotherapy dose increased the iAUC from 0.74 (bias 0.018, 95% CI 0.55-0.84) to 0.87 (bias 0.009, 95% CI 0.71-0.98). The likelihood ratio test comparing the predictive performance of the model with EF to the model without EF was highly significant (p<0.001). The model with EF showed good calibration at 10-years follow-up (Figure 2). Net reclassification of cases who developed LVD40 did not improve significantly with the addition of initial EF (case cNRI 0.15, 95% CI -0.55-0.84, Table S4). However, for non-cases (who did not develop LVD40) reclassification improved significantly (non-case cNRI 0.50, 95% CI 0.40-0.60). A 10-year predicted risk ≤3% was present in 76.3% of CCS and achieved a high sensitivity (89.8%, 95% CI 70.6%-100%) and negative predictive value (99.5%, 95% CI 98.6%-100%) with a specificity of 76.2%, 95% CI 70.0%-82.5% and a positive predictive value of 12.0%, 95% CI 4.0%-20.0% (Table 3). Results of the inverse probability weighted sensitivity analysis were comparable to the main results and are shown in Table S5. In another sensitivity analysis, heart failure medication use and presence cardiovascular risk factors (hypertension, dyslipidemia and diabetes) at time of the initial EF were not associated with LVD40 and did not attenuate the association of initial EF with LVD40 (Table S6).

External validation

In the validation cohort, the cumulative incidence of LVD40 at 10-years follow-up after the first echocardiogram was 3.6% (7 events, 95% CI 0.7%-6.4%). With the model developed in the derivation cohort, individual 10-year LVD40 probabilities were calculated. The model showed good calibration up to a LVD40 probability of 5%, which represented 83.0% of the CCS (Figure 2). The iAUC increased from 0.72 (bias -0.003, 95% CI 0.70-0.77) to 0.86 (bias -0.003, 95% CI 0.83-0.89) in the model containing initial EF versus the model containing only anthracycline and chest-directed radiotherapy dose. A predicted 10-year probability ≤3% was present in 74.8% of the CCS and resulted in a sensitivity

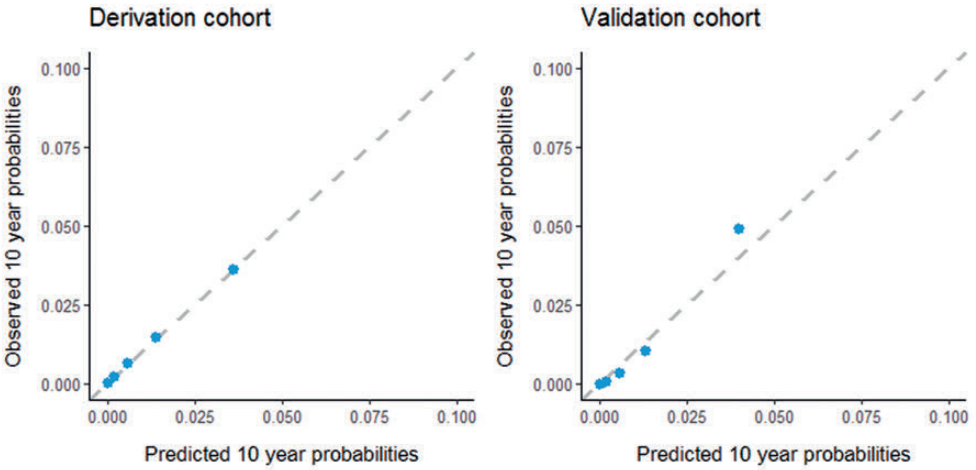


Figure 2. Calibration Plots. Agreement between the predicted 10-year probabilities of a left ventricular ejection fraction <40% (LVD40) obtained from the Cox regression model compared with the observed 10-year LVD40 probabilities in the derivation and the validation cohorts. Predictions from the final multivariable Cox regression model including ejection fraction are shown

of 85.1% (95% CI 57.8%-100%), specificity of 77.1% (95% CI 68.0%-86.2%), positive predictive value of 12.2% (95% CI 1.6%-22.8%) and negative predictive value of 99.3% (95% CI 97.9%-100%)(Table 3).

Predicted probabilities of LVD40 within 10 years in individual survivors with different predictor value combinations are shown in the Central Illustration. Survivors in the low and moderate risk group according to the IGHG surveillance guidelines with a preserved initial EF (EF 55%) had a predicted LVD40 probability ≤3.0%. In contrast, survivors in the low and moderate IGHG risk group with a mid-range EF (EF 48%) had a predicted LVD40 probability where the upper 95% CI was >3.0%. Our validated prediction model including a surveillance EF, cumulative anthracycline and chest-directed radiotherapy dose is accessible online at https://risk-of-cardiomyopathy.netlify.com/.

DISCUSSION

In this echocardiographic follow-up study of long-term CCS, we show in two independent cohorts that addition of an initial surveillance EF improves the 10-year prediction of LVD40 in CCS and accurately identifies low-risk survivors who are unlikely to develop LVD40 within 10 years. This may improve the current IGHG recommended risk-stratification for cardiomyopathy, which is based solely on anthracycline- and chest-directed radiotherapy dose to estimate risk.(11)

Previous echocardiographic follow-up studies in long-term CCS were generally limited in sample size (range n=28-115) and did not assess the additive predictive value of echocardiography together with cancer treatment exposures.(38, 39, 40, 41, 42, 43, 44) We demonstrate for the first time in a relatively large cohort of CCS with long-term follow-up, that a mid-range EF (EF 40-49%) is associated with an almost eight-fold increased risk for LVD40 compared to those with a preserved first EF (EF≥50%), a finding that is in line with the risk of asymptomatic LV dysfunction

Table 3. Time-dependent accuracy measures of the multivariable model including continuous EF at different predicted risks cut-offs for LVD40 at 10 years follow-up after the first echocardiogram.

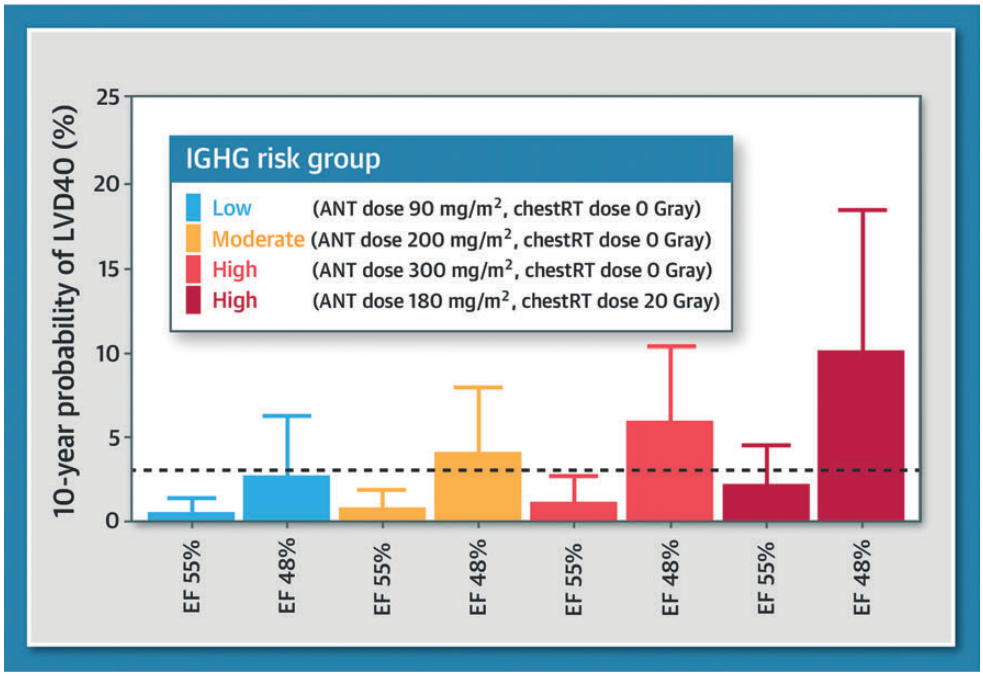
Cutoff* (%)	Actual risk* (%)	Positive test (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Derivation cohort: Amsterdam						
1.0	1.1	47.8	89.8 (70.6-100)	47.5 (40.2-54.8)	5.8 (1.9-9.8)	99.2 (97.7-100)
2.0	2.1	34.1	89.8 (70.6-100)	63.0 (55.9-70.0)	8.0 (2.6-13.5)	99.4 (98.3-100)
3.0	3.1	23.7	89.8 (70.6-100)	76.2 (70.0-82.5)	12.0 (4.0-20.0)	99.5 (98.6-100)
4.0	4.0	18.7	89.8 (70.6-100)	81.8 (76.1-87.4)	15.1 (5.2-25.0)	99.6 (98.7-100)
5.0	4.9	15.4	56.0 (23.4-88.6)	85.1 (79.9-90.3)	11.9 (1.9-22.0)	98.2 (96.4-100)
Validation cohort: Nijmegen						
1.0	0.7	47.2	100.0 (100-100)	59.0 (48.4-69.6)	8.3 (1.7-15.0)	100.0 (100-100)
2.0	1.9	31.7	100.0 (100-100)	71.1 (61.3-80.9)	11.4 (2.3-20.5)	100.0 (100-100)
3.0	3.3	25.2	85.1 (57.8-100)	77.1 (68.0-86.2)	12.2 (1.6-22.8)	99.3 (97.9-100)
4.0	4.9	20.2	85.1 (57.8-100)	81.9 (73.6-90.2)	14.9 (2.0-27.9)	99.3 (98.0-100)
5.0	6.6	17.0	85.1 (57.8-100)	88.0 (80.9-95.0)	20.8 (3.0-38.7)	99.4 (98.1-100)

* Predicted and actual cumulative incidences of LVD40 at 10-years from the first echocardiogram. Abbreviations: CI, confidence interval; EF, ejection fraction; LVD40, left ventricular dysfunction with EF<40%; NPV, negative predictive value; PPV, positive predictive value.

for development of symptomatic heart failure from other causes in the general population (RR 4.6, 95% CI 2.2-9.8).(6) The fact that 13.7% of the CCS in the derivation cohort and 5.5% of the CCS in the validation cohort had a mid-range EF, considerably higher than the LV dysfunction (EF<50%) prevalence of 1.7%-3.6% in the general population at age 50-62 years(6), suggests that a large group of relatively young CCS are already at an increased risk.

Implications for surveillance

The IGHG cardiomyopathy surveillance guidelines recommend echocardiographic surveillance once every five years in CCS treated with anthracyclines and/or chest-directed radiotherapy.(11) In the absence of long-term longitudinal echocardiographic follow-up data, these recommendations were based on simulation studies with relative risks of asymptomatic LV dysfunction for heart failure and treatment effects obtained from the general population.(45, 46)



Central Illustration. Refinement of the IGHG surveillance guideline risk groups using the EF measured with a surveillance echocardiogram. Predicted probabilities for developing left ventricular dysfunction with ejection fraction (EF) <40% within 10 years in individual fictional survivors. In colors the risk categories (low, moderate, or high) are presented according to the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG). In each IGHG risk category, the 10-year probability of left ventricular dysfunction with EF <40% is compared between a survivor with an initial surveillance EF of 48% and a survivor with an initial EF of 55%. Bars represent the risk estimate; error bars represent the 95% confidence interval.

Recently, it has been suggested in a simulation model that cardiomyopathy surveillance is cost-ineffective in the IGHG low-risk group, representing ~40% of the survivors.(47) Our results in two independent CCS cohorts also suggest that LVD40 is very unlikely in low-risk survivors according to the IGHG surveillance guideline as no LVD40 events occurred in this risk group during a median follow-up of 10.9 in the derivation cohort and 8.9 years in the validation cohort.

In addition, we demonstrate that a surveillance EF obtained at a median of 17 years (25th-75th percentile 13-22 years) after cancer diagnosis and a median age of 23 years (25th-75th percentile 20-28 years), in addition to anthracycline and chest-directed radiotherapy dose, accurately reclassifies 50% (95% CI 40-60%) of the CCS who will not develop LVD40 to a lower risk category. This means that an initial surveillance EF can refine the risk stratification as recommended by the IGHG surveillance guideline, resulting in reclassification of survivors in the IGHG moderate risk group to a group at low risk of LVD40 within 10 years (Central Illustration).

We were able to identify a large subgroup representing at least 75% of CCS in the derivation and validation cohort with a predicted risk ≤3% who were unlikely to develop LVD40 within 10 years

(NPV 99%, 95%CI 98-100%). This finding implicates that for low- risk CCS with a predicted risk ≤3%, obtaining the next surveillance echocardiogram within 10-years may be sufficient. It also means that only ~25% of the CCS population determined to be at higher risk need to be screened according to the current surveillance protocol, and that the yield of patients with LVD40 within the 10 year follow up period will be higher from 1 in 30 patients to 1 in 8 patients screened.

Limitations

Some limitations of our study should be considered. First, echocardiograms obtained before 1999 were unavailable for analysis and therefore the initial echocardiogram was obtained at varying time points after cancer diagnosis (25th-75th percentile 12-23 years) and age of the CCS (25th-75th percentile 20-30 years). This makes our results applicable to survivors with echocardiograms performed at these ages and years after diagnosis. Of note, age at baseline was not associated with LVD40 onset (HR 0.99, p=0.859). Second, the Teichholz EF is currently not preferred for calculating EF and limits of agreement with biplane EF were relatively large. However, there was 97% agreement between Teichholz EF and biplane EF on the outcome (LVD40) in our study. Third, the number of CCS who developed LVD40 was low which resulted in broad confidence intervals of our HR estimates. Fourth, selection bias may have been present in our study as the CCS included in the derivation cohort were treated with higher anthracycline doses compared to the CCS not included in the study. However, we confirmed our findings in a validation cohort of 218 CCS who received lower anthracycline doses (median 180 mg/m2) and in a sensitivity analysis that adjusted for the possible influence of selection bias. This underlines the generalizability of our findings to lower risk survivors. Lastly, other echocardiographic measurements such as diastolic dysfunction, valvular abnormalities and myocardial strain parameters were not evaluated in this study, although they may be useful.(48, 49) This is currently being assessed in the Dutch LATER cohort study.(50)

Conclusions

Our results demonstrate that EF measured with a surveillance echocardiogram at a median of 17 years (25th-75th percentile 13-22 years) from cancer diagnosis and a median age of 23 years (25th-75th percentile 20-28 years) has additional predictive value in the risk stratification for a therapeutically relevant decreased EF <40%. Our validated model and 10-year risk calculator can be used to classify 75% of CCS as low-risk for LVD40 within 10 years; less frequent surveillance may be appropriate in these survivors.

CLINICAL PERSPECTIVES

Competency in medical knowledge

In childhood cancer survivors at risk for heart failure, a prediction model that includes ejection fraction at approximately 13-22 years from cancer diagnosis, in addition to anthracycline and chest-directed radiotherapy dose, improves the 10-year prediction of ejection fraction (EF) < 40% (LVD40). In addition to the use of this model to identify a large subgroup of CCS with a predicted risk

≤3% for LVD40 within 10 years, we determined that a mid-range EF (EF 40-49%) is associated with an almost eight-fold increased risk for LVD40 compared to those with a preserved first EF (EF≥50%).

Translational outlook

Larger studies with longer follow-up are needed to assess whether follow-up surveillance echocardiograms can be performed at 10 year intervals or even longer periods of time. Moreover, other echocardiographic parameters, such as myocardial strain, should be studied to understand their predictive value in this population.

FUNDING

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REFERENCES

1. Gatta G, Botta L, Rossi S, Aareleid T, Bielska-Lasota M, Clavel J, et al. Childhood cancer survival in Europe 1999-2007: results of EUROCARE-5--a population-based study. The Lancet Oncology. 2014;15(1):35-47.
2. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, et al. Chronic health conditions in adult survivors of childhood cancer. The New England journal of medicine. 2006;355(15):1572-82.
3. Mulrooney DA, Yeazel MW, Kawashima T, Mertens AC, Mitby P, Stovall M, et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. BMJ (Clinical research ed). 2009;339:b4606.
4. Pal vd, H. J., Dalen v, E. C., Delden v, E., Dijk v, I. W., Kok WE, Geskus RB, et al. High risk of symptomatic cardiac events in childhood cancer survivors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30(13):1429-37.
5. Feijen EAM, Font–Gonzalez A, Van der Pal Helena JH, Kok Wouter EM, Geskus Ronald B, Ronckers Cécile M, et al. Risk and Temporal Changes of Heart Failure Among 5–Year Childhood Cancer Survivors: a DCOG–LATER Study. Journal of the American Heart Association. 2019;8(1):e009122.
6. Echouffo-Tcheugui JB, Erqou S, Butler J, Yancy CW, Fonarow GC. Assessing the Risk of Progression From Asymptomatic Left Ventricular Dysfunction to Overt Heart Failure: A Systematic Overview and Meta-Analysis. JACC: Heart Failure. 2016;4(4):237-48.
7. Mulrooney DA, Armstrong GT, Huang S, Ness KK, Ehrhardt MJ, Joshi VM, et al. Cardiac Outcomes in Adult Survivors of Childhood Cancer Exposed to Cardiotoxic Therapy: A Cross-sectional Study. Annals of internal medicine. 2016;164(2):93-101.
8. van der Pal HJ, van Dalen EC, Hauptmann M, Kok WE, Caron HN, van den Bos C, et al. Cardiac function in 5-year survivors of childhood cancer: a long-term follow-up study. Archives of internal medicine. 2010;170(14):1247-55.
9. Armenian SH, Mertens L, Slorach C, Venkataraman K, Mascarenhas K, Nathwani N, et al. Prevalence of anthracycline-related cardiac dysfunction in long-term survivors of adult-onset lymphoma. Cancer. 2018;124(4):850-7.
10. Armstrong GT, Joshi VM, Ness KK, Marwick TH, Zhang N, Srivastava D, et al. Comprehensive Echocardiographic Detection of Treatment-Related Cardiac Dysfunction in Adult Survivors of Childhood Cancer: Results From the St. Jude Lifetime Cohort Study. Journal of the American College of Cardiology. 2015;65(23):2511-22.
11. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2015;16(3):e123-36.
12. Sieswerda E, Postma A, van Dalen EC, van der Pal HJ, Tissing WJ, Rammeloo LA, et al. The Dutch Childhood Oncology Group guideline for follow-up of asymptomatic cardiac dysfunction in childhood cancer survivors. Ann Oncol. 2012;23(8):2191-8.
13. Silber JH, Cnaan A, Clark BJ, Paridon SM, Chin AJ, Rychik J, et al. Enalapril to Prevent Cardiac Function Decline in Long-Term Survivors of Pediatric Cancer Exposed to Anthracyclines. Journal of Clinical Oncology. 2004;22(5):820-8.
14. Cheuk DK, Sieswerda E, van Dalen EC, Postma A, Kremer LC. Medical interventions for treating anthracycline-induced symptomatic and asymptomatic cardiotoxicity during and after treatment for childhood cancer. The Cochrane database of systematic reviews. 2016(8):Cd008011.
15. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European journal of heart failure. 2016;18(8):891-975.

16. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Jr., Drazner MH, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology. 2013;62(16):e147-239.

17. Yusuf S, Pitt B, Davis CE, Hood WB, Jr., Cohn JN. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The New England journal of medicine. 1992;327(10):685-91.

18. Jong P, Yusuf S, Rousseau MF, Ahn SA, Bangdiwala SI. Effect of enalapril on 12-year survival and life expectancy in patients with left ventricular systolic dysfunction: a follow-up study. Lancet (London, England). 2003;361(9372):1843-8.

19. Pourier MS, Mavinkurve-Groothuis AMC, Loonen J, Bokkerink JPM, Roeleveld N, Beer G, et al. Is screening for abnormal ECG patterns justified in long-term follow-up of childhood cancer survivors treated with anthracyclines? Pediatric Blood and Cancer. 2017;64(3):e26243.

20. Mavinkurve-Groothuis AM, Groot-Loonen J, Bellersen L, Pourier MS, Feuth T, Bokkerink JP, et al. Abnormal NT-pro-BNP levels in asymptomatic long-term survivors of childhood cancer treated with anthracyclines. Pediatric blood & cancer. 2009;52(5):631-6.

21. Mavinkurve-Groothuis AM, Groot-Loonen J, Marcus KA, Bellersen L, Feuth T, Bokkerink JP, et al. Myocardial strain and strain rate in monitoring subclinical heart failure in asymptomatic long-term survivors of childhood cancer. Ultrasound in medicine & biology. 2010;36(11):1783-91.

22. Feijen EAM, Leisenring WM, Stratton KL, Ness KK, van der Pal HJH, van Dalen EC, et al. Derivation of Anthracycline and Anthraquinone Equivalence Ratios to Doxorubicin for Late-Onset Cardiotoxicity. JAMA oncology. 2019;5(6):864-71.

23. Kok JL, Teepen JC, van der Pal HJ, van Leeuwen FE, Tissing WJE, Neggers SJCMM, et al. Incidence of and Risk Factors for Histologically Confirmed Solid Benign Tumors Among Long-term Survivors of Childhood Cancer. JAMA oncology. 2019;5(5):671-80.

24. Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. The American journal of cardiology. 1976;37(1):7-11.

25. de Vet HCW, Mokkink LB, Terwee CB, Hoekstra OS, Knol DL. Clinicians are right not to like Cohen's κ. BMJ : British Medical Journal. 2013;346:f2125.

26. Stanton T, Leano R, Marwick TH. Prediction of all-cause mortality from global longitudinal speckle strain: comparison with ejection fraction and wall motion scoring. Circ Cardiovasc Imaging. 2009;2(5):356-64.

27. Gray RJ. A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. The Annals of Statistics. 1988;16(3):1141-54.

28. Grambsch PM, Therneau TM. Proportional Hazards Tests and Diagnostics Based on Weighted Residuals. Biometrika. 1994;81(3):515-26.

29. Marrie RA, Dawson NV, Garland A. Quantile regression and restricted cubic splines are useful for exploring relationships between continuous variables. Journal of clinical epidemiology. 2009;62(5):511-7.e1.

30. Royston P, Altman DG. External validation of a Cox prognostic model: principles and methods. BMC Med Res Methodol. 2013;13(1):33.

31. Vickers AJ, Cronin AM, Begg CB. One statistical test is sufficient for assessing new predictive markers. BMC Med Res Methodol. 2011;11:13-.

32. Heagerty PJ, Zheng Y. Survival Model Predictive Accuracy and ROC Curves. 2005;61(1):92-105.

33. Harrell FE, Jr, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the Yield of Medical Tests. JAMA. 1982;247(18):2543-6.

34. Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Statistics in medicine. 2011;30(1):11-21.

35. Blanche P, Dartigues J-F, Jacqmin-Gadda H. Estimating and comparing time-dependent areas under receiver operating characteristic

curves for censored event times with competing risks. Statistics in medicine. 2013;32(30):5381-97.

36. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. Epidemiology (Cambridge, Mass). 2004;15(5):615-25.
37. Elbl L, Hrstkova H, Tomaskova I, Blazek B, Michalek J. Long-term serial echocardiographic examination of late anthracycline cardiotoxicity and its prevention by dexrazoxane in paediatric patients. Eur J Pediatr. 2005;164(11):678-84.
38. Elbl L, Hrstkova H, Tomaskova I, Michalek J. Late anthracycline cardiotoxicity protection by dexrazoxane (ICRF-187) in pediatric patients: echocardiographic follow-up. Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer. 2006;14(2):128-36.
39. Sorensen K, Levitt GA, Bull C, Dorup I, Sullivan ID. Late anthracycline cardiotoxicity after childhood cancer: a prospective longitudinal study. Cancer. 2003;97(8):1991-8.
40. Rathe M, Carlsen NL, Oxhoj H, Nielsen G. Long-term cardiac follow-up of children treated with anthracycline doses of 300 mg/m2 or less for acute lymphoblastic leukemia. Pediatric blood & cancer. 2010;54(3):444-8.
41. Johnson GL, Moffett CB, Geil JD, Greenwood MF, Noonan JA. Late echocardiographic findings following childhood chemotherapy with normal serial cardiac monitoring. Journal of pediatric hematology/oncology. 1996;18(1):72-5.
42. Border WL, Sachdeva R, Stratton KL, Armenian SH, Bhat A, Cox DE, et al. Longitudinal Changes in Echocardiographic Parameters of Cardiac Function in Pediatric Cancer Survivors. JACC: CardioOncology. 2020;2(1):26-37.
43. Lipshultz SE, Lipsitz SR, Sallan SE, Dalton VM, Mone SM, Gelber RD, et al. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(12):2629-36.
44. Yeh JM, Nohria A, Diller L. Routine echocardiography screening for asymptomatic left ventricular dysfunction in childhood cancer survivors: a model-based estimation of the clinical and economic effects. Annals of internal medicine. 2014;160(10):661-71.
45. Wong FL, Bhatia S, Landier W, Francisco L, Leisenring W, Hudson MM, et al. Cost-effectiveness of the children's oncology group long-term follow-up screening guidelines for childhood cancer survivors at risk for treatment-related heart failure. Annals of internal medicine. 2014;160(10):672-83.
46. Ehrhardt MJ, Ward ZJ, Liu Q, Chaudhry A, Nohria A, Border W, et al. Cost-Effectiveness of the International Late Effects of Childhood Cancer Guideline Harmonization Group Screening Guidelines to Prevent Heart Failure in Survivors of Childhood Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2020:Jco2000418.
47. Gori M, Redfield MM, Calabrese A, Canova P, Cioffi G, De Maria R, et al. Is mild asymptomatic left ventricular systolic dysfunction always predictive of adverse events in high-risk populations? Insights from the DAVID-Berg study. 2018;20(11):1540-8.
48. Kalam K, Otahal P, Marwick TH. Prognostic implications of global LV dysfunction: a systematic review and meta-analysis of global longitudinal strain and ejection fraction. Heart (British Cardiac Society). 2014;100(21):1673-80.
49. Leerink JM, Feijen ELAM, van der Pal HJH, Kok WEM, Mavinkurve-Groothuis AMC, Kapusta L, et al. Diagnostic tools for early detection of cardiac dysfunction in childhood cancer survivors: Methodological aspects of the Dutch late effects after childhood cancer (LATER) cardiology study. American Heart Journal. 2020;219:89-98.

SUPPLEMENTAL MATERIAL

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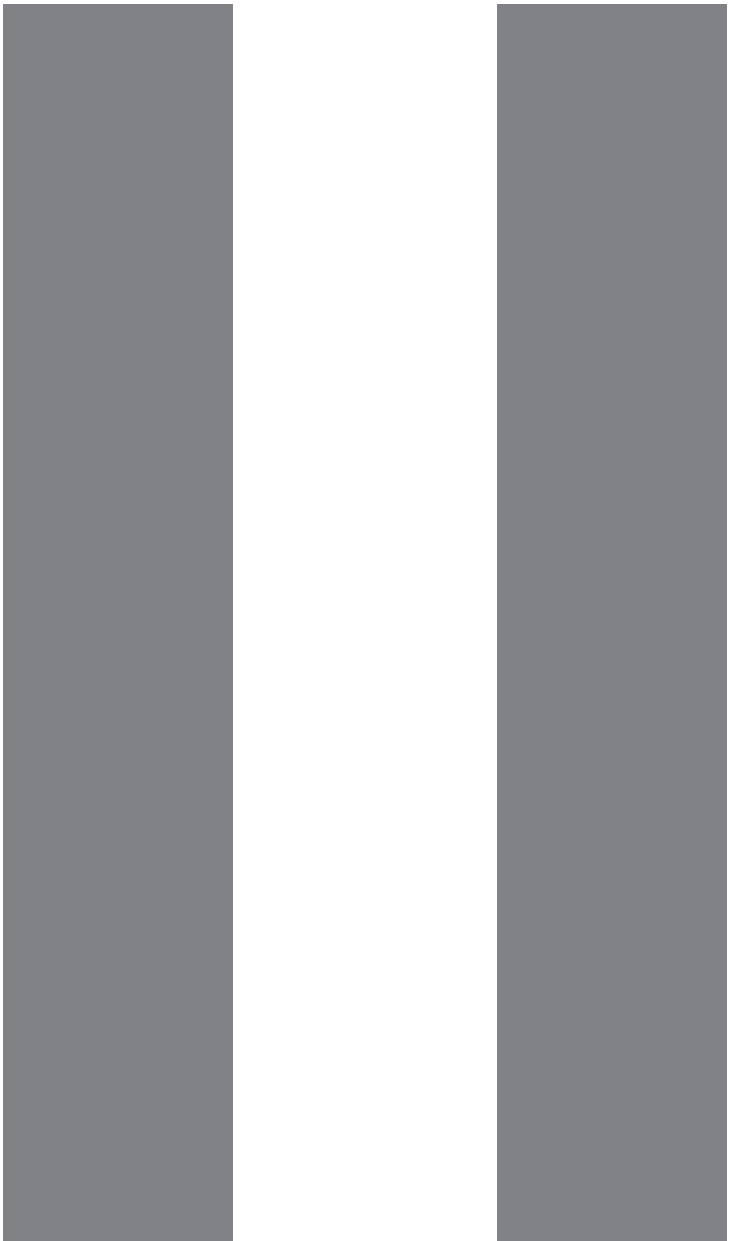

BLOOD BIOMARKERS TO DETECT CARDIAC DYSFUNCTION IN CHILDHOOD CANCER SURVIVORS

4

BIOMARKERS TO DIAGNOSE VENTRICULAR DYSFUNCTION IN CHILDHOOD CANCER SURVIVORS: A SYSTEMATIC REVIEW

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Heart 2019

ABSTRACT

Objective

To systematically review the literature and assess the diagnostic value of biomarkers in detection of late-onset left ventricular (LV) dysfunction in childhood cancer survivors (CCS) treated with anthracyclines.

Methods

We systematically searched the literature for studies that evaluated the use of biomarkers for detection of LV dysfunction in CCS treated with anthracyclines more than one year since childhood cancer diagnosis. LV dysfunction definitions were accepted as an ejection fraction <50 or <55% and/or a fractional shortening <28, <29 or <30%. Contingency tables were created to assess diagnostic accuracies of biomarkers for diagnosing LV dysfunction.

Results

Of 1362 original studies screened, eight heterogeneous studies evaluating four different biomarkers in mostly asymptomatic CCS were included. In four studies an abnormal N-terminal pro B-type natriuretic peptide (NT-proBNP, cut-off range 63 to 125 ng/L) had low sensitivity (maximally 22%) and a specificity of up to 97% for detection of LV dysfunction. For troponin levels, in five studies one patient had an abnormal troponin value as well as LV dysfunction, while in total 127 patients had LV dysfunction without troponin elevations above cut-off values (lowest 0.01 ng/mL). Two studies that evaluated brain natriuretic peptide and nitric oxide were underpowered to draw conclusions.

Conclusions

In individual studies, the diagnostic value of NT-proBNP for detection of LV dysfunction in CCS is limited. Troponins have no role in detecting late-onset LV dysfunction with cut-off values as low as 0.01 ng/mL. Further study on optimal NT-proBNP cut-off values for rule-out or rule-in of LV dysfunction is warranted.

KEY MESSAGES

What is already known about this subject?

Long-term childhood cancer survivors treated with anthracyclines are at risk for developing heart failure up to decades after anthracycline exposure and are therefore periodically screened for left ventricular dysfunction by guideline recommended echocardiograms. At present, biomarkers are not recommended for screening of LV dysfunction in long-term CCS.

What does this study add?

In this systematic review of eight studies we show that the biomarkers NT-proBNP and troponins have a limited diagnostic value for detection of late-onset left ventricular systolic dysfunction in CCS treated with anthracyclines. Other biomarkers are insufficiently studied to draw conclusions.

How might this impact on clinical practice?

The results of our systematic review should discourage the routine use of biomarkers in the screening for late-onset left ventricular systolic dysfunction in childhood cancer survivors treated with anthracyclines.

INTRODUCTION

Every year an estimated total of 20,000 children are diagnosed with cancer in Europe.(1) Due to better treatment options for childhood cancer the five-year survival rate has increased dramatically over the last decades and currently exceeds 80%.(2) As a result of this improved prognosis, a considerable part of these children nowadays become long-term survivors. It is estimated that 1 in 680 people is a childhood cancer survivor (CCS) in the United States.(3) Along with this growing number of CCS there is an increase in late effects of cancer therapies.(4)

Anthracycline derivatives are used in 60% of all children with cancer(5) and are well known for their dose dependent cardiotoxic side effects with the most important one being left ventricular (LV) dysfunction ultimately leading to congestive heart failure.(6, 7, 8) Three types of anthracycline related LV dysfunction are defined in time.(9) 1) Acute cardiotoxicity leading to a reversible decline in LV function within two weeks after anthracycline infusion. 2) Early onset progressive cardiotoxicity occurs during or within the first year after treatment. This cardiotoxicity is thought to be irreversible, related to cardiomyocyte damage resulting in progressive LV dysfunction. 3) Late-onset cardiotoxicity occurs more than one year after treatment and is thought to be caused by initial damage to cardiomyocytes resulting in harmful compensation mechanisms leading to LV dysfunction up to decades after anthracycline exposure.(9) This review will focus on late-onset LV dysfunction starting more than one year after anthracycline treatment in CCS.

Late-onset LV dysfunction ten to fifteen years after anthracycline treatment occurs in nearly 30% of this relatively young population of CCS, defined as a fractional shortening (FS) <30%.(7) Moreover, in 30 years one in eight has overt heart failure which requires treatment.(8) In the general population, early treatment of patients with asymptomatic LV dysfunction with angiotensin converting enzyme (ACE) inhibitors reduces mortality and incidence of heart failure.(10) Detecting LV dysfunction following anthracycline chemotherapy in the asymptomatic phase may reduce long-term morbidity and mortality as overt heart failure may be prevented by providing treatment with ACE inhibitors, although more evidence is still needed on the benefit of ACE inhibitors in the presence of LV dysfunction in this specific population.(6)

Echocardiography is the imaging modality of choice to detect LV dysfunction in long-term CCS. The current guidelines for the screening of LV dysfunction in CCS recommend echocardiography with a screenings interval of 5 years or shorter, depending on risk factors for LV dysfunction such as cumulative anthracycline dose (CAD), age during treatment, gender and concomitant radiotherapy.(6, 11) In the general population screening for asymptomatic LV dysfunction has been performed using natriuretic peptides (NT-proBNP and BNP).(12) Biomarkers such as natriuretic peptides show potential in early detection of LV dysfunction in CCS.(13, 14, 15) In children and adults, a rise in cardiac troponins during or shortly after anthracycline treatment, indicating cardiomyocyte damage, may serve as a predictor for future LV dysfunction.(16, 17) In late-onset cardiotoxicity the utility of biomarkers such as natriuretic peptides and troponins in detection of LV dysfunction is still evolving.(6, 14) In this systematic review we aimed to evaluate the diagnostic value of biomarkers to detect late-onset LV systolic dysfunction as measured by ejection fraction (EF) or FS, in long-term CCS treated with anthracyclines.

METHODS

Literature search and eligibility criteria

Pubmed, EMBASE and the Cochrane library were systematically searched for original studies on biomarkers and LV dysfunction in survivors of childhood cancer treated with anthracyclines more than one year after treatment. The full search strategy is provided in supplementary file 1. The reference list of included articles was manually screened for additional studies.

Two reviewers (SV, WK) independently screened these studies. Excluded were reviews, animal studies, studies with patients having their primary cancer diagnosis above 21 years of age, studies that lacked or did not define LV dysfunction by an EF or FS cut-off (see *definitions*) and studies with an unknown number of patients with LV dysfunction. Further excluded were studies that did not perform biomarker sampling and echocardiography within one month and studies with a duplicated patient cohort. Studies combining early (<1 years) and late-onset (>1 year) detections of LV dysfunction were included but were described separate in the tables.

Quality assessment

Critical appraisal of the included studies was performed by two reviewers using the Standards for Reporting Diagnostic Accuracy 2015 (STARD 2015) checklist(18)(Supplementary file 2). Uncertainties were discussed with a third person. This review was conducted following the criteria from the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guideline(19) (Supplementary file 3).

Definitions

Late-onset cardiotoxicity was defined as LV systolic dysfunction occurring more than one year after treatment. LV systolic dysfunction in studies was accepted as such when defined as an EF <50% or <55% and/or a FS <28%, <29% or <30% measured by echocardiography. Biomarker cut-off values were noted and defined as abnormal if stated in the study.

Data extraction

From the included studies number of patients, age at time of childhood cancer diagnosis, age at time of study, time since last treatment, gender, type of chemotherapy, CAD, radiotherapy exposure, cardiac symptoms or medication, LV dysfunction definition and prevalence, evaluated biomarkers, biomarker cut-off values and number of patients with an abnormal biomarker were manually collected by two investigators independently (JL, SV).

Statistical analysis of the data

To measure the diagnostic accuracy of each biomarker to detect LV dysfunction contingency tables were created and sensitivity (true positives/true positives+false negatives), specificity (true negatives/true negatives+false positives), positive predictive value (PPV, true positives/true positives+false positives), negative predictive value (NPV, true negatives/true negatives+false

negatives) were calculated. Likelihood ratios (LR+ and LR-) were calculated using the formulas: LR+=sensitivity/1-specificity and LR-=1-sensitivity/specificity. For an thorough overview on these metrics we refer the reader to one of the following articles.(20, 21, 22) Accompanying 95% confidence intervals were calculated by the efficient-score method.(23)

RESULTS

By searching the literature we identified 1494 studies (figure 1). In addition, three studies were manually found by searching the references. After removing 105 duplicate records, 1392 studies underwent title and abstract screening. Full-text screening was intended in 25 studies. However, of one study, a translation was requested but not received(24) and another study only had an abstract available.(25) Main reasons for exclusion of full-text studies were: An absent or different LV dysfunction definition not defined by an EF or FS cut-off, exclusion of patients with LV dysfunction, an unknown number of patients with LV dysfunction or time since anthracycline treatment of less than one year in all patients. Finally, eight cohort studies with in total 691 CCS at a median of 0.9 to 18.2 years since anthracycline treatment were included (median dose 165-480 mg/m2) evaluating four different biomarkers (table 1). Applying the STARD 2015 checklist, it was notable that none of

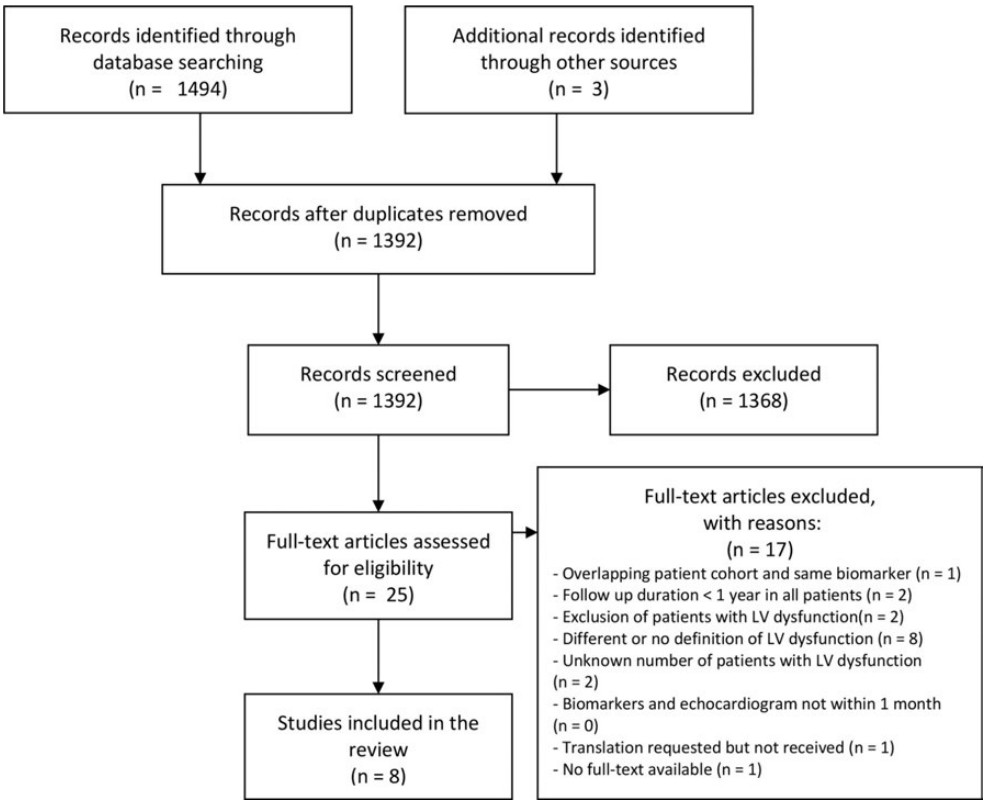

Figure 1. Study inclusion flow chart.

Table 1. Summary of the included studies on biomarkers for the detection of late-onset LV dysfunction

	n	Male sex	ANT type	CAD	Chest RT	HF; HF medication	Age at diagnosis	Age at study	Years since treatment	LVD definition	LVD	Biomarker
Early and late onset												
Guler, 2011	29	17 (59%)	DOX	310 (180-480)	6 (21%)	-	-	9.1 ± 3.7	0.9 (0.2-3.1)	EF<55 FS<30	10.3%	NO
Kismet, 2004	24	14 (58%)	DOX	480 (400-840)	4 (17%)	-	-	14 (3-31)	1.0 (0.1-14)	EF<55 FS<29	8.3%	cTnT
Late onset												
Aggarwal, 2007	63	37 (59%)	ANT	165 (45-520)	-	3;5	-	13.1 (6.5-26.5)	5.4 ± 4.1	FS<29	14.3%	BNP
Brouwer, 2011	277	155 (56%)	DAU, DOX	183 (50-600)	69 (25%)	11;17	8.8 (0-20.1)	27.5 (18-48)	18.2 (5.4-30.8)	FS<29	36.8%	cTnI, cTnT, NTproBNP
Mavinkurve-Groothuis, 2009	122	62 (51%)	DAU, DOX	180 (50-542)	7 (6%)	-	5.7 (0-14.4)	21 (5.0-39.4)	13.8 (5.0-28.7)	EF<55 FS<29	7.4%	cTnT, NTproBNP
Mladosievicova, 2012	36	19 (53%)	DAU, DOX, EPI	221	1 (3%)	-	8 (1-17)	22 (18-31)	11 (5-22)	EF<50	0%	NTproBNP
Pourier, 2015	64	38 (59%)	ANT	225 (85-450)	15 (23%)	-	5.8 (0.3-17.3)	16.7 (7.2–39.8)	8.3 (4.5-34.1)	EF<55 FS<29	10.9%	hs-cTnT, NTproBNP
Ylänen, 2015	76	34 (45%)	ANT	224 (80-454)	10 (13%)	-;4	3.8 (0-13.8)	14.3 (7.2-20.0)	7.1 (5-18)	3DEF<50 FS<28	13.3%	cTnI, cTnT, hs-cTnT, NTproBNP

Values are number (%), mean ± SD or median (range). Abbreviations: 3DEF, ejection fraction measured by 3-dimensional echocardiography; ANT, any anthracycline not further specified; CAD, cumulative anthracycline dose in mg/m2; DAU, daunorubicin; DOX, doxorubicin; EPI, epirubicin; HF, heart failure; RT, radiotherapy.

the studies were identified as a diagnostic accuracy study and no diagnostic accuracies of biomarkers for the diagnosis of LV dysfunction were reported. Also, three studies did not report biomarker cut-off values impairing the use of contingency tables in these studies(26, 27, 28) (Supplementary file 2). Three studies did not exclude symptomatic patients and patients on heart failure medication (15, 26, 29) and one study excluded patients with CAD <400 mg/m2 (table 1).(30)

NT-proBNP

Five studies with a total of 575 patients measured NT-proBNP levels in anthracycline exposed patients (table 2). Significant heterogeneity was present in median follow up duration (between 7.1 and 18.2 years) and in median CAD (ranged from 180 to 225 mg/m2). LV dysfunction was present in 0-37% of the study populations with one study having no patients with LV dysfunction.(28) Cut-off values for NT-proBNP levels varied from 63 ng/L in males and 116 ng/L in females(29) to 125 ng/L in males and females.(15) Cut-off values for children were defined in the studies as age and sex specific values previously reported by Nir(29, 31, 32) and Albers.(13, 33) Also, sex and age specific cut-off values defined by Fradley(34) (ranging from 42.5-106.4 ng/L in males and 111.0-215.9 ng/mL in females depending on age) were used.(32) Abnormal NT-proBNP values were seen in 5.3% up to 13% of the patients, we did not observe a relation with NT-proBNP cut-off values (table 2). In four of five studies patients with LV dysfunction were present and these studies were used for diagnostic accuracy analysis.(13, 15, 29, 32) Sensitivities (14-22%) and PPVs (13-50%) were low in the four studies, while demonstrating higher specificities (88-97%) and NPVs (65-90%). Likelihood ratios for having LV dysfunction with an abnormal NT-proBNP value (LR+) were 1.70-6.67 and likelihood ratios for having LV dysfunction with a normal NT-proBNP level (LR-) were 0.83-0.93 (table 2). Of note, confidence intervals of the diagnostic accuracy estimates were wide in all four studies (table 2). A meta-analysis was not performed because of heterogeneity in the presented studies.

Troponins

Five studies with a total of 423 patients were included using various troponin assays in the detection of LV dysfunction in CCS (table 3). Median CAD ranged from 180-480 mg/m2 and time since last treatment varied between 1-18.2 years. LV dysfunction prevalence ranged between 7.4% and 36.5%. Abnormal troponins (cut-off values for troponin T between 0.01 and 0.014 ng/mL , and for troponin I 0.03-0.04 ng/ml) were seen in only 5 of all 423 patients. Only one of these 5 patients showed LV dysfunction(30) and a troponin T > 0.01 ng/mL while in the total population LV dysfunction was present in 128 patients. Troponin testing in this single study had very limited sensitivity (50%; 95% CI 3, 97%) and PPV (33%; 95% CI 2, 87) with higher specificity (91%; 95% CI 69-98) and NPV (95%; 95% CI 74, 99). The LR+ and LR- for this study were 5.49 (95% CI 0.81, 37.32) and 0.55 (95% CI 0.14-2.22) respectively. Two studies used high sensitive troponin T measurements(29, 32) with the lowest cut-off value of 0.0135 ng/mL but no abnormal troponins were present in these studies.

Table 2. Diagnostic value of NT-proBNP for detection of LV dysfunction (LVD)

	n	Years since treatment	LVD	NT-proBNP cut-off, ng/L	Abnormal NT-proBNP	Sensitivity (95% CI)	Specificity (95% CI)	PPV, % (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
Late onset											
Brouwer, 2011	277	18.2 (5.4-30.8)	36.8%	125	32/262 (12.2%)	17% (10, 26)	90% (84, 94)	50% (32, 68)	65% (58, 71)	1.70 (0.89, 3.25)	0.93 (0.85, 1.01)
Mavinkurve-Groothuis, 2009	122	13.8 (5.0-28.7)	7.4%	M 84.6 F 152.2 C Albers*	16/122 (13.1%)	22% (4, 60)	88% (80, 93)	13% (2, 40)	93% (60, 98)	1.79 (0.48, 6.69)	0.89 (0.62, 1.26)
Mladosievicova, 2012	36	11 (5-22)	0%	M 75 F 105	4/36 (11.1%)	-	-	-	-	-	
Pourier, 2015	64	8.3 (4.5-34.1)	10.9%	M/F *Fradley C *Nir	5/64 (7.8%)	14% (1, 58)	93% (82, 98)	20% (1, 70)	90% (79, 96)	2.04 (0.26, 15.75)	0.92 (0.68, 1.25)
Ylänen, 2015	76	7.1 (5.0-18.0)	13.3%	M 63 F 116 C *Nir	4/76 (5.3%)	20% (4, 56)	97% (88, 99)	50% (9, 91)	89% (78, 95)	6.50 (1.03, 41.07)	0.83 (0.60, 1.13)

C, children; F, female; LR+, positive likelihood ratio; LR-, negative likelihood ratio; LVD=left ventricular dysfunction; NPV, negative predictive value; M, male; PPV, positive predictive value. * Albers 2006, age and sex specific values in children; Fradley 2011, age and sex specific values in adults; Nir 2009, age specific values in children.

Table 3. Diagnostic value of troponins for detection of LV dysfunction (LVD)

	n	Years since treatment	LVD	Type	Troponin cut-off(s) (ng/mL)	Abnormal troponin	LVD and abnormal troponin	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
Early and Late onset													
Kismet, 2004	24	1 (0.1-14)	8.3%	cTnT	0.010	2/24 (8.3%)	1	50% (3, 97)	91% (69, 98)	33.3% (2, 87)	95% (74, 99)	5.49 (0.81, 37.32)	0.55 (0.14, 2.22)
Late onset													
Brouwer, 2011	277	18.2 (5.4-30.8)	36.8%	cTnI, cTnT	0.04, 0.01	3/268 (1.1%)	0	-	-	-	-	-	-
Mavinkurve-Groothuis, 2009	122	13.8 (5.0-28.7)	7.4%	cTnT	0.01	0	0	-	-	-	-	-	-
Pourier, 2015	64	8.3 (4.5-34.1)	10.9%	hs-cTnT	0.0135	0	0	-	-	-	-	-	-
Ylänen, 2015	76	7.1 (5.0-18.0)	13.3%	cTnI, cTnT, hs-cTnT	0.0095, 0.03, 0.014	0	0	-	-	-	-	-	-

LR+, positive likelihood ratio; LR-, negative likelihood ratio; LVD, left ventricular dysfunction, NPV, negative predictive value; PPV, positive predictive value

BNP

Only one study was included studying BNP in 63 patients.(26) Higher BNP values were present in CCS with a FS<29% compared to CCS with a FS>29% (32.4 ± 34.9 vs. 15.6 ± 12.4 pg/mL, p<0.008) but no cut-off values or contingency tables were provided.(26)

Nitric Oxide

One study(27) measured plasma total nitrite levels, a stable product of nitric oxide (NO), 10.5 months (range 2-37.4) since last anthracycline treatment in 29 children. LV dysfunction defined as an EF<55% and/or FS<30% was present in 10.3% of the patients and this was related to significantly higher nitrite levels compared to matched healthy controls (92.35 ± 50.36 vs 59.26 ± 13.56 µmol/L, p=0.038).

DISCUSSION

In this systematic review we show that the diagnostic value of biomarkers to detect LV dysfunction in CCS is limited at the presented cut-off values and are overall not yet suited for either excluding (rule-out) or confirming (rule-in) LV dysfunction. Although biomarker screening has been advocated by some(35), we show that the current literature does not yet provide evidence to implement routine biomarker screening in the surveillance of CCS at risk for LV dysfunction.

NT-proBNP

In our review, NT-proBNP is the best studied biomarker for the detection of LV dysfunction in CCS with a limited diagnostic accuracy (table 2). Our finding of low sensitivities and PPVs and higher specificities and NPVs for detection of LV dysfunction in long-term CCS is in line with previous reviews.(6, 14) Considering the consequences of missing patients with LV dysfunction, the NPV of NT-proBNP must be at least 98% to rule-out LV dysfunction and defer an echocardiogram(36) Therefore, with the presented cut-off values, NT-proBNP is not useful to rule-out LV dysfunction. At the same cut-off values NT-proBNP is not yet suited for rule-in purposes, as specificity ranges from 88-97% implying significant rates of false positives.

The limited diagnostic accuracy of NT-proBNP for detection of LV dysfunction might be partly explained by the LV dysfunction definitions used in the included studies, similar to findings in the general population.(12) In the general population <65 years of age the diagnostic accuracy of NT-proBNP for detection of an EF<50% is very limited compared to the detection of an EF<40% (area under the curve of 0.88 and 0.56 respectively).(12) NT-proBNP might also prove more useful in diagnosing an EF<40% in CCS treated with anthracyclines. This is an interesting subject for future studies because diagnosing an EF<40% in CCS is meaningful as this has implications for initiating treatment with heart failure therapies.(37) Also, in the general population <65 years of age the optimal NT-proBNP cut-off value of 59 ng/L for diagnosing an EF<50%(12) is lower than the cut-off values used in the included studies and corresponded to a higher sensitivity of 62.2%, at the cost of a lower specificity of 61.3% compared to sensitivities and specificities reported in our review. In future studies separate optimal age and sex adjusted cut-off values for rule-out and

rule-in of LV dysfunction in CCS should be tested, as is also done for the diagnosis of acute heart failure in the emergency department.(36)

Optimal NT-proBNP cut-off values to rule-out and rule-in LV dysfunction may not only vary by age and sex, but also by individual pre-test probabilities of LV dysfunction because predictive values of a diagnostic test are dependent on the prevalence of disease.(20) We noticed a wide spread in the prevalence of LV dysfunction in the included studies ranging from 7.4% to 36.8%, probably due to patient selection, differences in CAD and differences in definitions of LV dysfunction (table 2). Indeed, NPVs were high (89 to 93%) in three of the four studies with the lowest prevalence of LV dysfunction (7.4-13.3%)(13, 29, 32) and lower (NPV 65%) in the study with the highest prevalence (36.8%).(15) To account for such heterogeneity in the populations, individual pre-test probabilities for LV dysfunction should be taken into consideration when using NT-proBNP to diagnose LV dysfunction in CCS and can be estimated by traditional risk factors for anthracycline related cardiotoxicity such as sex, age at diagnosis, follow-up duration and CAD and radiotherapy dose.(38) Subsequently, the likelihood ratio can be used to calculate individual post-test probabilities of LV dysfunction. Likelihood ratios above 10 or below 0.1 may be regarded as strong.(22) The likehood ratios we report for NT-proBNP are therefore moderate and need improvement before NT-proBNP testing can play a role in the surveillance for late-onset LV dysfunction in CCS.

Troponins

Troponins are markers for cardiomyocyte damage and may predict heart failure and cardiovascular death in the population using very low cut-off values.(39) Troponins measurements during or shortly after anthracycline treatment may be useful for prediction of future LV dysfunction. (16) For detection of late-onset LV dysfunction in CCS the position of troponins in detection of cardiotoxicity is less clear. In the five included studies troponins in the presence or absence of LV dysfunction are rarely elevated, even though cut-off values in these studies were as low as 0.01 ng/mL or 0.013 ng/ml with the newest high sensitivity assays. Therefore, there appears to be no potential in detecting LV dysfunction with the present troponin assays. This is in line with previous reports.(6, 14) Possibly troponins may be of use for risk stratification using very low cut-off values of troponin for development of LV dysfunction in long-term CCS.(39)

Other biomarkers

The few studies on BNP and NO were too limited in patient number to draw conclusions. Identification of new biomarkers for detection of late-onset LV dysfunction in an early stage in CCS treated with anthracyclines is an interesting subject for future studies. Especially, biomarkers that relate to the mechanisms of late-onset anthracycline induced LV dysfunction.

Strengths and limitations

Our systematic review provides a new overview of the literature in an emerging field of biomarkers with respect to the detection of LV dysfunction in CCS. However, some limitations must be mentioned. Based on the heterogeneity of the included studies regarding LV dysfunction definitions

and biomarker cut-off values performing a meta-analysis was not appropriate. An individual patient data analysis is needed to define optimal biomarker cut-off values with a uniform LV dysfunction definition and will be performed by us for NT-proBNP in the near future. The aim of our review was to compare biomarkers levels with the presence of LV systolic dysfunction as measured by EF or FS, while other parameters indicating milder forms of LV dysfunction such as diastolic function parameters, myocardial strain parameters and interstitial fibrosis measurements derived from cardiac MRI may also be compared to biomarker tests. However, we chose to compare biomarkers to the LV systolic dysfunction parameters EF and/or FS because these are the most widely used parameters for detection of cardiotoxicity in CCS(6, 11) with consequences for initiating heart failure therapies.(6, 37)

Implications for clinical practice and future research

Our results, showing that none of the biomarkers at present cut-off values can safely rule-in or rule-out LV dysfunction in CCS, discourages the routine use of biomarkers in the surveillance of CCS treated with anthracyclines. Furthermore, our review serves as an incentive for more research on optimal biomarker cut-off values and for the identification of new biomarkers that can accurately exclude or confirm LV dysfunction.

CONCLUSIONS

The biomarkers NT-proBNP and troponins have limited diagnostic value to detect late-onset LV dysfunction in CCS at the presented cut-off values and are not useful in the screening of long-term CCS treated with anthracyclines. Other biomarkers have not been sufficiently studied in long-term CCS to draw conclusions regarding their diagnostic value.

REFERENCES

1. Kowalczyk JR, Samardakiewicz M, Pritchard-Jones K, Ladenstein R, Essiaf S, Fitzgerald E, et al. European Survey on Standards of Care in paediatric oncology centres. European journal of cancer (Oxford, England : 1990). 2016;61:11-9.
2. Gatta G, Botta L, Rossi S, Aareleid T, Bielska-Lasota M, Clavel J, et al. Childhood cancer survival in Europe 1999-2007: results of EUROCARE-5--a population-based study. The Lancet Oncology. 2014;15(1):35-47.
3. Mariotto AB, Rowland JH, Yabroff KR, Scoppa S, Hachey M, Ries L, et al. Long-term survivors of childhood cancers in the United States. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18(4):1033-40.
4. Geenen MM, Cardous-Ubbink MC, Kremer LC, Bos vd, C., Pal vd, H. J. H., Heinen RC, et al. Medical Assessment of Adverse Health Outcomes in Long-term Survivors of Childhood Cancer. JAMA. 2007;297(24):2705-15.
5. Hudson MM, Ness KK, Gurney JG, Mulrooney DA, Chemaitilly W, Krull KR, et al. Clinical Ascertainment of Health Outcomes Among Adults Treated for Childhood Cancer. JAMA. 2013;309(22):2371-81.
6. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2015;16(3):e123-36.
7. van der Pal HJ, van Dalen EC, Hauptmann M, Kok WE, Caron HN, van den Bos C, et al. Cardiac function in 5-year survivors of childhood cancer: a long-term follow-up study. Archives of internal medicine. 2010;170(14):1247-55.
8. Pal vd, H. J., Dalen v, E. C., Delden v, E., Dijk v, I. W., Kok WE, Geskus RB, et al. High risk of symptomatic cardiac events in childhood cancer survivors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30(13):1429-37.
9. Pai VB, Nahata MC. Cardiotoxicity of Chemotherapeutic Agents Incidence, Treatment and Prevention. Drug Safety. 2000;22(4):264-302.
10. Yusuf S, Pitt B, Davis CE, Hood WB, Jr., Cohn JN. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The New England journal of medicine. 1992;327(10):685-91.
11. Sieswerda E, Postma A, van Dalen EC, van der Pal HJ, Tissing WJ, Rammeloo LA, et al. The Dutch Childhood Oncology Group guideline for follow-up of asymptomatic cardiac dysfunction in childhood cancer survivors. Ann Oncol. 2012;23(8):2191-8.
12. Costello-Boerrigter LC, Boerrigter G, Redfield MM, Rodeheffer RJ, Urban LH, Mahoney DW, et al. Amino-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide in the general community: determinants and detection of left ventricular dysfunction. Journal of the American College of Cardiology. 2006;47(2):345-53.
13. Mavinkurve-Groothuis AM, Groot-Loonen J, Bellersen L, Pourier MS, Feuth T, Bokkerink JP, et al. Abnormal NT-pro-BNP levels in asymptomatic long-term survivors of childhood cancer treated with anthracyclines. Pediatric blood & cancer. 2009;52(5):631-6.
14. Mavinkurve-Groothuis AM, Kapusta L, Nir A, Groot-Loonen J. The role of biomarkers in the early detection of anthracycline-induced cardiotoxicity in children: a review of the literature. Pediatric hematology and oncology. 2008;25(7):655-64.
15. Brouwer CA, Postma A, Vonk JM, Zwart N, van den Berg MP, Bink-Boelkens MT, et al. Systolic and diastolic dysfunction in long-term adult survivors of childhood cancer. European journal of cancer (Oxford, England : 1990). 2011;47(16):2453-62.
16. Lipshultz SE, Miller TL, Scully RE, Lipsitz SR, Rifai N, Silverman LB, et al. Changes in cardiac biomarkers during doxorubicin treatment of pediatric patients with high-risk acute lymphoblastic leukemia: Associations with long-term echocardiographic outcomes. Journal of Clinical Oncology. 2012;30(10):1042-9.

17. Cardinale D, Sandri MT, Colombo A, Colombo N, Boeri M, Lamantia G, et al. Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. Circulation. 2004;109(22):2749-54.

18. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ : British Medical Journal. 2015;351.

19. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ (Clinical research ed). 2009;339.

20. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ (Clinical research ed). 1994;309(6947):102.

21. Altman DG, Bland JM. Diagnostic tests. 1: Sensitivity and specificity. BMJ (Clinical research ed). 1994;308(6943):1552.

22. Deeks JJ, Altman DG. Diagnostic tests 4: likelihood ratios. BMJ (Clinical research ed). 2004;329(7458):168-9.

23. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Statistics in medicine. 1998;17(8):857-72.

24. Kismet E, Demirkaya E, Yurttutan N, Berberoglu S, Karademir S, Aydin HI, et al. Serum BNP levels in children treated with doxorubicin for their solid tumors. Gulhane Medical Journal. 2004;46(1):38-42.

25. Elbl L, Vasova I, Navratil M, Vorlicek J, Malaskova L, Spinar J. [Comparison of plasmatic levels of B-natriuretic peptide with echocardiographic indicators of left ventricle function after doxorubicin therapy]. Vnitrni lekarstvi. 2006;52(6):563-70.

26. Aggarwal S, Pettersen MD, Bhambhani K, Gurczynski J, Thomas R, L'Ecuyer T. B-type natriuretic peptide as a marker for cardiac dysfunction in anthracycline-treated children. Pediatric blood & cancer. 2007;49(6):812-6.

27. Guler E, Baspinar O, Cekmen M, Kilinc M, Balat A. Nitric oxide: a new biomarker of Doxorubicin toxicity in children? Pediatric hematology and oncology. 2011;28(5):395-402.

28. Mladosievicova B, Urbanova D, Radvanska E, Slavkovsky P, Simkova I. Role of NT-proBNP in detection of myocardial damage in childhood leukemia survivors treated with and without anthracyclines. Journal of experimental & clinical cancer research : CR. 2012;31:86.

29. Ylanen K, Poutanen T, Savukoski T, Eerola A, Vettenranta K. Cardiac biomarkers indicate a need for sensitive cardiac imaging among long-term childhood cancer survivors exposed to anthracyclines. Acta paediatrica (Oslo, Norway : 1992). 2015;104(3):313-9.

30. Kismet E, Varan A, Ayabakan C, Alehan D, Portakal O, Buyukpamukcu M. Serum troponin T levels and echocardiographic evaluation in children treated with doxorubicin. Pediatric blood & cancer. 2004;42(3):220-4.

31. Nir A, Lindinger A, Rauh M, Bar-Oz B, Laer S, Schwachtgen L, et al. NT-pro-B-type natriuretic peptide in infants and children: reference values based on combined data from four studies. Pediatric cardiology. 2009;30(1):3-8.

32. Pourier MS, Kapusta L, van Gennip A, Bokkerink JP, Loonen J, Bellersen L, et al. Values of high sensitive troponin T in long-term survivors of childhood cancer treated with anthracyclines. Clinica chimica acta; international journal of clinical chemistry. 2015;441:29-32.

33. Albers S, Mir TS, Haddad M, Laer S. N-Terminal pro-brain natriuretic peptide: normal ranges in the pediatric population including method comparison and interlaboratory variability. Clinical chemistry and laboratory medicine. 2006;44(1):80-5.

34. Fradley MG, Larson MG, Cheng S, McCabe E, Coglianese E, Shah RV, et al. Reference limits for N-terminal-pro-B-type natriuretic peptide in healthy individuals (from the Framingham Heart Study). The American journal of cardiology. 2011;108(9):1341-5.

35. Zamorano JL, Lancellotti P, Rodriguez Munoz D, Aboyans V, Asteggiano R, Galderisi M, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). European heart journal. 2016;37(36):2768-801.

36. Januzzi JL, van Kimmenade R, Lainchbury J, Bayes-Genis A, Ordonez-Llanos J, Santalo-Bel M, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study. European heart journal. 2006;27(3):330-7.

37. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European journal of heart failure. 2016;18(8):891-975.

38. Chow EJ, Chen Y, Kremer LC, Breslow NE, Hudson MM, Armstrong GT, et al. Individual prediction of heart failure among childhood cancer survivors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33(5):394-402.

39. deFilippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, et al. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. Jama. 2010;304(22):2494-502.

SUPPLEMENTAL MATERIAL

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5

DIAGNOSTIC TOOLS FOR EARLY DETECTION OF CARDIAC DYSFUNCTION IN CHILDHOOD CANCER SURVIVORS: METHODOLOGICAL ASPECTS OF THE DUTCH LATE EFFECTS AFTER CHILDHOOD CANCER (LATER) CARDIOLOGY STUDY

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ABSTRACT

Background

Cancer therapy-related cardiac dysfunction and heart failure are major problems in long-term childhood cancer survivors (CCS). We hypothesize that assessment of more sensitive echo- and electrocardiographic measurements, and/or biomarkers will allow for improved recognition of patients with cardiac dysfunction before heart failure develops, and may also identify patients at lower risk for heart failure.

Objective

To describe the methodology of the Dutch LATER cardiology study (LATER CARD).

Methods

The LATER CARD study is a cross-sectional study in long-term CCS treated with (potentially) cardiotoxic cancer therapies and sibling controls. We will evaluate 1) the prevalence and associated (treatment related) risk factors of subclinical cardiac dysfunction in CCS compared to sibling controls and 2) the diagnostic value of echocardiography including myocardial strain and diastolic function parameters, blood biomarkers for cardiomyocyte apoptosis, oxidative stress, cardiac remodeling and inflammation and ECG or combinations of them in the surveillance for cancer therapy-related cardiac dysfunction. From 2017 to 2020 we expect to include 1,900 CCS and 500 siblings.

Conclusions

The LATER CARD study will provide knowledge on different surveillance modalities for detection of cardiac dysfunction in long-term CCS at risk for heart failure. The results of the study will enable us to improve long-term follow-up surveillance guidelines for CCS at risk for heart failure.

INTRODUCTION

The long-term survival of childhood cancer has increased considerably over the last few decades. With a 5-year overall survival of more than 80%(1), the majority of childhood cancer patients nowadays will become long-term survivors. Unfortunately, the improved survival is accompanied by the occurrence of late adverse effects of treatment(2, 3). The cardiotoxic side effects of certain cancer treatments such as anthracyclines and chest directed radiotherapy are well-known and include heart failure, arrhythmias, coronary artery disease, valvular abnormalities and pericardial disease(4).

Of these cardiotoxic side effects, cancer therapy-related heart failure in childhood cancer survivors (CCS) is the most frequently encountered problem: almost 5% of all CCS develops clinical heart failure within 40 years after childhood cancer diagnosis(5). Moreover, mortality due to heart failure is six-fold higher in long-term CCS as compared to the general population and treatment related cardiac death is the leading cause of death after malignancies(6, 7).

Before clinical heart failure, a larger proportion of long-term CCS have a subclinical decline in left ventricular (LV) systolic function. The prevalence of subclinical LV systolic dysfunction varies in the literature, and is about 30% in different follow up periods when defined as a two-dimensional ejection fraction (EF) <50% or fractional shortening (FS) <30%(3, 8). In a recent study, prevalence of LV dysfunction was only 5.8% after a median follow up of 23 years but in that study LV systolic dysfunction was defined with more constraint as a three-dimensional EF <50%(9). The Dutch surveillance guideline for long-term CCS defines cancer therapy-related cardiac dysfunction as a FS <30% or EF<50%(10).

Established treatment related risk factors for heart failure and subclinical LV systolic dysfunction in CCS are higher cumulative anthracycline doses (in a non-linear fashion with no safe dose threshold), higher cumulative mitoxantrone dose and chest directed radiotherapy (especially in combination with anthracyclines)(2, 3, 11). The type of anthracycline analogue and anthracycline infusion duration might also play a role(12-16).

Surveillance for subclinical cardiac dysfunction may prevent further deterioration of LV function by timely initiating heart failure therapies(17, 18).

Currently, EF measured by two-dimensional echocardiography is the main parameter used in the surveillance for cancer therapy-related cardiac dysfunction and for clinical decision making in CCS(16, 19). However, the usefulness of EF is limited by a large variability of 10%(20).

In the general population myocardial strain imaging emerges as a valuable tool to detect subclinical LV dysfunction that is superior in predicting heart failure and all-cause mortality compared to two-dimensional EF(21-23). The prevalence of global longitudinal strain abnormalities in CCS with a preserved three-dimensional ejection fraction was 28% in a cohort study in 1,820 CCS at a median of 23 years after cancer diagnosis(9).

Another echocardiographic tool next to LV systolic dysfunction determined by 2D echocardiography is LV diastolic dysfunction which is encountered in 9-21% of long-term CCS, with severe dysfunctions mainly after (additional) chest directed radiotherapy(8, 9, 24). Also, diastolic dysfunction is a predictor of future heart failure in the general population(25, 26).

Studies have been performed that identified blood biomarkers that can detect subclinical cancer-treatment related cardiac dysfunction(16). However, blood biomarkers are not yet recommended in the surveillance for cardiac dysfunction in long-term CCS(10, 16). Recently, we reviewed the literature on blood biomarkers for the diagnosis of LV dysfunction in long-term CCS and showed that NT-proBNP and troponins have a limited diagnostic value, which underlines the need to find more accurate biomarkers(27).

The ability of ECG parameters to early detect cancer therapy-related cardiac dysfunction in CCS remains unknown(16, 28). Several studies in long-term CCS describe a high incidence and variety of electrocardiographic abnormalities(29). Major ECG abnormalities were predictive for cardiac and all-cause mortality in a large CCS cohort but were not compared with echocardiographic abnormalities(30). In a smaller long-term CCS cohort, ECG abnormalities (mainly conduction disorders, high amplitude R waves and sinus bradycardia) were not predictive for echocardiographic abnormalities(28). However, only one of these 340 CCS had evidence of systolic LV dysfunction with an EF<50% and strain parameters were not measured.

There are still gaps in knowledge that needs to be addressed in order to improve the surveillance for cardiomyopathy in CCS(16). These gaps include 1) the use of echocardiographic parameters for early detection of cardiomyopathy, 2) the accuracy of biomarkers and ECG parameters to diagnose subclinical cardiac dysfunction, 3) the cardiotoxicity of non-anthracycline containing chemotherapy, such as high-dose cyclophosphamide, ifosfamide and vincristine(3, 5, 13), 4) the role of genetics in the susceptibility for cardiomyopathy and 5) the combined use of blood biomarkers, ECG and echocardiography for the detection of subclinical cardiac dysfunction.

Considering these knowledge gaps, more information from echocardiography including myocardial strain, blood biomarkers (including genetics) and ECG measurements for the early detection of cardiac dysfunction in CCS and their associations with cancer treatment exposures is needed. In this paper we will describe the methodological aspects of the Dutch LATER cardiology study (LATER CARD) project that focusses on subclinical cardiac dysfunction in CCS who received (potential) cardiotoxic cancer treatment(s) as detected by echocardiographic parameters including myocardial strain, blood biomarkers and ECG parameters.

METHODS

Funding

The LATER CARD study is supported by grants from the Dutch Heart Foundation (CVON2015-21) and Kika/ODAS (grant 171 'DCOG LATER program').

Objectives

The main objectives of the LATER CARD study are to evaluate 1) the prevalence and associated (treatment related) risk factors of subclinical cardiac dysfunction in CCS compared to sibling controls and 2) the diagnostic value of echocardiography including myocardial strain and diastolic function parameters, blood biomarkers and ECG or combinations of them in the detection of cancer therapy-related cardiac dysfunction.

The LATER CARD study is subdivided into six work packages (WPs) with different objectives that support the main objective. WP1 consists of the data collection management that is required for the other WPs and does not address a specific research question. In WP2 we will study the prevalence and treatment related risk factors for subclinical systolic cardiac dysfunction including strain and diastolic LV dysfunction identified by echocardiography. WP3 concerns the prevalence of blood biomarker abnormalities and associated treatment related risk factors. In WP4 the prevalence and associated treatment related risk factors of abnormal ECG measurements will be studied. The diagnostic tools studied in WP 2, 3 and 4 will then be compared with respect to their diagnostic value in detecting subclinical cardiac dysfunction (see "Definitions" below). WP5 will study the gender differences in the prevalence of subclinical cardiac dysfunction, abnormal blood biomarker values and ECG abnormalities. In WP6 the results of all other WPs will be combined to formulate guideline recommendations for the cardiac surveillance of long-term CCS.

Study design

The Dutch LATER CARD study, is part of the Dutch Childhood Cancer Survivors Study LATER 2 study, a cross-sectional study of a retrospective nationwide cohort of 5-year CCS (Figure 1 and 2). The LATER study is a collaboration between 7 pediatric oncology centers in the Netherlands (Amsterdam University Medical Center (VU Medical Center and Academical Medical Center), Leiden University Medical Center, Erasmus Medical Center, University Medical Center Groningen, Radboudumc and University Medical Center Utrecht/Wilhelmina Children's Hospital/Princess Máxima Center for

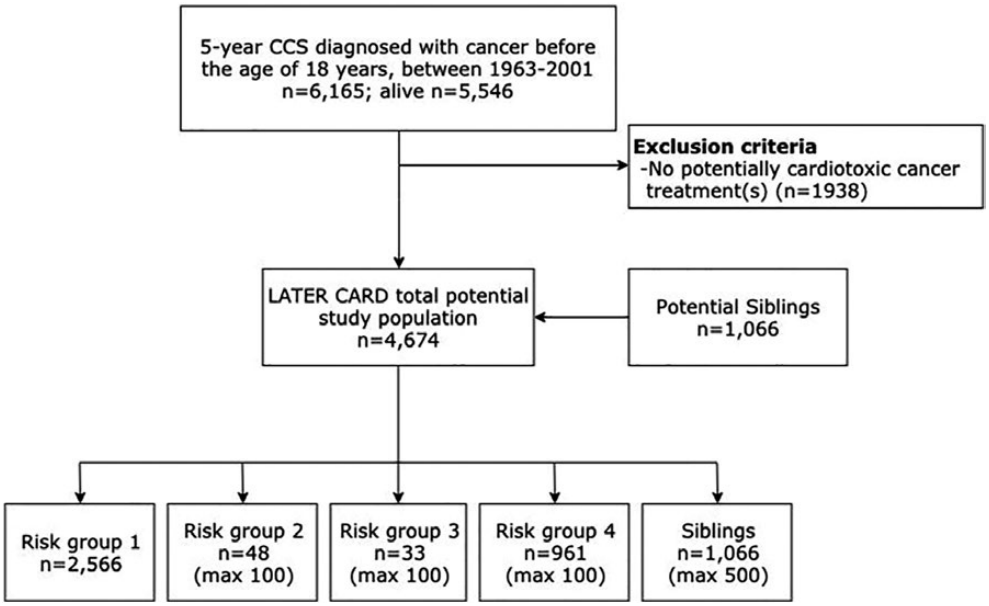

Figure 1. Flowchart of potentially eligible patients in the LATER CARD study. Cancer diagnosis dates of CCS in the LATER CARD study were between 1963 and 2001. Patient enrollment in the DCOG-LATER CARD study started in February 2017 and we are planning to include participants until March 2020.

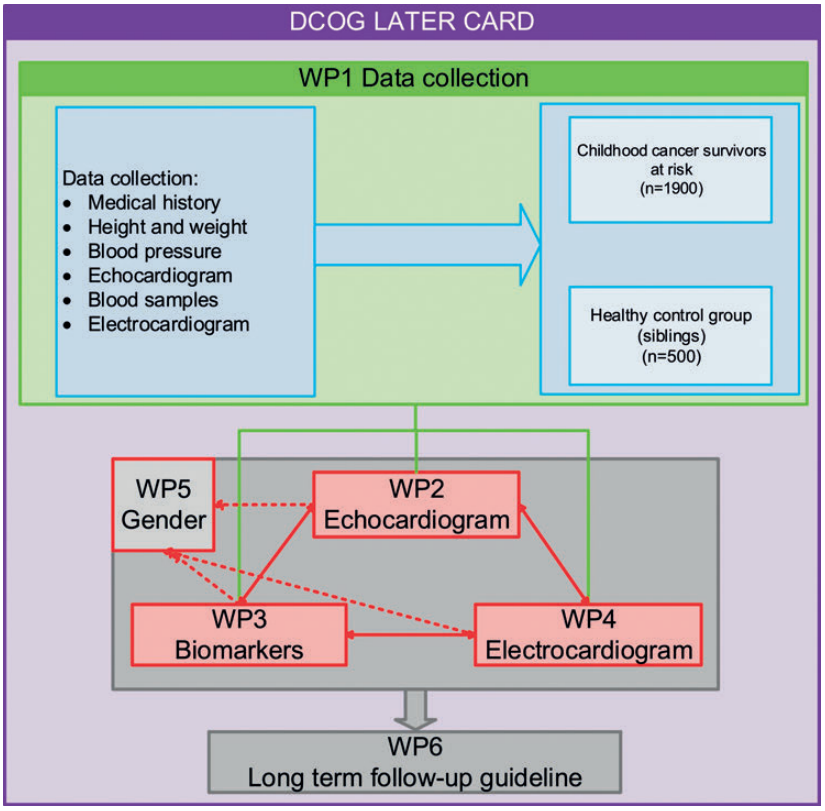

Figure 2. Study design of the LATER CARD study. The LATER CARD study is sub-divided in workpackages (WPs). WP1 consists of the data collection that is needed for the other WPs. The results of WP 2,3,4 and 5 will be used to improve the long-term follow-up guideline (WP6).

Pediatric Oncology) and includes a close collaboration with experts for specific health problems. The study protocol was approved by the medical ethic boards of all participating centers.

Study population

Informed consent is being obtained from all participants before study inclusion. The study population is obtained from the Dutch LATER nationwide cohort (n=6,165), including all 5-year CCS diagnosed before the age of 18 years, between 1/1/1963 and 12/31/2001 with a malignancy according to the third edition of the International Classification of Childhood Cancer(31). We only include CCS who were living in the Netherlands at the time of childhood cancer diagnosis and who were treated in one of the Dutch pediatric oncology centers.

In the LATER CARD study, we will include CCS from the LATER cohort who were treated with (potentially) cardiotoxic cancer treatments. The LATER CARD study will include four risk groups; *risk group 1 (no maximum number)*: CCS who received anthracyclines, mitoxantrone, or chest directed radiotherapy; *risk group 2 (max n=100)*: cyclophosphamide only (no anthracyclines, mitoxantrone,

or chest directed radiotherapy, ifosfamide or vincristine); *risk group 3 (max n=100)*: ifosfamide only (no anthracyclines, mitoxantrone, or chest directed radiotherapy, cyclophosphamide or vincristine); *risk group 4 (max n=100)*: vincristine only (no anthracyclines, mitoxantrone, or chest directed radiotherapy, ifosfamide or cyclophosphamide).

As a comparison group 500 healthy *siblings* recruited from the participants will be included (Figure 1). We chose for siblings as the comparison group because they are of approximately the same age and share a partially common background risk for cardiac disease based on genetic make-up and early life influences.

Study timeline

Patient enrollment in the LATER CARD study started in February 2017 and we are planning to include participants until 2020. We aim to finish all the analyses in January 2022. The specific timeline per WP is displayed in Figure 3.

Definitions

LV systolic dysfunction will be defined as a biplane EF<52% for males and a biplane EF<54% for females, in accordance with the European Association of Cardiovascular Imaging recommendations(32). Specific subgroups of LV dysfunction will be defined: Mid-range EF (EF 40-51% for males and EF 40-53% for females) and reduced EF (EF<40%).

LV diastolic dysfunction will be defined as grade >I diastolic dysfunction according to the European Association of Cardiovascular Imaging recommendations(33).

Abnormal myocardial strain values will be defined based on age, sex and vendor specific normal values and the values obtained in the sibling controls(34).

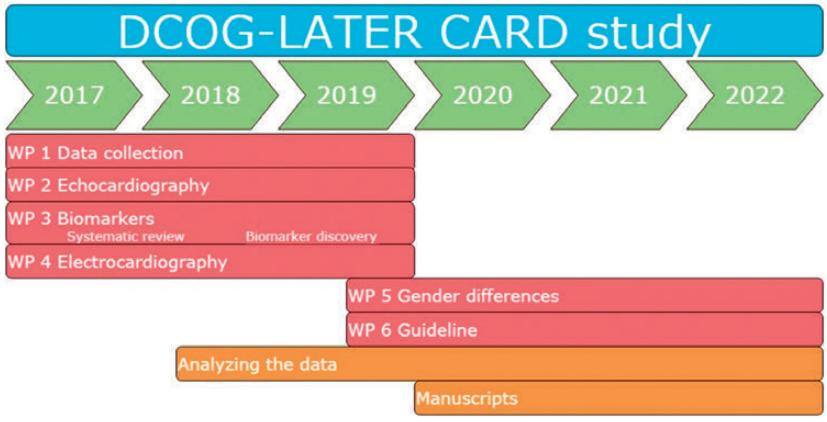

Figure 3. Timeline of the LATER CARD study and the different work packages (WP).

Data collection

The LATER CARD project is one of the 15 projects in the LATER study, each investigating different organ systems/topics (e.g. pulmonary, bone, cardiac, psychosocial, psychosexual). All study participants of the LATER CARD study will be invited once to the outpatient clinic in one of the pediatric oncology centers. A research physician will collect the following data for the LATER CARD study:1) Medical history including family history of cardiovascular diseases, presence of hypertension, diabetes, hypercholesterolemia and life style factors such as alcohol use and smoking. 2) A comprehensive medical history including questions regarding dyspnea, chest pain, palpitations, dizziness, fainting episodes and peripheral edema. 3) Diagnostic tests: height and weight, blood pressure, echocardiographic measurements including measurements from the previous echocardiogram (if available), blood for biomarker sampling and a resting ECG. All data will be anonymized and stored in a central database.

Echocardiography

A standardized echocardiogram, will be performed in all CCS and sibling controls included in the LATER CARD study in the participating centers. Standard measurements (including LV dimensions, LV mass, FS, biplane EF, right ventricular dimensions and function, systolic pulmonary artery pressure, valve abnormalities, and diastolic function measurements) will be performed by experienced sonographers. In one of the participating centers 3-dimensional EFs will be measured. Additional echocardiographic views are obtained for strain analysis in the corelab. All measurements will be anonymized and saved on disc (DICOM format). The analyses will centrally be interpreted by the echocardiography corelab in the Radboudumc in Nijmegen, the Netherlands. The standard measurements will be reviewed and corrected by the corelab and additional measurements will be performed (including radial, circumferential and longitudinal systolic strain and strain rate, biplane EF, left atrial volume index and diastolic parameters including tissue Doppler imaging).

Blood sampling

In all CCS and siblings included in the LATER CARD study, venous blood (divided in plasma and cell portions) will be collected and stored at -80 degrees Celsius in the LATER biobank in Utrecht. Blood sample storage will be available for future biomarker evaluation including the evaluation of genetic susceptibility for cardiac dysfunction.

A panel of 184 protein biomarkers from different biological processes will be evaluated in a case-control study in a subset of the LATER CARD study: markers of myocardial cellular damage/apoptosis, markers of hemodynamic load, markers of inflammation, markers of renal dysfunction/damage, markers of neurohormonal activation, markers of oxidative stress, markers of collagen turnover/remodeling, markers of platelet activation/thrombosis, markers of endothelial dysfunction, markers of anabolic status and markers of nutritional status (Supplementary Table 1)(35). N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and biomarkers identified in the case-control study will be evaluated in the LATER CARD cohort for their diagnostic value in detecting (subclinical) LV systolic dysfunction and LV diastolic dysfunction. Genetic analysis will include a genome wide

association study to identify susceptibility loci for cancer-therapy related cardiac dysfunction. In addition, we will evaluate the prevalence of genetic variants that are associated with dilated cardiomyopathy in CCS with cancer-therapy related cardiac dysfunction and their contribution in the risk-stratification of CCS.

ECG

In all CCS included in the LATER CARD study and sibling controls, a 12-lead resting ECG will be performed. ECGs will be stored centrally and will be analyzed by the corelab according to the Minnesota criteria(36).

Statistical analysis

Power analyses

The power calculations are performed with a power set at 80% and alpha set at 0.05. To detect a clinical significant difference in proportion of subjects with subclinical LV systolic dysfunction (assuming a proportion of 10% in CCS and 1.5% in the sibling controls) we need n=128 in each group. We assume that 13% of the CCS and 2.5% of the sibling controls will have abnormal NT-proBNP values, adjusted for age and sex(37, 38). Therefore, to detect a significant difference in proportion of abnormal NT-proBNP values between the CCS and the sibling controls we need n=108 in each group. To detect a clinically significant difference in proportion of major ECG abnormalities of 10% (estimated proportion in the sibling controls 2.8%(39)) between the CCS and the sibling controls we need n=112 in each group. However, because we aim to perform subgroup analyses according to risk factors (gender, cancer treatment) more CCS and sibling controls will be included.

Prevalence analyses

The prevalence of LV systolic dysfunction (defined as a biplane EF<52% for males and <54% for females(32)), LV diastolic dysfunction (grades according to the European Association of Cardiovascular Imaging(33)) and abnormal myocardial strain (age, sex and vendor based normal values(34)) will be reported and compared between the different risk groups and with the sibling controls with the X^2 test. Likewise, the prevalence of abnormal candidate blood biomarkers and major and minor ECG abnormalities (according to the Minnesota criteria(36)) will be reported.

Risk factor analyses

Risk factor analyses for LV systolic dysfunction, LV diastolic dysfunction, myocardial strain abnormalities, abnormal blood biomarkers findings and abnormal ECG measurements will be evaluated using separate multivariable logistic regression models including cancer treatment(s), follow-up time, sex, age at diagnosis, lifestyle factors (including smoking, alcohol consumption) and comorbidities (including hypertension, overweight, diabetes, hypercholesterolemia). The association between cancer treatment exposures and echocardiographic measurements (including EF, strain and diastolic function), blood biomarker values and ECG measurements will be evaluated with multivariable linear regression models.

Diagnostic accuracy analyses

The association between echocardiographic indices, blood biomarker values and ECG parameters will be tested in linear regression models. The diagnostic value of blood biomarkers and ECG parameters to detect LV systolic dysfunction, LV diastolic dysfunction or strain abnormalities will be evaluated with cut-off points derived from receiver operating characteristic curves. Optimal cut-off points for confirming or excluding the presence of LV systolic dysfunction, LV diastolic dysfunction or strain abnormalities will be reported with the sensitivity, specificity, positive predictive value and negative predictive value."

Subgroup analyses

Subgroup analyses will be performed in CCS without a (previous) diagnosis of congestive heart failure (as defined by the European Society of Cardiology heart failure guideline)(19). To detect early markers for subclinical cardiac dysfunction subgroup analyses will be performed in asymptomatic CCS with a normal LV function at the previous echocardiogram (within 5 years, if available) and without symptoms of heart failure. Additional subgroup analyses will be performed for males and females to evaluate gender differences in prevalence and risk factors for cardiac dysfunction. Non-normally distributed variables will be log-transformed or tested for with the use of non-parametric tests. Two-sided p-values <0.05 will be considered as statistically significant.

PRELIMINARY RESULTS

Study population

Table 1 presents the characteristics of all eligible CCS and sibling controls for the LATER CARD study who were alive on January 1, 2017. The eligible study cohort includes 3,608 CCS and 1,066 siblings, the majority of CCS had a primary diagnosis of leukemia, lymphoma, renal tumors, bone or soft tissue sarcomas. There are 2,566 eligible CCS in *risk group 1*, 48 eligible CCS in *risk group 2*, 33 eligible CCS in *risk group 3* and 961 eligible CCS in *risk group 4* (Figure 1).

Inclusion

We expect to include 1,900 of the 3,608 CCS and 500 of the 1,066 siblings (total study population=2,400) in a consecutive order. Currently (May 2019), we have collected data of 1283 CCS and 189 siblings.

Expected results

With the LATER CARD study, we will report the prevalence and relative risk of subclinical cardiac dysfunction in CCS compared to sibling controls based on echocardiographic imaging (including abnormal myocardial strain and LV diastolic dysfunction parameters), blood biomarkers and ECG parameters and their associations with treatment and gender related risk factors. Furthermore, we will determine the value of myocardial strain, blood biomarkers and ECG parameters in the diagnosis of LV dysfunction and their prognostic usefulness in the surveillance of long-term CCS

Table 1. Patient, cancer and treatment characteristics of eligible 5-year survivors for the DCOG-LATER CARD cohort and sibling controls

Characteristics	Chilhood cancer survivors	Sibling controls
n	3.608	1.066
Sex		
Female	1,530 (42.4%)	452 (42.4%)
Male	2,078 (57.6%)	614 (57.6%)
Primary childhood cancer (ICCC)		
Leukemias, myeloprofiferative diseases and myelodysplastic diseases	1,641 (45.5%)	-
Lymphomas and reticulo endothelial neoplasms	783 (21.7%)	-
CNS and miscellaneous intracranial and intraspinal neoplasms	150 (4.2%)	-
Neuroblastoma and other peripheral nervous cell tumors	85 (2.4%)	-
Retinoblastoma	1 (0.0%)	-
Renal tumors	430 (11.9%)	-
Hepatic tumors	36 (1.0%)	-
Bone tumors	226 (6.3%)	-
Soft tissue and other extraosseous sarcomas	185 (5.1%)	-
Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	61 (1.7%)	-
Other malignant epithelial neoplasms and malignant melanomas	8 (0.2%)	-
Other and unspecified malignant neoplasms	2 (0.0%)	-
Age at cancer diagnosis (year)		
0-4	1,663 (46.1%)	-
5-9	989 (27.4%)	-
10-14	749 (20.8%)	-
15-17	207 (5.7%)	-
Cancer treatment period		
1963-1979	442 (12.3%)	-
1980-1989	1,149 (31.8%)	-
1990-2001	2,017 (55.9%)	-
Overall treatment modality		
Risk group 1: Anthracyclines/ mitoxantrone and/ or chest RT	2566 (71.1%)	-
Risk group 2: Cyclofosfamide only	48 (1.3%)	-
Risk group 3: Ifosfamide only	33 (0.9%)	-
Risk group 4: Vincristine only	961 (26.6%)	-

* Percentage of the total DCOG-LATER CARD cohort including sibling controls. Chest RT = radiation therapy involving the heart region

at risk for heart failure (from subgroup analyses of patients with a prior normal echocardiogram). In addition, we expect to provide evidence on the cardiotoxicity of cyclophosphamide, ifosfamide and vincristine. Hereby the LATER CARD study will form the basis of an improved surveillance guideline in long-term CCS.

DISCUSSION

In the LATER CARD study, we will investigate the single and joint contributions of diagnostic tools to detect cancer therapy-related cardiac dysfunction in a large long-term CCS cohort treated with

cardiotoxic cancer therapies. The diagnostic tools (myocardial strain, diastolic function parameters including tissue Doppler measurements, blood biomarkers and ECG) that we will study are scarcely investigated in large long-term CCS cohorts(9, 16, 27, 28, 30) and were not previously studied in relationship with each other or compared with sibling controls.

Two-dimensional EF, measured with echocardiography, is the most frequently used parameter in the surveillance of long-term CCS for cancer therapy-related cardiac dysfunction but is limited by its reproducibility, load dependency and inability to detect subtle changes in EF(21, 40). From studies in adult oncology patients, we know that early detection of subclinical cardiac dysfunction is necessary to prevent cardiac deterioration, by initiating treatment with heart failure medication(18). Adopting this view, sensitive markers are needed that are able to detect early signs of cardiac deterioration before heart failure symptoms occur. Although, evidence of the diagnostic tools (EF, longitudinal strain, diastolic function, NT-proBNP and high-sensitive troponins) on future development of heart failure in long-term CCS is lacking, they are established predictors for heart failure in the general population(23, 26, 41, 42). Therefore, by extrapolating these findings to CCS we expect that the diagnostic tools evaluated in the LATER CARD study can identify CCS at higher risk for development of heart failure, and thus bring benefit from more frequent surveillance and/or early treatment initiation. Importantly, the results may also identify CCS at low risk for heart failure in whom we can decrease the surveillance frequency. In addition, with sub-group analyses of patients with a normal prior echocardiogram we may infer the role of these diagnostic tools in the natural history of cancer therapy-related cardiac dysfunction.

A limitation of the LATER CARD study is that it is of cross-sectional nature and therefore may not be able to validate the findings in predicting future cardiac dysfunction in those patients that do not have cardiac dysfunction or only minor signs of them. The study is therefore mainly able to discern those patients with early or progressed LV dysfunction from those with no dysfunction and therefore mainly to diagnose or exclude the presence of clinically important LV dysfunction with either of the diagnostic tools. As the study is conducted in a large cohort of CCS who previously received regular surveillance by echocardiography according to prevailing guidelines(10), we may be able to identify which markers are markers of progression for cardiac dysfunction and provide additional evidence for risk assessments. Still, future follow up studies of our cohort will be needed to confirm the value of the described diagnostic tools as early markers for clinical heart failure in long-term CCS.

Eventually, the results of the LATER CARD study will provide the evidence to improve long-term follow up guidelines, which we aim to complete at the end of this project. Furthermore, this project will form the basis for future prospective follow-up studies that will increase the knowledge on the predictive value of the described tests. This project will be carried out by a multidisciplinary team of caregivers and researchers in the field of cancer therapy-related cardiac dysfunction in long-term CCS. This team will enable the implementation of the improved guideline recommendations in a broad field of health professionals involved in the care for CCS.

CONCLUSION

The Dutch LATER CARD study will investigate diagnostic tools to detect subclinical cardiac dysfunction in long-term CCS treated with potential cardiotoxic cancer therapies. This will be an important study to investigate the relationship between clinical data, echocardiographic, blood biomarker and ECG measurements to detect cardiac dysfunction in a large nationwide cohort of CCS. The results will form the basis of an improved long-term follow up guideline to ultimately prevent heart failure in this population.

REFERENCES

1. Gatta G, Zigon G, Capocaccia R, Coebergh JW, Desandes E, Kaatsch P, et al. Survival of European children and young adults with cancer diagnosed 1995-2002. European journal of cancer. 2009;45(6):992-1005.
2. Pal vd, H. J., Dalen v, E. C., Delden v, E., Dijk v, l. W., Kok WE, Geskus RB, et al. High risk of symptomatic cardiac events in childhood cancer survivors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30(13):1429-37.
3. van der Pal HJ, van Dalen EC, Hauptmann M, Kok WE, Caron HN, van den Bos C, et al. Cardiac function in 5-year survivors of childhood cancer: a long-term follow-up study. Archives of internal medicine. 2010;170(14):1247-55.
4. Mulrooney DA, Yeazel MW, Kawashima T, Mertens AC, Mitby P, Stovall M, et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. BMJ (Clinical research ed). 2009;339:b4606.
5. Feijen EAM, Font-Gonzalez A, Van der Pal Helena JH, Kok Wouter EM, Geskus Ronald B, Ronckers Cécile M, et al. Risk and Temporal Changes of Heart Failure Among 5-Year Childhood Cancer Survivors: a DCOG-LATER Study. Journal of the American Heart Association. 2019;8(1):e009122.
6. Fidler MM, Reulen RC, Henson K, Kelly J, Cutter DJ, Levitt GA, et al. Population-Based Long-Term Cardiac-Specific Mortality among 34,489 Five-Year Survivors of Childhood Cancer in Great Britain. Circulation. 2017.
7. Mertens AC, Liu Q, Neglia JP, Wasilewski K, Leisenring W, Armstrong GT, et al. Cause-specific late mortality among 5-year survivors of childhood cancer: the Childhood Cancer Survivor Study. Journal of the National Cancer Institute. 2008;100(19):1368-79.
8. Brouwer CA, Postma A, Vonk JM, Zwart N, van den Berg MP, Bink-Boelkens MT, et al. Systolic and diastolic dysfunction in long-term adult survivors of childhood cancer. European journal of cancer (Oxford, England : 1990). 2011;47(16):2453-62.
9. Armstrong GT, Joshi VM, Ness KK, Marwick TH, Zhang N, Srivastava D, et al. Comprehensive Echocardiographic Detection of Treatment-Related Cardiac Dysfunction in Adult Survivors of Childhood Cancer: Results From the St. Jude Lifetime Cohort Study. Journal of the American College of Cardiology. 2015;65(23):2511-22.
10. Sieswerda E, Postma A, van Dalen EC, van der Pal HJ, Tissing WJ, Rammeloo LA, et al. The Dutch Childhood Oncology Group guideline for follow-up of asymptomatic cardiac dysfunction in childhood cancer survivors. Ann Oncol. 2012;23(8):2191-8.
11. Smith LA, Cornelius VR, Plummer CJ, Levitt G, Verrill M, Canney P, et al. Cardiotoxicity of anthracycline agents for the treatment of cancer: systematic review and meta-analysis of randomised controlled trials. BMC cancer. 2010;10:337.
12. Feijen EA, Leisenring WM, Stratton KL, Ness KK, van der Pal HJ, Caron HN, et al. Equivalence Ratio for Daunorubicin to Doxorubicin in Relation to Late Heart Failure in Survivors of Childhood Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33(32):3774-80.
13. Lipshultz SE, Franco VI, Miller TL, Colan SD, Sallan SE. Cardiovascular disease in adult survivors of childhood cancer. Annual review of medicine. 2015;66:161-76.
14. Dalen v EC, Michiels EMC, Caron HN, Kremer LC. Different anthracycline derivates for reducing cardiotoxicity in cancer patients (Review). Cochrane DB Syst Rev. 2010(5).
15. van Dalen EC, van der Pal HJ, Kremer LC. Different dosage schedules for reducing cardiotoxicity in people with cancer receiving anthracycline chemotherapy. The Cochrane database of systematic reviews. 2016;3:Cd005008.
16. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2015;16(3):e123-36.

17. Yusuf S, Pitt B, Davis CE, Hood WB, Jr., Cohn JN. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The New England journal of medicine. 1992;327(10):685-91.

18. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. Circulation. 2015;131(22):1981-8.

19. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European journal of heart failure. 2016;18(8):891-975.

20. Thavendiranathan P, Grant AD, Negishi T, Plana JC, Popovic ZB, Marwick TH. Reproducibility of echocardiographic techniques for sequential assessment of left ventricular ejection fraction and volumes: application to patients undergoing cancer chemotherapy. Journal of the American College of Cardiology. 2013;61(1):77-84.

21. Plana JC, Galderisi M, Barac A, Ewer MS, Ky B, Scherrer-Crosbie M, et al. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. European heart journal cardiovascular Imaging. 2014;15(10):1063-93.

22. Stanton T, Leano R, Marwick TH. Prediction of all-cause mortality from global longitudinal speckle strain: comparison with ejection fraction and wall motion scoring. Circ Cardiovasc Imaging. 2009;2(5):356-64.

23. Kalam K, Otahal P, Marwick TH. Prognostic implications of global LV dysfunction: a systematic review and meta-analysis of global longitudinal strain and ejection fraction. Heart (British Cardiac Society). 2014;100(21):1673-80.

24. Klein Hesselink MS, Bocca G, Hummel YM, Brouwers AH, Burgerhof JGM, van Dam E, et al. Diastolic Dysfunction is Common in Survivors of Pediatric Differentiated Thyroid Carcinoma. Thyroid : official journal of the American Thyroid Association. 2017;27(12):1481-9.

25. Lam CS, Lyass A, Kraigher-Krainer E, Massaro JM, Lee DS, Ho JE, et al. Cardiac dysfunction and noncardiac dysfunction as precursors of heart failure with reduced and preserved ejection fraction in the community. Circulation. 2011;124(1):24-30.

26. Kane GC, Karon BL, Mahoney DW, et al. Progression of left ventricular diastolic dysfunction and risk of heart failure. JAMA. 2011;306(8):856-63.

27. Leerink JM, Verkleij SJ, Feijen EAM, Mavinkurve-Groothuis AMC, Pourier MS, Ylanen K, et al. Biomarkers to diagnose ventricular dysfunction in childhood cancer survivors: a systematic review. Heart. 2018.

28. Pourier MS, Mavinkurve-Groothuis AM, Loonen J, Bokkerink JP, Roeleveld N, Beer G, et al. Is screening for abnormal ECG patterns justified in long-term follow-up of childhood cancer survivors treated with anthracyclines? Pediatric blood & cancer. 2017;64(3).

29. Brouwer CA, Gietema JA, van den Berg MP, Bink-Boelkens MT, Elzenga NJ, Haaksma J, et al. Low-dose anthracyclines in childhood Acute Lymphoblastic Leukemia (ALL): no cardiac deterioration more than 20 years post-treatment. Journal of cancer survivorship : research and practice. 2007;1(4):255-60.

30. Mulrooney DA, Soliman EZ, Ehrhardt MJ, Lu L, Duprez DA, Luepker RV, et al. Electrocardiographic abnormalities and mortality in aging survivors of childhood cancer: A report from the St Jude Lifetime Cohort Study. Am Heart J. 2017;189:19-27.

31. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Seminars in Radiation Oncology. 2003;13(3):176-81.

32. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European

Association of Cardiovascular Imaging. European Heart Journal - Cardiovascular Imaging. 2015;16(3):233-71.

33. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, 3rd, Dokainish H, Edvardsen T, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2016;29(4):277-314.
34. Takigiku K, Takeuchi M, Izumi C, Yuda S, Sakata K, Ohte N, et al. Normal range of left ventricular 2-dimensional strain: Japanese Ultrasound Speckle Tracking of the Left Ventricle (JUSTICE) study. Circulation journal : official journal of the Japanese Circulation Society. 2012;76(11):2623-32.
35. Braunwald E. Biomarkers in heart failure. The New England journal of medicine. 2008;358(20):2148-59.
36. Prineas. The Minnesota code manual of electrocardiographic findings. Standards and procedures for measurements and classification. Littleton, MA John Wright, PSG Inc; 1982.
37. Mavinkurve-Groothuis AM, Groot-Loonen J, Bellersen L, Pourier MS, Feuth T, Bokkerink JP, et al. Abnormal NT-pro-BNP levels in asymptomatic long-term survivors of childhood cancer treated with anthracyclines. Pediatric blood & cancer. 2009;52(5):631-6.
38. Raymond I, Groenning BA, Hildebrandt PR, Nilsson JC, Baumann M, Trawinski J, et al. The influence of age, sex and other variables on the plasma level of N-terminal pro brain natriuretic peptide in a large sample of the general population. Heart (British Cardiac Society). 2003;89(7):745-51.
39. De Bacquer D, De Backer G, Kornitzer M. Prevalences of ECG findings in large population based samples of men and women. Heart (British Cardiac Society). 2000;84(6):625-33.
40. Jacobs LD, Salgo IS, Goonewardena S, Weinert L, Coon P, Bardo D, et al. Rapid online quantification of left ventricular volume from real-time three-dimensional echocardiographic data. European heart journal. 2006;27(4):460-8.
41. de Lemos JA, Drazner MH, Omland T, Ayers CR, Khera A, Rohatgi A, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. Jama. 2010;304(22):2503-12.
42. Willeit P, Kaptoge S, Welsh P, Butterworth AS, Chowdhury R, Spackman SA, et al. Natriuretic peptides and integrated risk assessment for cardiovascular disease: an individual-participant-data meta-analysis. The Lancet Diabetes & Endocrinology. 2016;4(10):840-9.

SUPPLEMENTAL MATERIAL

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6

DEVELOPMENT AND INTERNAL VALIDATION OF A CARDIAC BIOMARKER BASED DIAGNOSTIC MODEL FOR CARDIAC DYSFUNCTION IN ADULT SURVIVORS OF CHILDHOOD CANCER: A REPORT FROM THE DUTCH CHILDHOOD CANCER SURVIVOR STUDY

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Submitted

ABSTRACT

Importance

Life-long echocardiographic surveillance is currently performed in childhood cancer survivors at risk for heart failure. Previous studies reported a limited diagnostic accuracy of N-terminal pro B-type natriuretic peptide (NT-proBNP) and high-sensitive cardiac troponin T (Hs-cTnT) to detect left ventricular (LV) dysfunction in survivors. Combining cardiac biomarkers with clinical characteristics may however improve diagnostic accuracy.

Objective

To develop and internally validate a diagnostic model that combines cardiac biomarkers with clinical characteristics for ruling-in or ruling-out LV dysfunction in childhood cancer survivors.

Design

Prospectively designed, multicenter, cross-sectional study.

Setting

Long-term survivorship outpatient clinics in the Netherlands.

Participants

Childhood cancer survivors treated with cardiotoxic cancer treatments, ≥5 years from cancer diagnosis, without a previous diagnosis of cardiomyopathy; and sibling controls.

Main outcomes and measures

Diagnostic accuracy of a multivariable model including NT-proBNP, Hs-cTnT and clinical characteristics for three degrees of LV dysfunction measured with echocardiography (LVEF<54% in females or <52% in males; LVEF<50%; LVEF<45%). Prespecified criteria were used for rule-in (positive predictive value ≥75% and specificity ≥90%) and rule-out (negative predictive value ≥98% and sensitivity ≥90%).

Results

Included were 1334 childhood cancer survivors (median age 34.2 years) and 278 siblings (median age 36.8 years). NT-proBNP was abnormal for age-and sex in 22.1% of survivors compared to 5.4% of siblings (p<0.001). A Hs-cTnT>10 ng/L was uncommon in survivors (5.9%) and siblings (5.0%). Diagnostic models improved by adding NT-proBNP and Hs-cTnT to clinical characteristics (C-statistic from 0.69 to 0.74 for LVEF<50%) and were more accurate for more severe LV dysfunction (C-statistic from 0.78 to 0.84 for LVEF<45%). Rule-in of any LV dysfunction was not possible due to low specificity. A LVEF<50% (prevalence 10.9%) could be ruled-out in 18.5% of survivors with high sensitivity (95.9%, 95% CI 90.7-100%) and negative predictive value (97.7%, 95.4-100%). A LVEF<45%

(prevalence 3.4%) could be ruled out in 52.5% of survivors with moderate to high sensitivity (88.4%, 75.6-97.8%) and high negative predictive value (99.2%, 98.6-99.8%).

Conclusions and relevance

A diagnostic model for LV dysfunction including NT-proBNP, Hs-cTnT and clinical characteristics can be used to reduce the number of unnecessary surveillance echocardiograms in childhood cancer survivors. External validation is needed.

KEY POINTS

Question

Can a cardiac biomarker based diagnostic model be used to rule-in or rule-out LV dysfunction in childhood cancer survivors treated with cardiotoxic cancer therapies?

Findings

In this cross-sectional study, a diagnostic model including NT-proBNP, Hs-cTnT and clinical characteristics was superior compared to biomarkers only or clinical characteristics only and could rule-out a LVEF <50% in 18.5% of survivors with a sensitivity of 95.9% and negative predictive value of 97.7%.

Meaning

After external validation the diagnostic model may be used to reduce the number of unnecessary surveillance echocardiograms in childhood cancer survivors.

INTRODUCTION

Cardiovascular disease is a major concern among the growing number of long-term childhood cancer survivors (CCS).[1,2] Almost 11% of CCS who have been treated with anthracyclines, mitoxantrone and/or chest-directed radiotherapy develops heart failure within 40 years from cancer diagnosis.[3] Life-long echocardiographic surveillance is currently recommended to prevent or delay heart failure by early detection of left ventricular (LV) dysfunction, with surveillance intervals depending on cumulative anthracycline, mitoxantrone and chest-directed radiotherapy doses.[4,5]

The role of cardiac biomarkers in the long-term surveillance of CCS is uncertain. Cardiac biomarkers might serve as a cost-effective triage test to conduct or postpone an echocardiogram. If LV dysfunction can be ruled-out with a blood biomarker test, an echocardiogram can be deferred to the next surveillance time point. Previous studies reported a limited diagnostic accuracy of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and (high sensitive) cardiac troponins (Hs-cTnT) to detect LV dysfunction in long-term CCS[6,7], which led to a recommendation not to use cardiac biomarkers for surveillance in CCS.[4,5] However, unresolved questions remain. First, previous studies investigated cardiac biomarkers as a single diagnostic test for detection of LV dysfunction[6], while combining biomarkers with clinical information might improve diagnostic performance.[8] Second, no studies in CCS investigated biomarker cutoff concentrations specific for rule-out or rule-in of LV dysfunction which may improve diagnostic performance.[6] Third, it is unclear in CCS whether cardiac biomarkers may be better suited to diagnose more severely abnormal LV function, a finding that a previous study in the general population acknowledged.[9]

In this cross-sectional multicenter study, we developed and internally validated diagnostic models that combine cardiac biomarkers with clinical characteristics to rule-in or rule-out LV dysfunction in CCS without a previous diagnosis of cardiomyopathy.

METHODS

Study population

We conducted a multicenter cross-sectional study in CCS and siblings participating in the Dutch Childhood Cancer Survivor Study, LATER part 2 cardiology study (DCCSS LATER2 CARD). DCCSS LATER2 CARD is a multicenter study in ≥5-year CCS diagnosed with a malignancy before the age of 18 years and between 1/1/1963 and 12/31/2001 who were treated with cardiotoxic cancer treatments.[10] Participants visited the outpatient clinic between February 2016 and February 2020 for questionnaires, physical examination, blood sampling and echocardiography. For the present study, we included CCS treated with anthracyclines, mitoxantrone and/or chest-directed radiotherapy without a previous diagnosis of cardiomyopathy to reflect a surveillance population. As the most recent surveillance guideline no longer recommends surveillance in low-risk CCS (anthracycline dose <100 mg/m2 and chest directed radiotherapy dose <15 Gray), we conducted a secondary analysis where we excluded these low-risk CCS. Siblings of the CCS were included as controls. We excluded CCS and siblings who were pregnant, had a history of heart transplantation or severe congenital heart disease interfering with echocardiographic measurement of LV function.

Ethics

The study was approved by the medical ethics board of all participating centers and included the storage of blood samples. Informed consent was obtained from all participants.

Clinical characteristics

We obtained patient and cancer treatment characteristics from the central database of the study. Cumulative anthracycline or anthraquinone dose was calculated using doxorubicin equivalents.[11] Radiotherapy dose received by the heart was calculated with a standardized protocol (see supplements). Medical history, cardiac medication uses and cardiac symptoms were obtained from questionnaires. Self-reported heart failure, hypertension and diabetes were considered present if participants also reported to use medications for the condition. All participants underwent a physical examination at time of blood sampling to obtain body mass index (BMI), heart rate and blood pressure.

Blood biomarkers

We measured NT-proBNP, Hs-cTnT and creatinine in fasting serum samples at the Erasmus Medical Center, the Netherlands. Fasting serum samples were obtained from participants within 1 year from the qualifying echocardiogram (89% were obtained at the same day). Samples were centrifuged at 3000xg for 10 minutes, shipped on dry ice to the central biobank and stored at -80 degrees Celsius. The assay range is 5-35,000 ng/L for NT-proBNP (Cobas e601, Roche Diagnostics, Mannheim, Germany) and 3-10,000 ng/L for Hs-cTnT (Cobas e602, Roche Diagnostics). Biomarker values below the limit of detection (NT-proBNP n=111, Hs-cTnT n=543) were set at the limit of detection divided by the square root of 2. An abnormal NT-proBNP was defined as the 97,5 percentile value exceeding age and sex specific normal values in the Framingham Heart Study cohort obtained with the quantile regression method (Table S1).[12] An abnormal Hs-cTnT was defined as a Hs-cTnT ≥10 ng/L, in line with a previous study in CCS.[7] Glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[13]

Echocardiograms

We measured echocardiographic parameters including biplane LV ejection fraction (LVEF) in a core lab blinded for blood biomarker results and patient characteristics.[14] We studied the following LV dysfunction definitions: LVEF<54% for females and <52% for males[15], LVEF<50% and LVEF<45%.

Missing values

Missing values were present in cardiac biomarkers (<5% for all), cardiotoxic cancer treatment doses (≤1% for all), traditional cardiovascular risk factors (self-reported history of hypertension, diabetes, dyslipidemia, smoking; 8-10%) and LVEF (17%). We assumed these to be missing at random and imputed these 20-times using predictive mean matching.[16] The imputation model included all variables considered in the diagnostic models, as well as other measures of LV function (fractional shortening, mitral annular plane systolic excursion) to improve LVEF imputations. Analyses were

performed on each imputed dataset and results pooled using Rubin's rules.[16] We compared the results to a complete case analysis.

Statistical analyses

Median biomarker concentrations were compared between CCS and siblings with the Wilcoxon rank sum test. The proportion of CCS with abnormal biomarker levels was compared to siblings with the Fisher exact test. We visualized the association of cardiac biomarker concentrations with LVEF using local polynomial regression fitting.

We predefined criteria for rule-out (negative predictive value (NPV) ≥98% and sensitivity of ≥90%), and rule-in (positive predictive value (PPV) ≥75% and specificity ≥90%) of LV dysfunction based on previous studies on the diagnosis of heart failure among dyspneic patients.[8,17] We compared findings in the three aforementioned categories of LV dysfunction.

We calculated diagnostic test accuracies (sensitivity, specificity, positive predictive value (PPV) and NPV) of multiple cutoff concentrations of NT-proBNP (normal values according to age and sex[12] and a range from 10 to 600 ng/L) and concentrations of Hs-cTnT (range 3 to 14 ng/L).

Next, we developed and internally validated multivariable logistic regression models to estimate the probability of three categories of LV dysfunction. The first model considered only clinical predictors: sex, age at diagnosis, age at study, anthracycline dose, mitoxantrone dose, chest-directed radiotherapy dose, history of hypertension, hypercholesterolemia, diabetes and smoking, and BMI, heart rate and systolic blood pressure at time of blood sampling. Backwards selection was applied using a p-value of 0.05 to obtain the final clinical model with sex, age at diagnosis, age at study and cardiotoxic treatments always included in the model. In the second model, we added NT-proBNP, Hs-cTnT and eGFR to the final clinical model and tested for improvement in model fit with the pooled Wald test for multiple imputed data.[16] We tested for potential non-linear associations with the use of restricted cubic splines.[18] We assessed diagnostic performance with various measures (sensitivity, specificity, PPV, NPV, C-statistic and decision curve analysis[19]). We assessed calibration with calibration plots. We internally validated the models using 500 bootstrap resamples in each of the imputed datasets to adjust for optimism and to calculate 95% confidence intervals (95% CI). All analyses were conducted in R. A p-value <0.05 was considered statistically significant.

RESULTS

Study population

Of the 6165 CCS in the DCCSS LATER cohort, 2986 CCS met the eligibility criteria for the LATER2 CARD study (Figure S1). Of these CCS, 1605 (54%) participated in the LATER2 CARD study. Participants were more frequently female (48%) compared to non-participants (39%) but there were no clinically relevant differences between participants and non-participants in other patient and treatment characteristics (Table S2). After excluding CCS with a previous diagnosis of cardiomyopathy (n=51) and CCS not treated with anthracyclines, mitoxantrone or chest-directed radiotherapy (n=220), 1334 CCS were included in the present study. A total of 278 siblings were included as controls (Figure S1). Characteristics of the included CCS and siblings are presented in Table 1. CCS were slightly younger

(median age 34.2 years, IQR 28.5-41.5 versus 36.8 years, IQR 29.1-43.7) and there were less women among CCS compared to siblings (46.9% versus 59.7%).

Table 1. Characteristics of participating childhood cancer survivors and siblings.

Characteristic	Survivors n=1334	Siblings n=278
Female (%)	625 (46.9)	166 (59.7)
Age at diagnosis (years) (median [IQR])	6.3 [3.2, 11.3]	NA
Age at study (years) (median [IQR])	34.2 [28.5, 41.5]	36.8 [29.1, 43.7]
Primary cancer diagnosis (%)		
Leukemias, myeloproliferative diseases and myelodysplastic diseases	537 (40.3)	0 (0.0)
Lymphomas and reticuloendothelial neoplasms	342 (25.6)	0 (0.0)
CNS and miscellaneous intracranial and intraspinal neoplasms	43 (3.2)	0 (0.0)
Neuroblastoma and other peripheral nervous cell tumors	50 (3.7)	0 (0.0)
Retinoblastoma	0 (0.0)	0 (0.0)
Renal tumors	150 (11.2)	0 (0.0)
Hepatic tumors	12 (0.9)	0 (0.0)
Bone tumors	112 (8.4)	0 (0.0)
Soft tissue and other extraosseous sarcomas	71 (5.3)	0 (0.0)
Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	12 (0.9)	0 (0.0)
Other malignant epithelial neoplasms and malignant melanomas	4 (0.3)	0 (0.0)
Other and unspecified malignant neoplasms	1 (0.1)	0 (0.0)
NA	0 (0.0)	278 (100.0)
IGHG risk group (%)		
low risk	306 (22.9)	0 (0.0)
moderate risk	596 (44.7)	0 (0.0)
high risk	432 (32.4)	0 (0.0)
NA	0 (0.0)	278 (100.0)
Anthracycline dose (mg/m2) (%)		
No anthracyclines	202 (15.1)	278 (100.0)
1-100	207 (15.5)	0 (0.0)
100-250	603 (45.2)	0 (0.0)
>250	302 (22.6)	0 (0.0)
missing	20 (1.5)	0 (0.0)
Mitoxantrone dose (mg/m2) (%)		
No Mitoxantrone	1258 (94.3)	278 (100.0)
1-40	42 (3.1)	0 (0.0)
>40	28 (2.1)	0 (0.0)
missing	6 (0.4)	0 (0.0)
Chest RT dose (Gray) (%)		
No Chest RT	896 (67.2)	278 (100.0)
1-15	277 (20.8)	0 (0.0)
15-30	99 (7.4)	0 (0.0)
>30	52 (3.9)	0 (0.0)
missing	10 (0.7)	0 (0.0)

Table 1. (continued)

Characteristic	Survivors n=1334	Siblings n=278
Hypertension (%)		
No	1138 (85.3)	228 (82.0)
Yes	68 (5.1)	2 (0.7)
missing	128 (9.6)	48 (17.3)
Diabetes (%)		
No	1183 (88.7)	231 (83.1)
Yes	27 (2.0)	0 (0.0)
missing	124 (9.3)	47 (16.9)
Hypercholesterolemia (%)		
No	1152 (86.4)	227 (81.7)
Yes	44 (3.3)	1 (0.4)
missing	138 (10.3)	50 (18.0)
Smoking (%)		
No	859 (64.4)	140 (50.4)
Yes	364 (27.3)	83 (29.9)
missing	111 (8.3)	55 (19.8)
BMI (median [IQR])	24.0 [21.6, 26.9]	25.2 [23.0, 27.8]
Systolic blood pressure (mmHg) (median [IQR])	123.0 [114.0, 134.0]	120.0 [112.0, 131.0]
Diastolic blood pressure (mmHg) (median [IQR])	76.0 [69.0, 82.0]	74.0 [67.0, 81.0]
Heart rate (/min) (median [IQR])	70.0 [62.0, 79.0]	63.0 [56.7, 70.1]
eGFR (ml/min/1.73 m2) (median [IQR])	107.8 [92.4, 125.8]	103.8 [89.5, 123.2]
NT-proBNP (ng/L) (median [IQR])	42.3 [25.4, 84.6]	33.8 [16.9, 59.2]
Abnormal NTproBNP for age and sex (%)		
No	996 (74.7)	232 (83.5)
Yes	295 (22.1)	15 (5.4)
missing	43 (3.2)	31 (11.2)
Hs-cTnT (ng/L) (median [IQR])	4.0 [2.2, 5.0]	3.0 [2.4, 5.0]
Abnormal Hs-cTnT (>=10 ng/L) (%)		
No	1214 (91.0)	233 (83.8)
Yes	77 (5.8)	14 (5.0)
missing	43 (3.2)	31 (11.2)

Cardiac biomarkers in CCS compared to siblings

The median NT-proBNP concentration was significantly higher in CCS compared to siblings (42.3 ng/L, IQR 25.4-84.6, versus 33.8 ng/L, IQR 16.9-59.2, respectively, p<0.001)) and abnormal NT-proBNP levels for age and sex were more frequent among CCS compared to siblings (22.1% versus 5.4%, p<0.001) (Table 1). The median Hs-cTnT concentration was not significantly different in CCS (4.0 ng/L, IQR 2.2-5.0, p=0.812) compared to siblings (3.0 ng/L, IQR 2.4-5.0) and an abnormal Hs-cTnT level ≥10 ng/L was rare in both CCS (5.8%) and siblings (5.0%, p=1.00)(Table 1).

Diagnostic accuracy of cardiac biomarkers only

Of the 1334 CCS, 23.2% had a LVEF<54% (females) or LVEF<52% (males), 10.9% had a LVEF<50% and 3.4% had a LVEF<45%. Visual inspection of local polynomial regression curves of both biomarkers with LVEF showed increasing NT-proBNP and Hs-cTnT concentrations in CCS with lower LVEFs, especially when LVEF decreases to <50% (Figure S2). The univariable diagnostic accuracy of abnormal NT-proBNP or abnormal Hs-cTnT was too limited for either rule-out or rule-in of any category of LV dysfunction (Table 2), also when using lower cutoff concentrations for rule-out or higher cutoff concentrations for rule-in (Table S3).

Diagnostic accuracy of cardiac biomarkers in combination with clinical characteristics

Clinical characteristics included in the final diagnostic model after backwards selection were sex, age at diagnosis, age at study, anthracycline dose, mitoxantrone dose, chest-directed radiotherapy dose and heart rate. For all three categories of LV dysfunction, adding NT-proBNP and Hs-cTnT, but not eGFR, to the clinical characteristics improved diagnostic performance compared to clinical characteristics only, NT-proBNP only or Hs-cTnT only (pooled Wald test p<0.001 for all). Coefficients of the final diagnostic models are presented in Table S4 and can be used to calculate predicted probabilities of LV dysfunction for each survivor.

Discrimination of the combined diagnostic model was better for more severe LV dysfunction with optimism corrected C-statistics of 0.70 (95% CI 0.67-0.74), 0.74 (95% CI 0.69-0.78) and 0.84 (95% CI 0.78-0.90) for LVEF<54% (females) or LVEF<52% (males), LVEF<50% and LVEF<45%, respectively (Figure 1, Table 2). The models were well calibrated (Figure 2). Decision curve analysis showed that the diagnostic models combining clinical characteristics with cardiac biomarkers achieved a higher net reduction in unnecessary echocardiograms compared to cardiac biomarkers only or clinical characteristics only across a range of threshold probabilities (Figure S3).

For ruling-out LVEF<54% (females) or LVEF<52% (males), a predicted probability threshold of 8.0% by the diagnostic model had a sensitivity of 99.3% (95% CI 97.6-100%) and a NPV of 94.6% (95% CI 84.1-100%). However, at this predicted probability threshold only 2.6% of survivors could be ruled-out (Table 2). For ruling-out LVEF<50%, a predicted probability threshold of 4.3% had a high sensitivity of 95.9% (95% CI 90.7-100%) and a NPV of 97.7% (95% CI 95.4-100%) and ruled-out a LVEF<50% in 18.5% of survivors (Table 2). Even though Hs-cTnT significantly improved overall performance of the diagnostic model, we were interested whether a model including only clinical characteristics and NT-proBNP, without Hs-cTnT, would suffice for rule-out purposes. As can be seen in Table 2, sensitivity and NPV of this simplified model were comparable, however 16.6% of survivors could be ruled-out compared to 18.5% with the full model. For ruling-out LVEF<45%, a predicted probability threshold of 1.5% ruled out 52.5% of survivors with high NPV of 99.2% (95% CI 98.6-99.8%) and a sensitivity of 88.4% (95% CI 75.6-97.8%) that was below our predefined sensitivity of 90% for rule-out (Table 2). The wide 95% CI of the sensitivity and the relatively large optimism of 3.1% in the bootstrap validation suggest the model was underpowered.

The diagnostic models were not useful for rule-in because at PPVs ³75% and specificities ³90%, only 0.9%, 1.1% and 1.8% of survivors had LVEF<54% (females) or LVEF<52% (males), LVEF<50% and LVEF<45%, respectively (Figure S4). A complete cases analysis gave comparable results (Table S5).

Table 2. Optimism adjusted diagnostic accuracy measures of cardiac biomarkers and clinical characteristics to detect LV dysfunction in childhood cancer survivors treated with cardiotoxic cancer treatments.

Predictor	C-statistic	Cutoff	Ruled out	Sensitivity	Specificity	PPV	NPV
LVEF <52% in males, <54% in females (prevalence 23.2%)							
NT-proBNP	0.63 (0.59-0.66)	Age/sex	77.0%	35.2 (29.6-40.8)	80.7 (78.2-83.2)	35.5 (29.6-41.3)	80.5 (77.8-83.3)
Hs-cTnT	0.55 (0.51-0.60)	10 ng/L	94.1%	10.5 (6.8-14.1)	95.4 (94.1-96.8)	40.7 (28.8-52.7)	78.0 (75.3-80.6)
Clinical[a]	0.68 (0.65-0.72)	8.0%[b]	2.0%	100 (98.6-100)	2.6 (0.5-5.8)	23.6 (22.6-25.1)	99.8 (89.9-100)
Clinical+NT-proBNP	0.70 (0.66-0.73)	8.0%[b]	1.9%	99.7 (98.3-100)	2.4 (0.6-5.9)	23.6 (22.6-25.2)	97.5 (88.9-100)
Clinical+NT-proBNP+Hs-cTnT	0.70 (0.67-0.74)	8.0%[b]	2.6%	99.3 (97.6-100)	3.2 (0.9-6.8)	23.7 (22.6-25.1)	94.6 (84.1-100)
LVEF <50% (prevalence 10.9%)							
NT-proBNP	0.63 (0.58-0.69)	Age/sex	77.0%	44.9 (36.2-53.7)	79.7 (77.3-82.1)	21.3 (16.3-26.4)	92.2 (90.3-94.1)
Hs-cTnT	0.64 (0.59-0.69)	10 ng/L	94.1%	15.6 (9.2-22.1)	95.2 (94.0-96.5)	28.7 (17.8-39.6)	90.2 (88.3-92.1)
Clinical[a]	0.69 (0.65-0.74)	4.3%[b]	12.4%	97.2 (92.0-100)	13.5 (6.5-21.8)	12.1 (11.2-12.9)	97.5 (94.9-100)
Clinical+NT-proBNP	0.72 (0.68-0.77)	4.3%[b]	16.6%	95.9 (90.6-99.3)	18.1 (10.0-26.8)	12.5 (11.4-13.6)	97.2 (94.9-100)
Clinical+NT-proBNP+Hs-cTnT	0.74 (0.69-0.78)	4.3%[b]	18.5%	95.9 (90.7-100)	20.3 (12.1-29.3)	12.8 (11.8-13.9)	97.7 (95.4-100)
LVEF <45% (prevalence 3.4%)							
NT-proBNP	0.75 (0.65-0.84)	Age/sex	77.0%	65.0 (50.3- 79.7)	78.5 (76.2-80.8)	9.7 (6.3-13.1)	98.4 (97.6- 99.3)
Hs-cTnT	0.75 (0.67-0.83)	10 ng/L	94.1%	24.5 (10.9-38.0)	94.7 (93.5-96.0)	14.1 (5.9-22.4)	97.2 (96.3- 98.2)
Clinical[a]	0.78 (0.70-0.85)	1.5%[b]	38.0%	90.9 (80.0-98.0)	39.1 (28.0-46.6)	5.0 (4.1-5.9)	99.2 (98.3-100)
Clinical+NT-proBNP	0.84 (0.77-0.90)	1.5%[b]	48.6%	90.7 (77.8-100)	50.0 (39.6-58.4)	6.1 (4.9-7.2)	99.3 (98.6-100)
Clinical+NT-proBNP+Hs-cTnT	0.84 (0.78-0.90)	1.5%[b]	52.5%	88.4 (75.6-97.8)	54.0 (43.7-62.7)	6.4 (5.0-7.7)	99.2 (98.6-99.8)

[a]Clinical characteristics: sex, age at diagnosis, age at study, anthracycline dose, mitoxantrone dose, chest-directed radiotherapy dose, heart rate.

[b]Cutoff probability of LV dysfunction from the multivariable logistic regression model.

95% confidence intervals are shown between brackets.

Abbreviations: Hs-cTnT=high sensitive cardiac troponin T, LV=left ventricular, NT-proBNP=N-terminal pro B-type natriuretic peptide B, NPV=negative predictive value, PPV=positive predictive value.

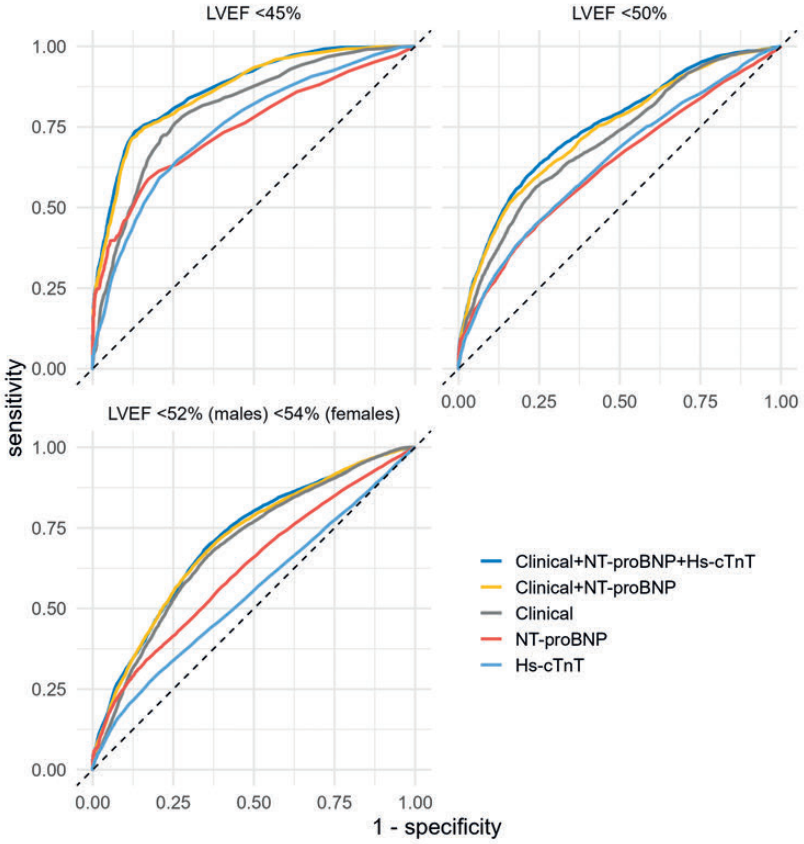

Figure 1. Receiver operating characteristic curves of diagnostic models including cardiac biomarkers alone, clinical characteristics alone and cardiac biomarkers in combination with clinical characteristics for left ventricular dysfunction in childhood cancer survivors. Abbreviations: LVEF=left ventricular ejection fraction, NT-proBNP=N-terminal pro B-type natriuretic peptide, Hs-cTnT=high sensitive cardiac troponin T.

Secondary analysis in moderate and high risk CCS

As the recently updated cardiomyopathy surveillance guideline[5] no longer recommends echocardiographic surveillance in low-risk CCS who were treated with a low anthracycline dose (<100mg/m2) and/or a low chest-directed radiotherapy dose (<15 Gray), we also tested the performance of the diagnostic model after excluding low-risk CCS. The results of this secondary analysis are presented in Table S6. In moderate- and high-risk CCS (n=1028), the model was still able to rule-out a LVEF<50% in 13.1% of these CCS with high NPV (97.4%, 95% CI 94.7-100%) and high sensitivity (96.9%, 95% CI 91.4-100%). A LVEF <45% could be ruled out in 41.0% of these moderate and high risk CCS with high NPV (99.2, 95% CI 98.3-100%) and high sensitivity of 92.3%, with a wide 95% CI (80.5-100%).

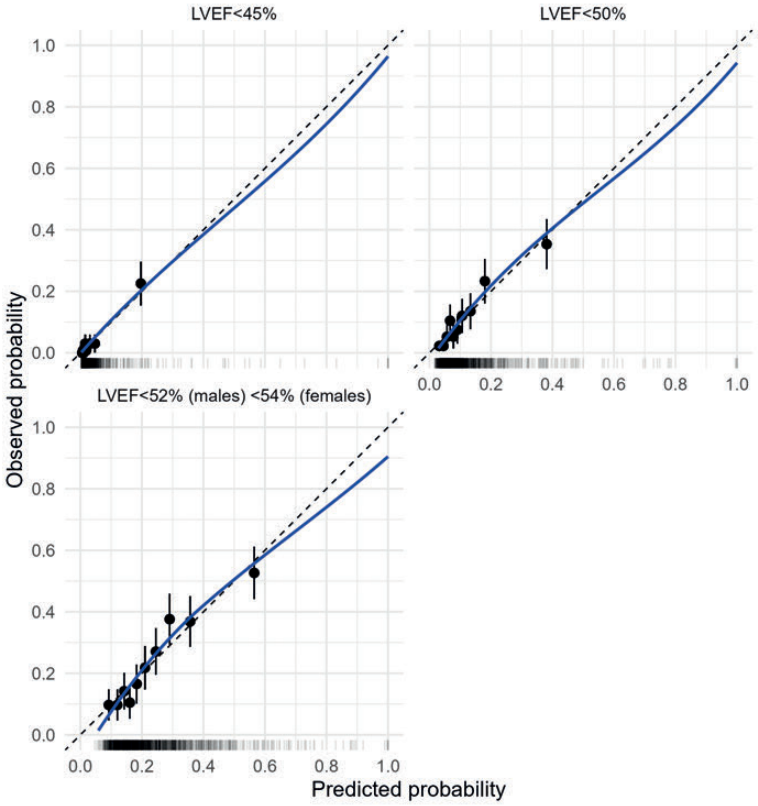

Figure 2. Calibration plots of diagnostic models including cardiac biomarkers and clinical characteristics for left ventricular dysfunction in childhood cancer survivors. Abbreviations: LVEF=left ventricular ejection fraction.

DISCUSSION

In this multicenter cohort study, we developed and internally validated diagnostic models for three categories of LV dysfunction using clinical characteristics in combination with cardiac biomarkers (NT-proBNP and Hs-cTnT) in adult survivors of childhood cancer. We demonstrate for the first time that cardiac biomarkers may be useful for the diagnosis of LV dysfunction when combined with clinical characteristics. If our findings can be validated in an independent cohort, this combined diagnostic model may be clinically useful for ruling-out LVEF<50% in 18.5% of survivors in whom an echocardiogram can potentially be deferred.

Diagnostic accuracy of NT-proBNP alone for LV dysfunction was low, despite the finding that 22% of CCS treated with cardiotoxic therapies had an age- and sex-defined abnormal NT-proBNP level at the age of 34 years compared to 5% siblings, which is in line with a previous report from the St Jude Life cohort in CCS of similar age.[7] This prevalence is high when also considering the association of abnormal NT-proBNP levels with cardiac mortality in CCS[7] and in the general population.[20] We confirm the results of previous studies in CCS[6,7] and the general population[9], which

showed that natriuretic peptides as single predictors have a too limited diagnostic accuracy for rule-in or rule-out of LV dysfunction on an echocardiogram, defined as a LVEF <50% to <55% and/or a fractional shortening <28% to <30%. We extend these findings in CCS by showing that higher or lower NT-proBNP cutoff concentrations are also not useful for rule-in or rule-out of LV dysfunction.

The finding of a higher prevalence of abnormal troponin T values (5.8%) than previously reported (0.6%) in CCS of similar age in the St Jude Life cohort [7] can be explained by the use of a high sensitive assay in our study, enabling a higher precision at troponin T concentrations around the cut-off level of 10 ng/L.[21] Still, abnormal Hs-cTnT concentrations were uncommon in CCS and not different from siblings. While the diagnostic value of Hs-cTnT for LV dysfunction was very limited in univariate analysis at any cutoff concentration corroborating previous reports[6,7,22], Hs-cTnT was significantly associated with LV dysfunction in the multivariable diagnostic model and improved its diagnostic performance.

The multivariable diagnostic model that combined cardiac biomarkers with clinical characteristics to estimate the probability of LV dysfunction in CCS was mainly useful for rule-out of LV dysfunction, and performed better for more severely abnormal LV function similar to what has been demonstrated for NT-proBNP in the general population.[9] While the diagnostic model was not able to accurately rule-out LVEF<54% (females) or LVEF<52% (males), we showed that it was useful to rule-out LVEF<50% in 18.5% of CCS. We expected that the model would be even better for ruling-out LVEF<45%, with a NPV of 99.2%. However, sensitivity (88.4%) was too limited for rule-out. The low number of events (3.4%), the wide confidence interval (75.6-97.8%) and the relatively large bootstrap optimism of 3.1% in the internal validation indicate that the model for LVEF<45% was underpowered. Future studies including more survivors with LVEF<45% are needed to clarify this issue.

As for clinical use, the diagnostic model may be used as a triage test before conducting a surveillance echocardiogram to reduce the burden to both CCS and echocardiography laboratories and to potentially reduce cardiomyopathy surveillance costs. If our results can be validated in an external cohort, it could impact current cardiomyopathy surveillance practices, as an echocardiogram can be safely deferred in 18.5% of CCS. Importantly, the recently updated IGHG cardiomyopathy surveillance guideline no longer recommends surveillance in low-risk survivors treated with an anthracycline dose <100mg/m^2 and/or a chest-directed radiotherapy dose <15 Gray.[5] We showed in a secondary analysis that the diagnostic model also performs well with respect to rule-out of LV dysfunction after excluding the low-risk group. For rule-out of LVEF<50%, it resulted in somewhat fewer patients in whom an echocardiogram may be deferred (13.1% instead of 18.5%).

Strengths and limitations

The present study was prospectively designed, included a large number of CCS, with reliable LVEF measurements from a core lab, and was conducted in multiple centers in the Netherlands, improving the generalizability of the results. Limitations of the study are also present. First, 17% of CCS had a missing biplane LVEF. We used multiple imputation to mitigate potential bias arising from these missing values and compared the results to a complete case analysis which gave comparable results. Second, the number of survivors with LVEF<45% was low (3.4%) which may have caused overfitting

of the diagnostic model for LVEF<45% as described above. Third, although we internally validated our results using bootstrapping techniques, our findings should still be externally validated. Finally, we recognize that there may be an interest in the diagnostic accuracy of cardiac biomarkers for abnormalities in global longitudinal strain, diastolic dysfunction and/or valve dysfunction. However, the main reason to concentrate on detecting LVEF is that at present only LVEF impacts on the decision to initiate heart failure treatment in asymptomatic patients.[23]

CONCLUSIONS

This study demonstrates that a diagnostic model including cardiac biomarkers and clinical characteristics is superior to using cardiac biomarkers only or clinical characteristic only and may be clinically useful to triage survivors for echocardiography by ruling-out LVEF<50% in 18.5% of CCS.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Mulrooney DA, Hyun G, Ness KK, et al. Major cardiac events for adult survivors of childhood cancer diagnosed between 1970 and 1999: report from the Childhood Cancer Survivor Study cohort. *BMJ (Clinical research ed)*. Jan 15 2020;368:l6794. doi:10.1136/bmj.l6794
2. van der Pal HJ, van Dalen EC, van Delden E, et al. High risk of symptomatic cardiac events in childhood cancer survivors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. May 1 2012;30(13):1429-37. doi:10.1200/JCO.2010.33.4730
3. Feijen E, Font-Gonzalez A, Van der Pal HJH, et al. Risk and Temporal Changes of Heart Failure Among 5-Year Childhood Cancer Survivors: a DCOG-LATER Study. *Journal of the American Heart Association*. Jan 8 2019;8(1):e009122. doi:10.1161/jaha.118.009122
4. Armenian SH, Hudson MM, Mulder RL, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. *The Lancet Oncology*. Mar 2015;16(3):e123-36. doi:10.1016/s1470-2045(14)70409-7
5. Ehrhardt MJ, Leerink JM, Mulder RL, et al. Systematic review and updated recommendations for cardiomyopathy surveillance for survivors of childhood, adolescent, and young adult cancer from the International Late Effects of Childhood Cancer Guideline Harmonization Group. *The Lancet Oncology*. 2023; doi:10.1016/S1470-2045(23)00012-8
6. Leerink JM, Verkleij SJ, Feijen EAM, et al. Biomarkers to diagnose ventricular dysfunction in childhood cancer survivors: a systematic review. *Heart (British Cardiac Society)*. 2019;105(3):210. doi:10.1136/heartjnl-2018-313634
7. Dixon SB, Howell CR, Lu L, et al. Cardiac biomarkers and association with subsequent cardiomyopathy and mortality among adult survivors of childhood cancer: A report from the St. Jude Lifetime Cohort. *Cancer*. 2021;127(3):458-466. doi:https://doi.org/10.1002/cncr.33292
8. Lee KK, Doudesis D, Anwar M, et al. Development and validation of a decision support tool for the diagnosis of acute heart failure: systematic review, meta-analysis, and modelling study. *BMJ (Clinical research ed)*. 2022;377:e068424. doi:10.1136/bmj-2021-068424
9. Costello-Boerrigter LC, Boerrigter G, Redfield MM, et al. Amino-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide in the general community: determinants and detection of left ventricular dysfunction. *Journal of the American College of Cardiology*. Jan 17 2006;47(2):345-53. doi:10.1016/j.jacc.2005.09.025
10. Leerink JM, Feijen E, van der Pal HJH, et al. Diagnostic tools for early detection of cardiac dysfunction in childhood cancer survivors: Methodological aspects of the Dutch late effects after childhood cancer (LATER) cardiology study. *Am Heart J*. Jan 2020;219:89-98. doi:10.1016/j.ahj.2019.10.010
11. Feijen EAM, Leisenring WM, Stratton KL, et al. Derivation of Anthracycline and Anthraquinone Equivalence Ratios to Doxorubicin for Late-Onset Cardiotoxicity. *JAMA oncology*. 2019;5(6):864-871. doi:10.1001/jamaoncol.2018.6634
12. Fradley MG, Larson MG, Cheng S, et al. Reference limits for N-terminal-pro-B-type natriuretic peptide in healthy individuals (from the Framingham Heart Study). *The American journal of cardiology*. Nov 01 2011;108(9):1341-5. doi:10.1016/j.amjcard.2011.06.057
13. Inker LA, Eneanya ND, Coresh J, et al. New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race. *The New England journal of medicine*. Nov 4 2021;385(19):1737-1749. doi:10.1056/NEJMoa2102953
14. Merkx R, Leerink JM, Feijen E, et al. Echocardiography protocol for early detection of cardiac dysfunction in childhood cancer survivors in the multicenter DCCSS LATER 2 CARD study: Design, feasibility, and reproducibility. *Echocardiography*. May 20 2021; doi:10.1111/echo.15081
15. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults:

An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *European Heart Journal - Cardiovascular Imaging*. 2015;16(3):233-271. doi:10.1093/ehjci/jev014

16. Buuren Sv. Flexible imputation of missing data. 2018;
17. Januzzi JL, van Kimmenade R, Lainchbury J, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study. *European heart journal*. Feb 2006;27(3):330-7. doi:10.1093/eurheartj/ehi631
18. Harrell F. *REGRESSION MODELING STRATEGIES : with applications to linear models, logistic and ordinal ... regression, and survival analysis*. SPRINGER; 2016.
19. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. *Med Decis Making*. Nov-Dec 2006;26(6):565-74. doi:10.1177/0272989x06295361
20. Dietl A, Stark K, Zimmermann ME, et al. NT-proBNP Predicts Cardiovascular Death in the General Population Independent of Left Ventricular Mass and Function: Insights from a Large Population-Based Study with Long-Term Follow-Up. *PloS one*. 2016;11(10):e0164060. doi:10.1371/journal.pone.0164060
21. Apple FS, Collinson PO. Analytical characteristics of high-sensitivity cardiac troponin assays. *Clinical chemistry*. Jan 2012;58(1):54-61. doi:10.1373/clinchem.2011.165795
22. Slieker MG, Fackoury C, Slorach C, et al. Echocardiographic Assessment of Cardiac Function in Pediatric Survivors of Anthracycline-Treated Childhood Cancer. *Circ Cardiovasc Imaging*. Dec 2019;12(12):e008869. doi:10.1161/circimaging.119.008869
23. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *European journal of heart failure*. Aug 2016;18(8):891-975. doi:10.1002/ejhf.592

SUPPLEMENTAL METHODS

Chest-directed radiotherapy dose calculation

Based on the available information on the radiotherapy field(s) (location) from the letter of the pediatric radiation oncologist, each treatment was assigned to one or more body compartments, including head, neck, spine, thorax, abdominopelvic, upper- and lower extremities. Total body irradiation (TBI) was considered separately. Validation of radiotherapy data was performed by experts in radiotherapy. We calculated the total maximum prescribed dose as the maximum dose to the smallest field, consisting of the sum of the full-field dose (primary) and the boost dose. Furthermore, all our calculations include radiotherapy doses for both the primary tumor and any recurrences. If the same body part was re-irradiated the respective doses were summed to derive the maximum dose to the smallest field. In case the recurrence treatment was given as a non-overlapping field in the same body part (e.g., for primary tumor and recurrences or metastases both in the lungs for example), the dose to the field with the highest dose was assigned as body compartment dose for our study. In DCCSS LATER 2 CARD we focused on thorax, spine and abdominopelvic fields and TBI as they possibly involve the heart region. Specific fields exposing the body compartments are shown in the table below. In collaboration with MD Anderson Cancer Center (University of Texas, United States) and Institut Gustave Roussy (Villejuif, France) we estimated the mean dose received by the whole heart after total spine or abdominopelvic radiotherapy by using radiation dose reconstruction methods [1,2]. Based on a subset of 110 survivors, we derived percentages of dose received by the whole heart, by dividing the total prescribed dose and the estimated mean whole heart dose. As a result, we used 55% of the maximum prescribed spine dose and 10% of the maximum prescribed abdominopelvic dose to estimate the dose received by the whole heart. Furthermore, we used 100% of the maximum prescribed thorax dose to estimate the dose received by the whole heart. If more than one of abovementioned body compartments were irradiated, the highest dose was assigned as the dose received on the heart region. Finally, we added 100% of the total prescribed TBI dose estimate to the final radiotherapy dose on the heart region.

Uniform radiotherapy body compartment classification system

RT body compartments	Childhood cancer-specific treatment fields
Spine	Craniospinal
	Total spine
	Spine, thoracic region
	Spine, lumbar region
	Spine, sacral region
	Spine, not otherwise specified
Thorax	Thorax
	Mantle field
	Mantle field without mediastinal
	Scapula left
	Scapula right
	Scapula both sides
	Scapula, side unknown
	Ribs, sternum, clavicle
	Mediastinal
	Parasternal
	Axilla
	Supraclavicular
Abdominopelvic	Abdominal
	Liver
	Spleen
	Paraaortic field
	Paraaortic field plus spleen
	Inverted-Y field
	Inverted-Y field plus spleen
	Pelvis (including iliacal field)
	Parailliacal field
	Inguinal field

SUPPLEMENTAL FIGURES

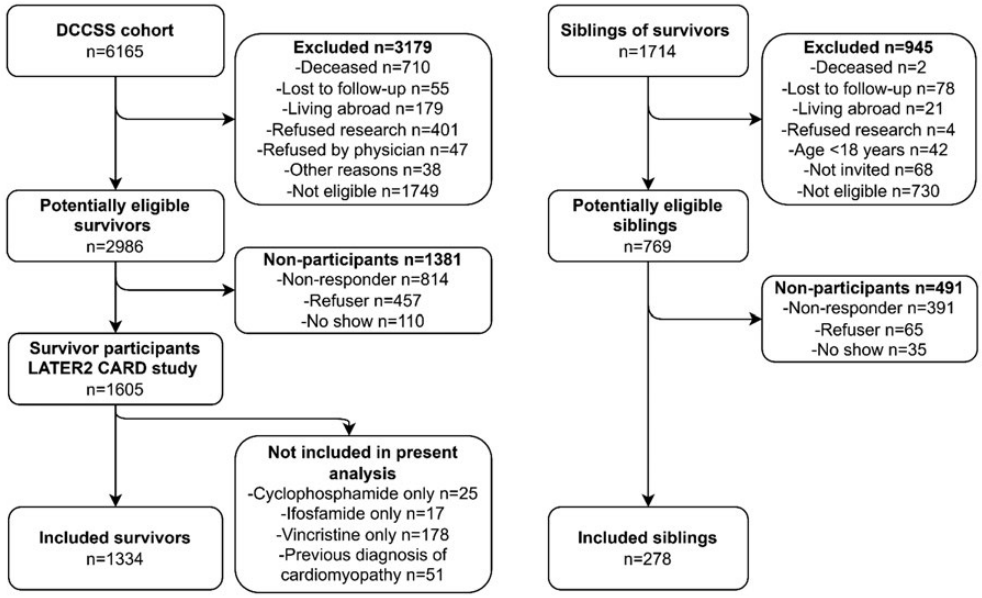

Figure S1. Patient inclusion flowchart.

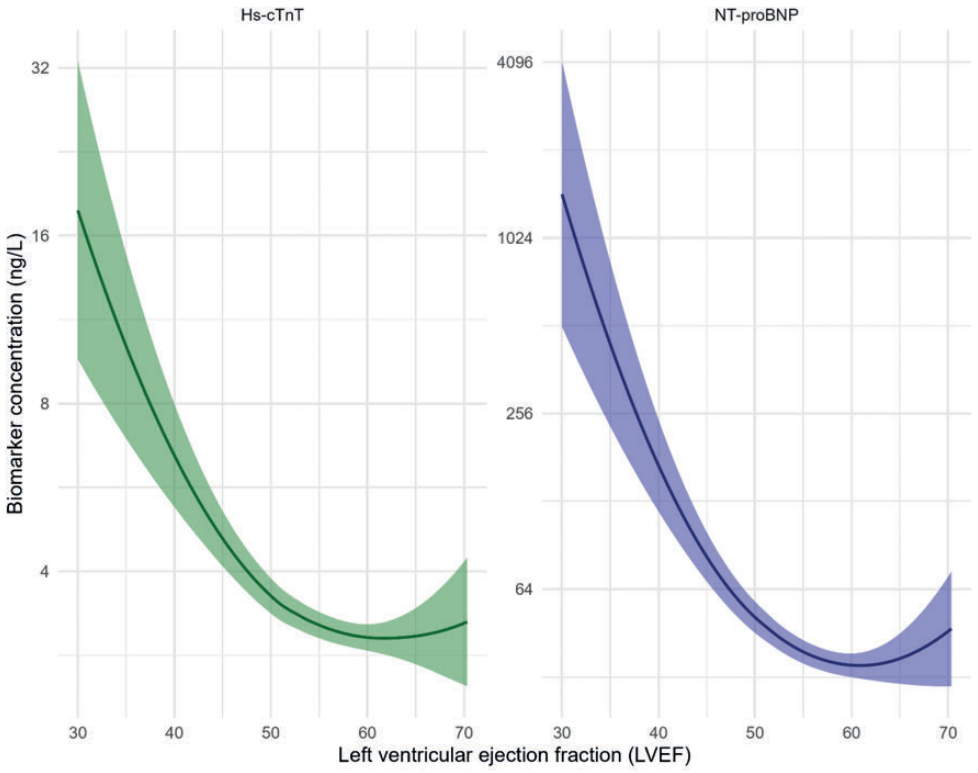

Figure S2. Local polynomial regression lines showing the association of cardiac biomarker levels with left ventricular ejection fraction (LVEF) in childhood cancer survivors treated with cardiotoxic cancer treatments. Abbreviations: Hs-cTnT=high sensitive cardiac troponin T, NTproBNP=N-terminal pro B-type natriuretic peptide.

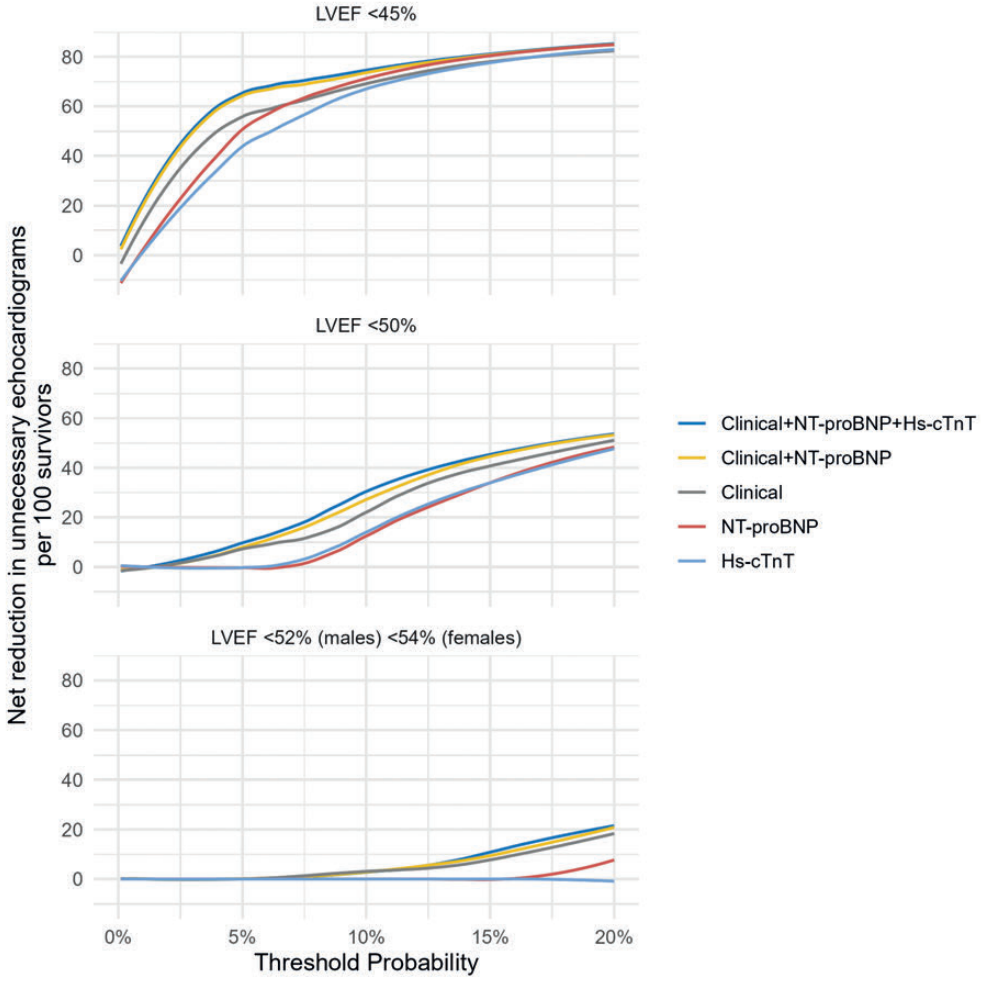

Figure S3. Decision curve analysis of diagnostic models including cardiac biomarkers alone, clinical characteristics alone and cardiac biomarkers in combination with clinical characteristics for left ventricular dysfunction in childhood cancer survivors. The net reduction in unnecessary echocardiograms per 100 survivors is shown (y-axis) at a range of threshold probabilities for left ventricular dysfunction (x axis). Abbreviations: LVEF=left ventricular ejection fraction, NT-proBNP=N-terminal pro B-type natriuretic peptide, Hs-cTnT=high sensitive cardiac troponin T.

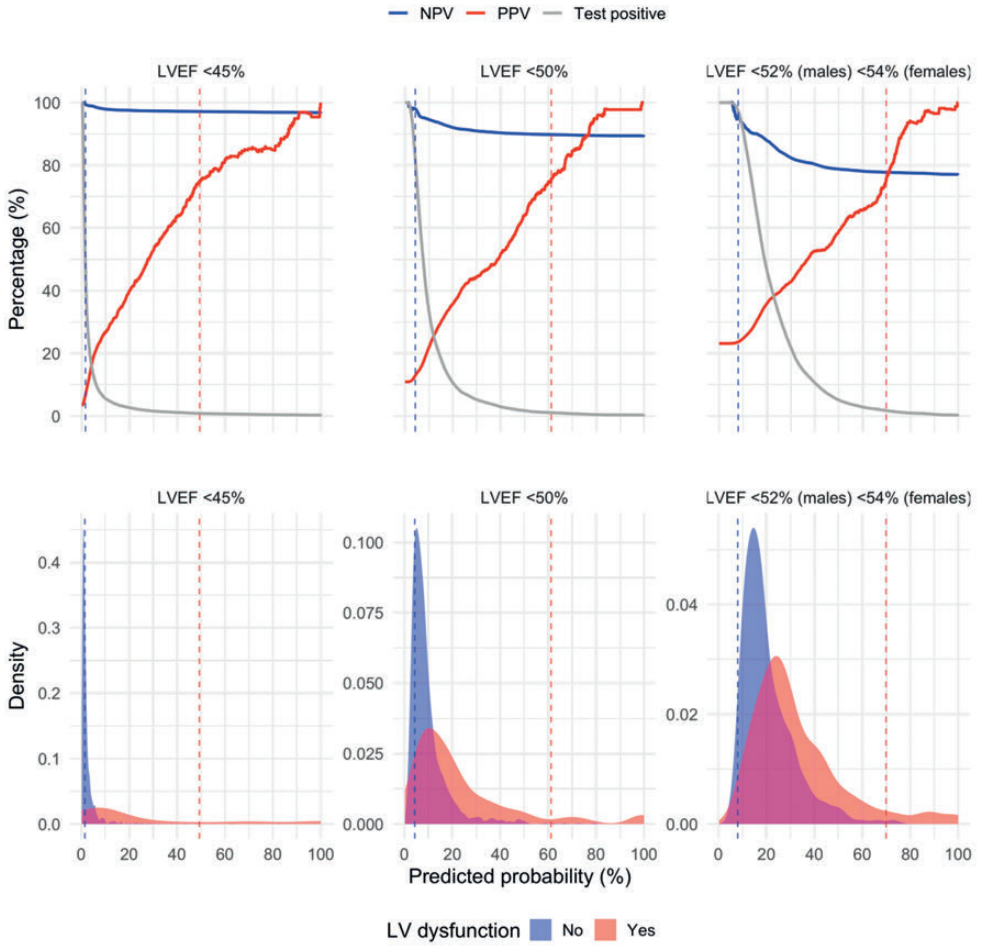

Figure S4. Performance of the diagnostic model including cardiac biomarkers and clinical characteristics for detecting left ventricular (LV) dysfunction in childhood cancer survivors. Top: negative predictive value (NPV), positive predictive value (PPV) and proportion test positive (y-axis) by predicted probability of LV dysfunction (x-axis). The blue vertical dashed line indicates the threshold for rule-out (NPV ³98% and sensitivity ³90%). The red vertical dashed line indicates the threshold for rule-in (PPV ³75% and specificity ³90%). Bottom: density plot of predicted probability of LV dysfunction.

SUPPLEMENTAL TABLES

Table S1. Abnormal NT-proBNP cut-points defined by 97.5th percentile limit of normal by age and sex (from the Framingham Heart Study[4]) Age Group (Years).

Age (years)	Males (97.5th percentile, ng/L)	Females (97.5th percentile, ng/L)
20-24	42.5	111.0
25-29	48.5	122.1
30-34	55.3	134.3
35-39	63.0	147.6
40-44	71.8	162.4
45-49	81.9	178.5
50-54	93.3	196.3
55-59	106.4	215.9

Table S2. Characteristics of participants compared to non-participants.

Characteristics	Participants (n=1605)	%	Non-participants (n=1381)	%
Sex				
Male	832	52%	842	61%
Female	773	48%	539	39%
Transgender	0	0%	0	0%
Missing	0	0%	0	0%
Primary childhood cancer				
Leukemias, myeloproliferative diseases and myelodysplastic diseases	678	42%	588	43%
Lymphomas and reticulo-endothelial neoplasms	375	23%	340	25%
Central nervous system and miscellaneous intracranial and intraspinal neoplasms	54	3%	67	5%
Neuroblastoma and other peripheral nervous cell tumors	54	3%	42	3%
Retinoblastoma	1	0%	0	0%
Renal tumors	189	12%	117	8%
Hepatic tumors	12	1%	22	2%
Bone tumors	125	8%	107	8%
Soft tissue and other extraosseous sarcomas	79	5%	70	5%
Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	31	2%	21	2%
Other malignant epithelial neoplasms and malignant melanomas	6	0%	6	0%
Other and unspecified malignant neoplasms	1	0%	1	0%
Missing	0		0	
Age at diagnosis, years				
0-4	693	43%	578	42%
5-9	457	28%	380	28%
10-14	358	22%	323	23%
15-17	97	6%	97	7%
Missing	0		3	

Table S2. (continued)

Characteristics	Participants (n=1605)	%	Non-participants (n=1381)	%
Treatment period				
1963-1969	16	1%	8	1%
1970-1979	196	12%	153	11%
1980-1989	488	30%	411	30%
1990-1999	714	44%	666	48%
2000-2001	191	12%	143	10%
Missing	0		0	
Years since cancer diagnosis at invitation				
10-19	357	22%	292	21%
20-29	652	41%	605	44%
30-39	462	29%	386	28%
>40	134	8%	98	7%
Missing	0		0	
Therapy				
Chemotherapy only	930	58%	837	61%
Radiotherapy only	32	2%	47	3%
Chemotherapy and radiotherapy	642	40%	497	36%
Missing	1		0	

Table S3. Diagnostic accuracy of NT-proBNP, Hs-cTnT for a range of cutoff concentrations to detect left ventricular dysfunction in childhood cancer survivors treated with cardiotoxic cancer treatments.

Biomarker	Cutoff	Abnormal test	Sensitivity	Specificity	NPV	PPV
LVEF <52% (males) <54% (females)						
NT-proBNP	10 ng/L	87.4%	92.1%	14.0%	85.5%	24.4%
NT-proBNP	20 ng/L	76.4%	84.5%	26.0%	84.8%	25.6%
NT-proBNP	30 ng/L	65.0%	76.8%	38.6%	84.6%	27.4%
NT-proBNP	40 ng/L	55.9%	68.8%	47.9%	83.6%	28.5%
NT-proBNP	50 ng/L	48.8%	62.4%	55.3%	83.0%	29.6%
NT-proBNP	60 ng/L	37.0%	49.4%	66.7%	81.4%	30.9%
NT-proBNP	70 ng/L	32.5%	44.9%	71.2%	81.1%	32.0%
NT-proBNP	80 ng/L	28.7%	41.2%	75.1%	80.9%	33.2%
NT-proBNP	90 ng/L	24.3%	37.1%	79.5%	80.7%	35.2%
NT-proBNP	100 ng/L	22.4%	36.0%	81.7%	80.9%	37.3%
NT-proBNP	110 ng/L	18.9%	32.5%	85.1%	80.7%	39.7%
NT-proBNP	120 ng/L	17.2%	29.9%	86.7%	80.4%	40.3%
NT-proBNP	130 ng/L	15.8%	28.6%	88.1%	80.4%	42.1%
NT-proBNP	140 ng/L	14.8%	27.1%	88.9%	80.2%	42.4%
NT-proBNP	150 ng/L	13.3%	25.5%	90.4%	80.1%	44.5%
NT-proBNP	300 ng/L	3.9%	9.7%	97.9%	78.3%	58.3%
NT-proBNP	400 ng/L	2.4%	6.6%	98.9%	77.8%	63.6%
NT-proBNP	500 ng/L	1.9%	6.2%	99.4%	77.9%	74.4%
NT-proBNP	600 ng/L	1.4%	5.1%	99.7%	77.7%	84.6%

Table S3. (continued)

Biomarker	Cutoff	Abnormal test	Sensitivity	Specificity	NPV	PPV
Hs-cTnT	3 ng/L	59.4%	62.5%	41.5%	78.6%	24.4%
Hs-cTnT	4 ng/L	50.4%	54.9%	51.0%	79.0%	25.2%
Hs-cTnT	5 ng/L	33.5%	39.2%	68.3%	78.9%	27.2%
Hs-cTnT	6 ng/L	22.6%	30.3%	79.7%	79.1%	31.0%
Hs-cTnT	7 ng/L	15.5%	22.5%	86.6%	78.8%	33.6%
Hs-cTnT	8 ng/L	10.6%	17.5%	91.5%	78.6%	38.2%
Hs-cTnT	9 ng/L	7.6%	13.3%	94.1%	78.3%	40.2%
Hs-cTnT	10 ng/L	5.9%	10.5%	95.4%	78.0%	40.7%
Hs-cTnT	11 ng/L	4.3%	7.6%	96.7%	77.6%	40.8%
Hs-cTnT	12 ng/L	3.4%	6.3%	97.5%	77.6%	43.3%
Hs-cTnT	13 ng/L	2.9%	6.0%	98.0%	77.6%	47.2%
Hs-cTnT	14 ng/L	2.4%	4.6%	98.3%	77.4%	45.1%
LVEF<50%	**Cutoff**	**Abnormal test**	**Sensitivity**	**Specificity**	**NPV**	**PPV**
Nt-proBNP	10 ng/L	87.4%	90.8%	13.0%	92.0%	11.3%
Nt-proBNP	20 ng/L	76.4%	84.7%	24.6%	92.9%	12.1%
Nt-proBNP	30 ng/L	65.0%	77.3%	36.5%	92.9%	13.0%
Nt-proBNP	40 ng/L	55.9%	69.9%	45.8%	92.5%	13.6%
Nt-proBNP	50 ng/L	48.8%	65.3%	53.3%	92.6%	14.6%
Nt-proBNP	60 ng/L	37.0%	54.4%	65.1%	92.1%	16.1%
Nt-proBNP	70 ng/L	32.5%	49.5%	69.6%	91.8%	16.6%
Nt-proBNP	80 ng/L	28.7%	46.9%	73.6%	91.9%	17.8%
Nt-proBNP	90 ng/L	24.3%	42.5%	77.9%	91.7%	19.1%
Nt-proBNP	100 ng/L	22.4%	41.5%	79.9%	91.8%	20.2%
Nt-proBNP	110 ng/L	18.9%	36.9%	83.3%	91.5%	21.3%
Nt-proBNP	120 ng/L	17.2%	34.2%	84.9%	91.3%	21.8%
Nt-proBNP	130 ng/L	15.8%	31.9%	86.2%	91.2%	22.1%
Nt-proBNP	140 ng/L	14.8%	28.8%	87.0%	90.9%	21.3%
Nt-proBNP	150 ng/L	13.3%	27.1%	88.4%	90.8%	22.2%
NT-proBNP	300 ng/L	3.9%	12.4%	97.2%	90.0%	34.9%
NT-proBNP	400 ng/L	2.4%	10.4%	98.6%	90.0%	47.3%
NT-proBNP	500 ng/L	1.9%	9.6%	99.0%	89.9%	54.4%
NT-proBNP	600 ng/L	1.4%	8.8%	99.5%	89.9%	69.4%
Hs-cTnT	3 ng/L	59.4%	76.0%	42.6%	93.6%	14.0%
Hs-cTnT	4 ng/L	50.4%	67.8%	51.7%	92.9%	14.7%
Hs-cTnT	5 ng/L	33.5%	51.0%	68.7%	92.0%	16.6%
Hs-cTnT	6 ng/L	22.6%	41.8%	79.7%	91.8%	20.1%
Hs-cTnT	7 ng/L	15.5%	31.9%	86.5%	91.2%	22.5%
Hs-cTnT	8 ng/L	10.6%	25.3%	91.2%	90.9%	25.9%
Hs-cTnT	9 ng/L	7.6%	19.0%	93.8%	90.4%	27.2%
Hs-cTnT	10 ng/L	5.9%	15.6%	95.2%	90.2%	28.7%
Hs-cTnT	11 ng/L	4.3%	12.0%	96.6%	90.0%	30.1%
Hs-cTnT	12 ng/L	3.4%	10.8%	97.5%	89.9%	34.6%
Hs-cTnT	13 ng/L	2.9%	10.0%	97.9%	89.9%	37.2%
Hs-cTnT	14 ng/L	2.4%	7.9%	98.3%	89.7%	36.3%
LVEF <45%	**Cutoff**	**Abnormal test**	**Sensitivity**	**Specificity**	**NPV**	**PPV**
NT-proBNP	10 ng/L	87.4%	92.4%	12.7%	97.9%	3.6%

Table S3. (continued)

Biomarker	Cutoff	Abnormal test	Sensitivity	Specificity	NPV	PPV
NT-proBNP	20 ng/L	76.4%	91.2%	24.1%	98.7%	4.1%
NT-proBNP	30 ng/L	65.0%	87.8%	35.8%	98.8%	4.6%
NT-proBNP	40 ng/L	55.9%	83.1%	45.0%	98.7%	5.1%
NT-proBNP	50 ng/L	48.8%	81.9%	52.4%	98.8%	5.8%
NT-proBNP	60 ng/L	37.0%	67.3%	64.1%	98.2%	6.2%
NT-proBNP	70 ng/L	32.5%	65.4%	68.7%	98.2%	6.9%
NT-proBNP	80 ng/L	28.7%	64.9%	72.6%	98.3%	7.8%
NT-proBNP	90 ng/L	24.3%	62.3%	77.0%	98.3%	8.8%
NT-proBNP	100 ng/L	22.4%	61.9%	79.0%	98.3%	9.5%
NT-proBNP	110 ng/L	18.9%	61.4%	82.6%	98.4%	11.1%
NT-proBNP	120 ng/L	17.2%	58.6%	84.3%	98.3%	11.7%
NT-proBNP	130 ng/L	15.8%	56.0%	85.7%	98.2%	12.2%
NT-proBNP	140 ng/L	14.8%	51.2%	86.5%	98.0%	11.9%
NT-proBNP	150 ng/L	13.3%	50.5%	88.0%	98.0%	13.0%
NT-proBNP	300 ng/L	3.9%	29.9%	97.1%	97.5%	26.5%
NT-proBNP	400 ng/L	2.4%	25.1%	98.4%	97.4%	35.7%
NT-proBNP	500 ng/L	1.9%	24.8%	98.9%	97.4%	44.1%
NT-proBNP	600 ng/L	1.4%	22.4%	99.4%	97.3%	55.6%
Hs-cTnT	3 ng/L	59.4%	88.4%	41.6%	99.0%	5.1%
Hs-cTnT	4 ng/L	50.4%	83.4%	50.8%	98.9%	5.7%
Hs-cTnT	5 ng/L	33.5%	67.5%	67.7%	98.3%	6.9%
Hs-cTnT	6 ng/L	22.6%	61.1%	78.7%	98.3%	9.3%
Hs-cTnT	7 ng/L	15.5%	47.8%	85.7%	97.9%	10.6%
Hs-cTnT	8 ng/L	10.6%	37.4%	90.3%	97.6%	12.1%
Hs-cTnT	9 ng/L	7.6%	33.7%	93.3%	97.5%	15.1%
Hs-cTnT	10 ng/L	5.9%	24.5%	94.7%	97.2%	14.1%
Hs-cTnT	11 ng/L	4.3%	17.5%	96.1%	97.0%	13.8%
Hs-cTnT	12 ng/L	3.4%	16.3%	97.1%	97.0%	16.4%
Hs-cTnT	13 ng/L	2.9%	13.8%	97.5%	96.9%	16.1%
Hs-cTnT	14 ng/L	2.4%	13.8%	98.0%	97.0%	19.8%

Hs-cTnT=High sensitive cardiac troponin T, LVEF=left ventricular ejection fraction, NT-proBNP=N-terminal pro B-type natriuretic peptide B, NPV=negative predictive value, PPV=positive predictive value.

Table S4. Coefficients of clinical characteristics and cardiac biomarkers included in the multivariable logistic regression model for left ventricular dysfunction.

	LVEF<52% (males) LVEF<54% (females)		LVEF<50%		LVEF<45%	
Factor	**Log OR**	**P-value**	**Log OR**	**P-value**	**Log OR**	**P-value**
Intercept	-4.095	<0.001	-5.131	<0.001	-7.607	<0.001
Female versus male sex	0.311	0.088	-0.532	0.033	-0.815	0.077
Age at diagnosis, per 1 year	-0.029	0.131	-0.025	0.356	-0.021	0.637
Age at study, per 1 year	-0.01	0.345	-0.011	0.434	0.008	0.761
Anthracycline dose, per 1 mg/m^2	0.002	<0.001	0.003	0.001	0.003	0.019
Mitoxantrone dose, per 1 mg/m^2	0.006	0.183	0.009	0.091	0.025	<0.001
Chest RT dose, per 1 Gray	0.017	0.029	0.014	0.13	0.007	0.66
Heart rate, per 1 bpm	0.032	<0.001	0.035	<0.001	0.036	0.008
NT-proBNP, per 1 ng/L	0.003	0.002	0.003	0.002	0.004	0.001
Hs-cTnT, per 1 ng/L	0.052	0.022	0.071	0.005	0.06	0.050

Abbreviations: BMI=body mass index, chest RT=chest-directed radiotherapy dose, CI=confidence interval, Hs-cTnT=high sensitive cardiac troponin T, NT-proBNP=N-terminal pro B-type natriuretic peptide B, OR=odds ratio.

Table S5. Complete case analysis: Diagnostic accuracy measures of cardiac biomarkers and clinical characteristics to detect LV dysfunction in childhood cancer survivors treated with cardiotoxic cancer treatments.

Predictor	C-statistic	Cutoff	Ruled out	Sensitivity	Specificity	PPV	NPV
LVEF <52% in males, <54% in females (prevalence 22.9%)							
Clinical+NT-proBNP+Hs-cTnT	0.71 (0.68-0.75)	8.0%[b]	3.6%	99.6 (98.7-99.6)	4.6 (3.2-6.1)	23.7 (23.4-24.1)	97.4 (91.4-100)
LVEF <50% (prevalence 10.8%)							
Clinical+NT-proBNP+Hs-cTnT	0.75 (0.70-0.80)	4.3%[b]	21.5%	95.5 (91.1-99.1)	23.6 (21.0-26.5)	13.3 (12.7-14.0)	97.8 (95.7-99.5)
LVEF <45% (prevalence 3.5%)							
Clinical+NT-proBNP+Hs-cTnT	0.86 (0.84-0.94)	1.5%[b]	63.5%	88.2 (76.5-97.1)	65.3 (62.4-68.4)	8.1 (7.0-9.1)	99.4 (98.8-99.9)

[a]Clinical characteristics: sex, age at diagnosis, age at study, anthracycline dose, mitoxantrone dose, chest-directed radiotherapy dose, heart rate.

[b]Cutoff probability of LV dysfunction from the multivariable logistic regression model.

95% confidence intervals are shown between brackets.

Abbreviations: Hs-cTnT=high sensitive cardiac troponin T, LV=left ventricular, NT-proBNP=N-terminal pro B-type natriuretic peptide B, NPV=negative predictive value, PPV=positive predictive value.

Table S6. Secondary analysis in moderate and high risk CCS (n=1028). Optimism adjusted diagnostic accuracy measures of the final diagnostic model including cardiac biomarkers (NT-proBNP and Hs-cTnT) and clinical characteristics[a].

Outcome	C-statistic	Cutoff	Ruled out	Sensitivity	Specificity	PPV	NPV
LVEF <52% in males, <54% in females (prevalence 23.8%)	0.71 (0.67-0.75)	8.4%[b]	2.3%	99.2 (97.6-100)	2.8 (0.1-8.8)	24.3 (23.3-25.5)	93.6 (80.5-100)
LVEF <50% (prevalence 12.1%)	0.72 (0.67-0.77)	4.5%[b]	13.1%	96.9 (91.4-100)	14.5 (9.6-20.8)	13.5 (12.5-14.3)	97.4 (94.7-100)
LVEF <45% (prevalence 4.1%)	0.84 (0.76-0.90)	1.5%[b]	41.0%	92.3 (80.5-100)	42.6 (30.0-53.6)	6.5 (4.9-7.7)	99.2 (98.3-100)

[a]Clinical characteristics: sex, age at diagnosis, age at study, anthracycline dose, mitoxantrone dose, chest-directed radiotherapy dose, heart rate.

[b]Cutoff probability of LV dysfunction from the multivariable logistic regression model.

95% confidence intervals are shown between brackets.

Abbreviations: Hs-cTnT=high sensitive cardiac troponin T, LVEF=left ventricular ejection fraction, NT-proBNP=N-terminal pro B-type natriuretic peptide B, NPV=negative predictive value, PPV=positive predictive value.

REFERENCES SUPPLEMENTAL MATERIAL

1. Howell RM, Smith SA, Weathers RE, Kry SF, Stovall M. Adaptations to a Generalized Radiation Dose Reconstruction Methodology for Use in Epidemiologic Studies: An Update from the MD Anderson Late Effect Group. *Radiat Res*. 2019;192:169-188. doi: 10.1667/rr15201.1
2. Alziar I, Bonniaud G, Couanet D, Ruaud JB, Vicente C, Giordana G, Ben-Harrath O, Diaz JC, Grandjean P, Kafrouni H, et al. Individual radiation therapy patient whole-body phantoms for peripheral dose evaluations: method and specific software. *Phys Med Biol*. 2009;54:N375-383. doi: 10.1088/0031-9155/54/17/n01
3. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. *Med Decis Making*. 2006;26:565-574. doi: 10.1177/0272989x06295361
4. Fradley MG, Larson MG, Cheng S, McCabe E, Coglianese E, Shah RV, Levy D, Vasan RS, Wang TJ. Reference limits for N-terminal-pro-B-type natriuretic peptide in healthy individuals (from the Framingham Heart Study). *The American journal of cardiology*. 2011;108:1341-1345. doi: 10.1016/j.amjcard.2011.06.057

7

EXTRACELLULAR MATRIX REMODELING IN ANIMAL MODELS OF ANTHRACYCLINE-INDUCED CARDIOMYOPATHY: A META-ANALYSIS

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ABSTRACT

As in other cardiomyopathies, extracellular matrix (ECM) remodeling plays an important role in anthracycline-induced cardiomyopathy. To understand the pattern and timing of ECM remodeling pathways we conducted a systematic review in which we describe protein and mRNA markers for ECM remodeling that are differentially expressed in the hearts of animals with anthracycline-induced cardiomyopathy. We included 68 studies in mice, rats, rabbits and pigs with follow-up of 0.1-8.2 human equivalent years after anthracycline administration. Using meta-analysis, we found 29 proteins and 11 mRNAs that were differentially expressed in anthracycline-induced cardiomyopathy compared to controls. Collagens, matrix metalloproteinases (MMPs), inflammation markers, transforming growth factor ß signaling markers and markers for cardiac hypertrophy were upregulated, whereas the protein kinase B (AKT) pro-survival pathway was downregulated. Their expression patterns over time from single time-point studies were studied with meta-regression using human equivalent years as the time scale. Connective tissue growth factor showed an early peak in expression but remained upregulated at all studied time-points. Brain natriuretic peptide (BNP) and MMP9 protein levels increased in studies with longer follow-up. Significant associations were found for higher atrial natriuretic peptide with interstitial fibrosis and for higher BNP and MMP2 protein levels with left ventricular systolic function.

INTRODUCTION

Anthracyclines are a class of chemotherapeutic agents used to treat various types of cancer and they have contributed to a significant improvement in survival of cancer patients.(1) However, anthracycline treatment is associated with left ventricular dysfunction and heart failure in a dose dependent manner and can occur up to decades after exposure.(2)

Understanding the harmful mechanisms leading to anthracycline-induced cardiomyopathy and their timing in the transition to heart failure is critical to develop strategies for early detection, prevention and treatment. While the exact mechanism remains unclear, multiple processes have been identified to be involved in anthracycline-induced cardiotoxicity.(3, 4) A major role is attributed to inhibition of topoisomerase 2 activity, a nuclear enzyme required for DNA transcription and replication, which is followed by the formation of reactive oxygen species, mitochondrial dysfunction and apoptosis of cardiomyocytes.(5)

As a central theme in cardiac remodeling and response to excess loading or injury, a hypertrophic response occurs within cardiomyocytes, but also in cardiac fibroblasts, which are ubiquitously present in the heart.(6) Excess loading conditions may trigger transforming growth factor ß (TGFß) induced fibroblast activation, which results in excessive production of extracellular matrix (ECM) components and ventricular dysfunction.(7) Irrespective of its cause, cardiomyocyte injury by itself triggers an inflammatory reaction, which also induces fibroblast activation.(6, 7, 8, 9). In anthracycline-induced cardiomyopathy both inflammatory and adverse ECM remodeling processes are present.(4, 10, 11, 12, 13, 14) Several transcriptomic analysis in animal and in vitro studies have reported differential expression in genes related to the innate immune system, TGFß signaling and collagen turnover.(10, 15, 16, 17, 18) Understanding the pathways and timing of ECM remodeling in anthracycline-induced cardiomyopathy is urgently needed to identify potential targets for treatment and blood markers that may be used during surveillance. In this systematic review we had 3 objectives: 1) to find which ECM remodeling markers are significantly upregulated or downregulated in the hearts of animals with anthracycline-induced cardiomyopathy compared to control animals; 2) to delineate possible temporal expression patterns of ECM remodeling markers, and 3) to find associations of ECM remodeling marker levels with interstitial fibrosis, left ventricular systolic function and/or apoptosis.

METHODS

This systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.(19) The protocol was registered in PROSPERO (ID: CRD42020161338), an international database of systematic review protocols.

Search strategy, eligibility criteria and risk of bias assessment

Pubmed and EMBASE were systematically searched for studies measuring markers for ECM remodeling in animals with anthracycline-induced cardiomyopathy (search strategy in Supplementary Material). The reference list of included articles was screened for additional studies. Two authors independently screened studies and included studies with in vivo administration of

anthracyclines and with analysis of ECM proteins or mRNAs in heart tissue after at least 2 doses of anthracyclines, as this more closely reflects the situation in humans. Excluded were studies without anthracycline exposure, in vitro studies, studies with another concomitant cardiotoxic intervention, conference abstracts, studies not describing ECM remodeling markers and studies written in other languages than English, French or German. Discrepancies between the reviewers were discussed and resolved in group. Risk of bias was assessed with the systematic review center for laboratory animal experimentation risk of bias checklist by two authors.(20)

Data extraction

Data was extracted with a predefined form. Data only available in graphs was extracted with Adobe Acrobat Pro. Extracted were the number of animals, age at start of anthracyclines, time of sampling after the first anthracyclines dose, the anthracycline derivative and the cumulative dose in mg/kg. The mean and standard deviation of ECM remodeling markers, left ventricular ejection fraction (LVEF) or fractional shortening (FS), interstitial fibrosis area and cardiomyocyte apoptosis were extracted. If the standard deviation was not reported, it was calculated from the standardized error of the meanusing the formula: standard deviation=standardized error of the mean*√(number of animals).

Age equivalency and follow-up comparison between animal species

To compare follow-up time after first anthracycline injection between animal species we converted animal age and follow-up duration to human equivalent years based on the maximum life span of each animal species obtained from the anAge longevity database (mice 4.0 years, rats 4.2 years, rabbits 9 years, pigs 27 years, humans 90 years) (https://genomics.senescence.info/species/). Separate analyses were performed for small animals (mice, rats, rabbits) and pigs due to the differences in body mass and life expectancy.

Objective 1: ECM remodeling markers in anthracycline-induced cardiomyopathy animals compared to control animals

We defined markers for ECM remodeling as proteins or mRNAs implicated in ECM remodeling as described by the authors of each study or as described in the literature. For every ECM remodeling marker described in each study, we calculated the ratio of the means (ROM) by dividing mean expression in cardiomyopathy animals by mean expression in control animals. A ROM >1 indicates a higher mean expression in anthracycline-induced cardiomyopathy whereas a ROM <1 indicates higher expression in controls.(21) We used a random effects meta-analysis to pool the ROM of each marker across studies and considered markers with a ROM>1.2 or <0.83 and a p-value <0.05 as significantly up- or downregulated, respectively. We used the Hartung and Knapp method to estimate p-values and 95% confidence intervals, as it has been shown to be more accurate in situations with moderate to substantial inter-study heterogeneity.(22) For presentation, we classified proteins and mRNAs into pathways as described in the literature and Reactome (reactome.org). Protein quantity (measured with Western blot) and gelatinase activity (measured with zymography) of MMP2 and

MMP9 were analyzed together to increase power since we did not observe differences in temporal trends when we analyzed them separately. mRNA expression of MMPs was measured in studies with quantitative polymerase chain reaction.

Objective 2: Temporal expression patterns of ECM remodeling markers

We used meta-regression to study temporal patterns in ECM marker levels after anthracycline injection. Meta-regression is a method to study the effect of an exploratory variable (time in our study) on the effect estimate of each study (ROM in our study).(21) In this analysis, we only included ECM markers measured at ≥5 unique time-points, either within the same study or in different studies that measured the same marker.

Objective 3: Association of ECM remodeling markers with fibrosis, LV function, and apoptosis

We also used meta-regression to study the association of ECM marker levels with 1) myocardial interstitial fibrosis area (quantified with the standardized mean difference (SMD)), 2) LV systolic function (LVEF or FS) quantified with the SMD) and 3) cardiomyocyte apoptosis detected with terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL, quantified with the SMD). The SMD represents the difference in means between cardiomyopathy and control animals divided by the standard deviation, which is useful when measurements are on a different scale.(21) Only markers with ≥5 measurements were analyzed. Meta-analysis and meta-regression were performed in R studio version 3.6.1 using the "metafor" package. A two sided p-value <0.05 was considered statistically significant. P-values were not corrected for multiple testing in this hypothesis generating study.

Assessment of inter-study heterogeneity

Heterogeneity was quantified with the I^2 statistic. An I^2 between 0–25% reflects very low heterogeneity, 25–50% reflects low heterogeneity, 50–75% reflects moderate heterogeneity; and an I^2 >75% reflects substantial heterogeneity.(21) As substantial heterogeneity was expected in animal studies and this review is hypothesis generating, we also describe the concordance in the direction of the effect (i.e., upregulation or downregulation in all studies).

RESULTS

Overview of included studies in the systematic review

Using the search terms indicated in the supplementary material, we identified 915 original studies by searching PubMed and Embase. In addition, we identified 10 studies from expert knowledge and by reviewing the references of the studies (Figure 1). After exclusion of 809 studies based on title and abstract, we screened the full text of 116 studies. Main exclusion reasons were a single anthracycline dose (n=19), in vitro experiments in cell lines (n=16) and no marker for ECM remodeling studied (n=8). We included in total 68 studies in mice (19 studies, 312 animals), rats (40 studies, 752 animals), rabbits (6 studies, 96 animals) and pigs (3 studies, 43 animals) in the systematic review. Study

characteristics are shown in Table S1 and are summarized in Table 1. The cumulative anthracycline dose ranged from 2.3 mg/kg to 40.0 mg/kg with a dose of 15.0 mg/kg most commonly used. Protein or mRNA levels were measured in heart tissue at 2 days up to 20 weeks after the first anthracycline injection corresponding to a human equivalent follow-up of 0.1 to 8.2 years. In the majority of

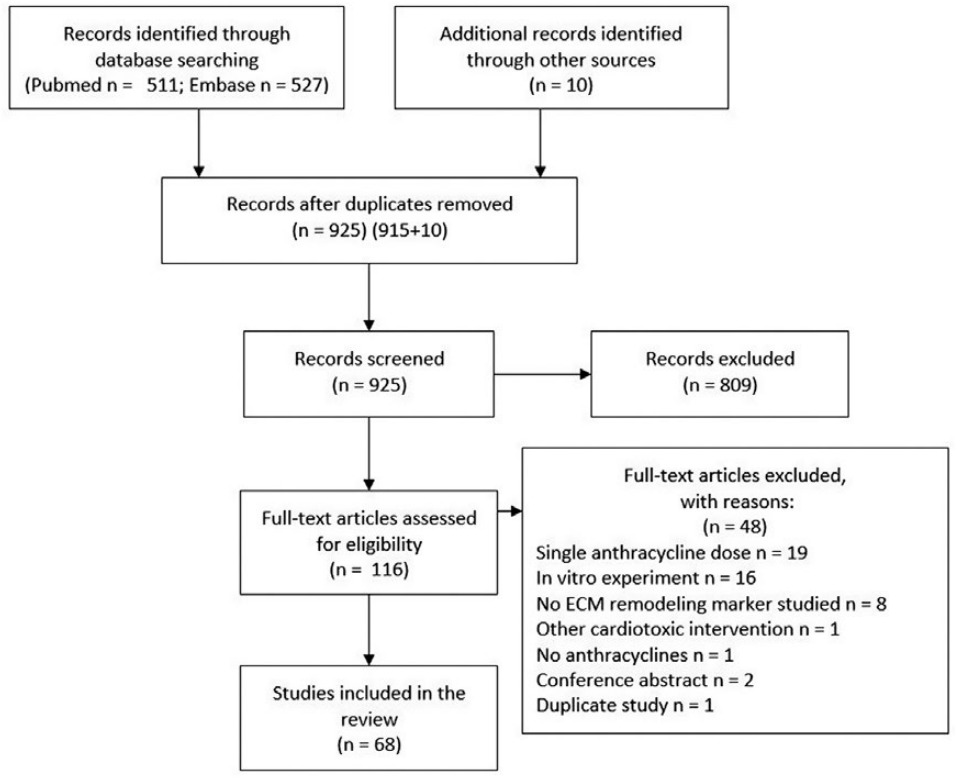


Figure 1. PRISMA flowchart of study inclusion in the systematic review. ECM=extracellular matrix.

Table 1. Summary of the included studies stratified by animal species (characteristics per study are shown in Table S1).

Characteristic	Mice 19 studies	Rats 40 studies	Rabbits 6 studies	Pigs 3 studies
Number of animals	312	752	96	43
Median anthracycline dose, mg/kg (IQR)	15.0 (12.0, 24.0)	15.0 (12.0, 17.1)	30.0 (21.0, 30.0)	2.9 (2.6, 3.5)
Median weeks after injection (range)	5.0 (1.0, 18.0)	4.0 (0.3, 20.0)	10.0 (8.0, 20.0)	8.6 (4.3, 16)
Human equivalent follow-up in years, median (range)	2.2 (0.4, 7.8)	1.6 (0.1, 8.2)	1.9 (1.5, 3.8)	0.5 (0.3, 1.0)
LVEF and/or FS measurement	14 studies	18 studies	3 studies	3 studies
Fibrosis area measurement	13 studies	21 studies	5 studies	1 studies
Proteins/mRNAs measured	Proteins n=44, mRNAs n=19			

Abbreviations: IQR=inter quartile range, LVEF=left ventricular ejection fraction, FS=fractional shortening.

studies, cardiomyopathy severity was assessed with cardiac function measurements (LVEF or FS). Histologic examination revealed interstitial fibrosis, cytoplasmic vacuolization and loss of myofibrils in most of the studies (Table S2).(23)

Objective 1: ECM remodeling marker expression in anthracycline-induced cardiomyopathy compared to control hearts

Results of the meta-analysis in 65 studies in mice, rats and rabbits are shown in Figure 2. We identified 30 proteins and 12 mRNAs that were significantly up- or downregulated in anthracycline-induced cardiomyopathy as compared to controls (random effect p-value <0.05 and ROM >1.20 or <0.83). We classified them in 6 pathways: 1) collagen synthesis, 2) matrix metalloproteinases, 3) transforming growth factor ß (TGFß) signaling, 4) protein kinase B (AKT) signaling, 5) immune system and 6) cardiac hypertrophy. Markers upregulated more than 3-fold were TGFß1 (ROM 3.80, n=13 studies), CTGF (ROM 4.04, n=6), SMAD3 (ROM 3.27, n=7), MMP2 mRNA (ROM 3.23, n=9), collagen I mRNA (ROM 3.22, n=7) and GAL3 (mRNA ROM 9.46, protein ROM 5.78, n=1) (Figure 2). Markers of interest that were upregulated less than 3-fold were MMP9 (ROM 1.94, n=13), MMP2 (ROM 1.50, n=19), TNFβ (ROM 2.88, n=6) and IL6 (ROM 2.46, n=5 studies, p=0.03).

Due to the limited number of studies in pigs (3 studies) we report results descriptively. Goetzenich et al. studied matrix metalloproteinases at a human equivalent follow-up of ±0.3 years in pigs (mean LVEF 38%) treated with 5-7 intracoronary doses of 25mg doxorubicin (±2.9 mg/kg).(24) MMP2 and MMP1 activity, measured with fluorogenic assay, were increased 5 to 8-fold compared to controls, respectively. mRNA levels of MMP1 (4.9-fold), MMP2 (5.4-fold), MMP9 (4.7-fold) and membrane type-1 MMP (3.2-fold) were also significantly increased. The mRNA expression of MMP3, TIMP1 and collagen 1 were not significantly altered in this study.(24)

Gyongyosi et al. performed RNA sequencing in pigs with less severe cardiomyopathy (mean LVEF 45%, cumulative doxorubicin dose 180 mg/m^2, ±4.1 mg/kg) and with a slightly longer follow up of ±0.6 human equivalent years. In transcriptomic analysis, they showed differential expression of genes in the TGFß signaling pathway, ECM genes (fibroblast activation markers osteonectin and tenascin-c), DNA damage genes, collagen synthesis genes and growth factors. TIMP2 mRNA expression was significantly downregulated but mRNA levels of MMPs were not different in pigs with anthracycline-induced cardiomyopathy compared to controls.(18)

Galán-Arriola et al. studied pigs with doxorubicin-induced cardiomyopathy (mean LVEF 32.5%, cumulative dose 2.25 mg/kg) and found a significant increase compared to healthy controls in mitochondrial fragmentation and in interstitial fibrosis area at ±1.0 human equivalent years.(25)

Objective 2: Temporal changes in ECM remodeling markers

We used meta-regression to study temporal expression trends of proteins and mRNAs that were differentially expressed in our meta-analysis and were measured in at least 5 of the included studies. Results are presented in Table S3 and illustrated in Figure 3. We found 3 proteins of which expression in anthracycline-induced cardiomyopathy compared to control animals (ROM) changed significantly in studies with longer follow-up after anthracyclines. Connective tissue growth factor (CTGF)

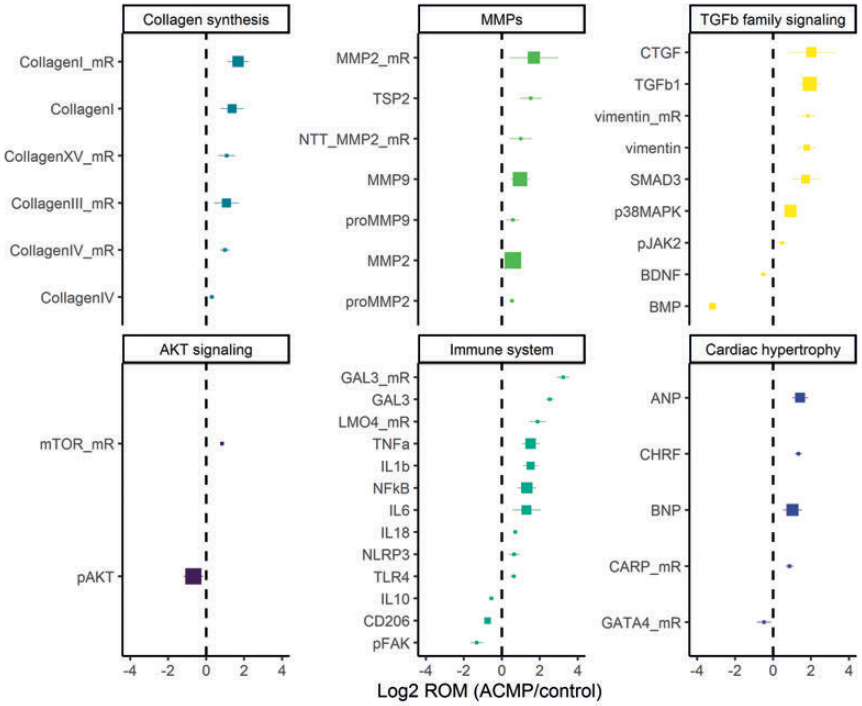


Figure 2. Differentially expressed proteins and mRNAs from the random effects meta-analysis of studies in mice, rats and rabbits with anthracycline-induced cardiomyopathy (ACMP). The x-axis indicates the log2 ratio of the means (ROM) from the random effects meta-analysis in ACMP compared to controls. Differentially expressed proteins and mRNAs (mR) with a ROM >1.2 or <0.83 and p-value <0.05 are shown. The size of each square is proportional with the number of studies in which the protein or mRNA was measured. Lines indicate the 95% confidence interval of the log2 ROM. MMP=matrix metalloproteinase, NTT=N-terminal truncated, TSP=thrombospondin, CTGF=connective tissue growth factor, TGFb1=transforming growth factor beta 1, SMAD=mothers against decapentaplegic homolog, p38 MAPK (p38 mitogen-activated protein kinases), pJAK2=phosphorylated janus kinase 2, BDNF=brain-derived neurotrophic factor, BMP=bone morphogenetic protein, mTOR=mechanistic target of rapamycin kinase, pAKT=phosphorylated protein kinase B, GAL3=galectin 3, LMO4=LIM domain transcription factor, TNFa=tumor necrosis factor alpha, IL=interleukin, NFkB=nuclear factor kappa B, NLRP3=NLR family pyrin domain containing 3, TLR4=toll-like receptor 4, CD206=cluster of differentiation factor 206/mannose receptor on macrophages, pFAK=phosphorylated focal adhesion kinase, ANP=atrial natriuretic peptide, BNP=brain natriuretic peptide, CHRF=cardiac hypertrophy-related factor, CARP=cardiac adriamycin-responsive protein, GATA4=GATA binding protein 4.

protein was measured in studies performed at 0.4-1.7 human equivalent years after anthracycline administration. CTGF levels were higher in anthracycline-induced cardiomyopathy compared to controls in all studies with a peak at 0.4 human equivalent years after anthracycline administration and a decrease in studies with longer follow-up (1.70 lower ROM per year, meta-regression p=0.03). MMP9 protein was studied at 0.2-5.2 years and levels were higher in anthracycline-induced

cardiomyopathy compared to control animals in studies with longer follow-up after anthracycline administration (0.32 higher ROM per year, p=0.04). Brain natriuretic peptide (BNP) was studied at 0.8-4.7 years and levels were higher in anthracycline-induced cardiomyopathy compared to control animals in studies with longer follow-up after anthracycline administration (0.32 higher ROM per year, p=0.04).

We observed consistent upregulation at ≥5 time points without a significant temporal trend in TGFß1 protein (0.8-4.7 human equivalent years), p38 mitogen-activated protein kinase (p38 MAPK) protein (0.8-3.9 years), tumor necrosis factor (TNFα) protein (0.8-4.7 years), nuclear factor-κB (NF-κB) protein (0.1-4.7 years), atrial natriuretic peptide (ANP) protein (0.4-4.1 years) and collagen I mRNA (0.8-8.2 years) (Figure 3, Table S3).

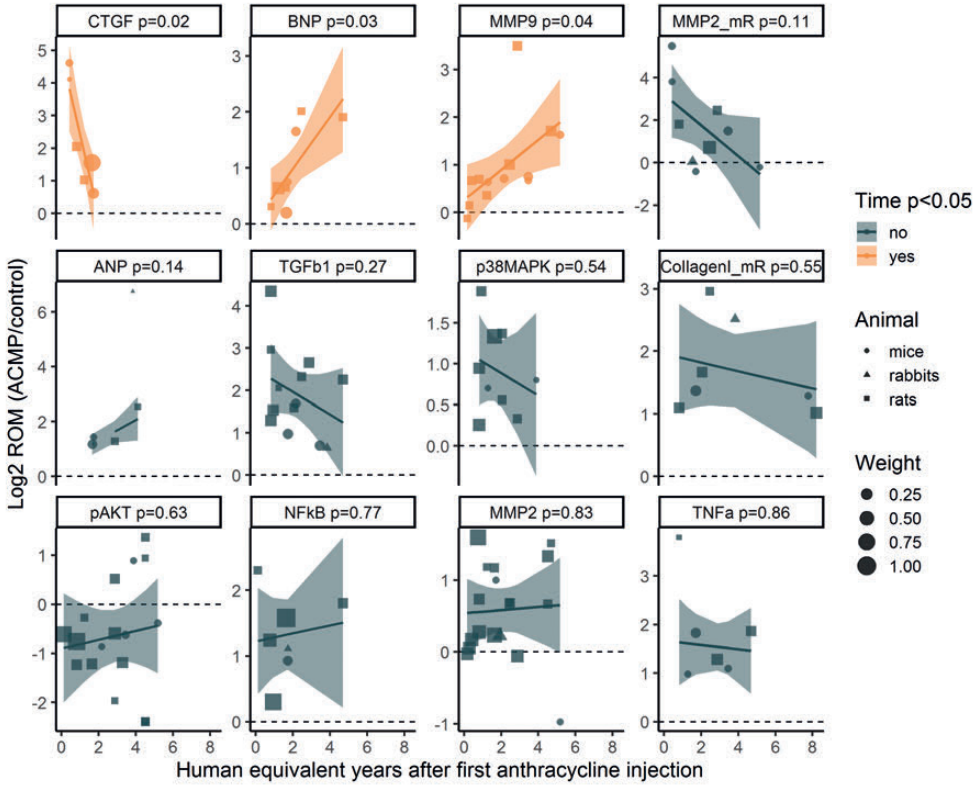


Figure 3. Temporal expression patterns of extracellular matrix related proteins in mouse, rat and rabbit models of anthracycline-induced cardiomyopathy (ACMP). Every dot represents one study where the size is proportional to the weight in the meta-regression. Lines and ribbons represent the meta-regression fit and 95% confidence interval. Proteins significantly association with time after first anthracycline injection in the meta-regression were connective tissue growth factor (CTGF, negative association, p=0.02), brain natriuretic peptide (BNP, positive association, p=0.03) and matrix metalloproteinase 9 (MMP9, positive association, p=0.04). ANP=atrial natriuretic peptide, MMP2=matrix metalloproteinase 2, mR=mRNA, NFκB=Nuclear factor-κB, TGFß=transforming growth factor beta, TNFα=tumor necrosis factor alpha, pAKT=phosphorylated protein kinase B, p38 MAPK=p38 mitogen-activated protein kinases.

Objective 3: Association of ECM remodeling markers with interstitial fibrosis, LV function and cardiomyocyte apoptosis

We studied associations of interstitial fibrosis area, LV systolic function and cardiomyocyte apoptosis with time after anthracycline administration (Figure 4) and with protein or mRNA levels (Table S3) using meta-regression of studies performed in mice, rats and rabbits.

Interstitial fibrosis area, investigated with Sirius red staining's in 38 studies, was more pronounced in anthracycline-induced cardiomyopathy as compared to control animals (SMD 5.70, p<0.0001) and increased in studies with longer follow-up (1.71 increase in SMD per human equivalent year, p=0.01) (Figure 4). Two studies, performed in rats and mice, reported a significant increase in interstitial fibrosis area as early as 0.4 human equivalent years after anthracyclines.(26, 27) Higher ANP protein levels were associated with a larger interstitial fibrosis area (0.76 higher ROM per 1 SMD increase in interstitial fibrosis area, p=0.01)(Table S3). The expression of TGFß1, CTGF, MMPs, NFkB and TNFβ were not significantly associated with interstitial fibrosis area.

LV systolic function, measured with LVEF or FS in 40 studies, was worse in anthracycline-induced cardiomyopathy compared to control animals (SMD -3.65, p<0.0001) and lower LV systolic function was observed in studies with longer follow-up (-0.77 SMD decrease per 1 human equivalent year, p=0.02)(Figure 4). LV measurements were performed in anesthetized animals in 36 studies and in 4 studies this was unclear. Higher MMP2 protein (p=0.02) and BNP protein (p=0.04) levels were associated with worse LV systolic function (Table S3).

Cardiomyocyte apoptosis, measured with TUNEL in 28 studies, was higher in anthracycline-induced cardiomyopathy compared to control animals (SMD 6.21, p<0.0001). This increase in apoptosis as measured with TUNEL was confirmed in 9 studies using caspase 3 (SMD 9.03, p=0.01). A decreasing trend in apoptosis was observed in studies with a longer follow-up after first anthracycline injection, although not statistically significant (p=0.44, Figure 4). The study with the longest follow up after anthracycline administration (56 days, 3.5 human equivalent years) in mice treated with a cumulative dose of 12 mg/kg, still demonstrated apoptosis of cardiomyocytes and vascular smooth muscle cells.(28) In meta-regression, higher mRNA expression of MMP2 (p=0.02) and higher protein levels of TGFß1 (p=0.03) were significantly associated with more cardiomyocyte apoptosis (Table S3).

Inter-study heterogeneity in marker expression

Significant heterogeneity in marker expression values was present between studies in most of the markers pooled in the meta-analysis (I^2>75%). However, the direction of the marker expression was the same in all studies except for MMP2 (downregulation in one study that used a higher cumulative dose of 24 mg/kg(29)) and phosphorylated AKT (upregulation in 3 studies(13, 30, 31)). We did not observe differences in anthracycline dose and/or animal species that could explain heterogeneity in AKT expression.

Risk of bias assessment

Risk of bias was unknown in the majority of the included studies (Table S4). Random group allocation was performed in 34/68 studies and blinding of the outcome assessor and investigators

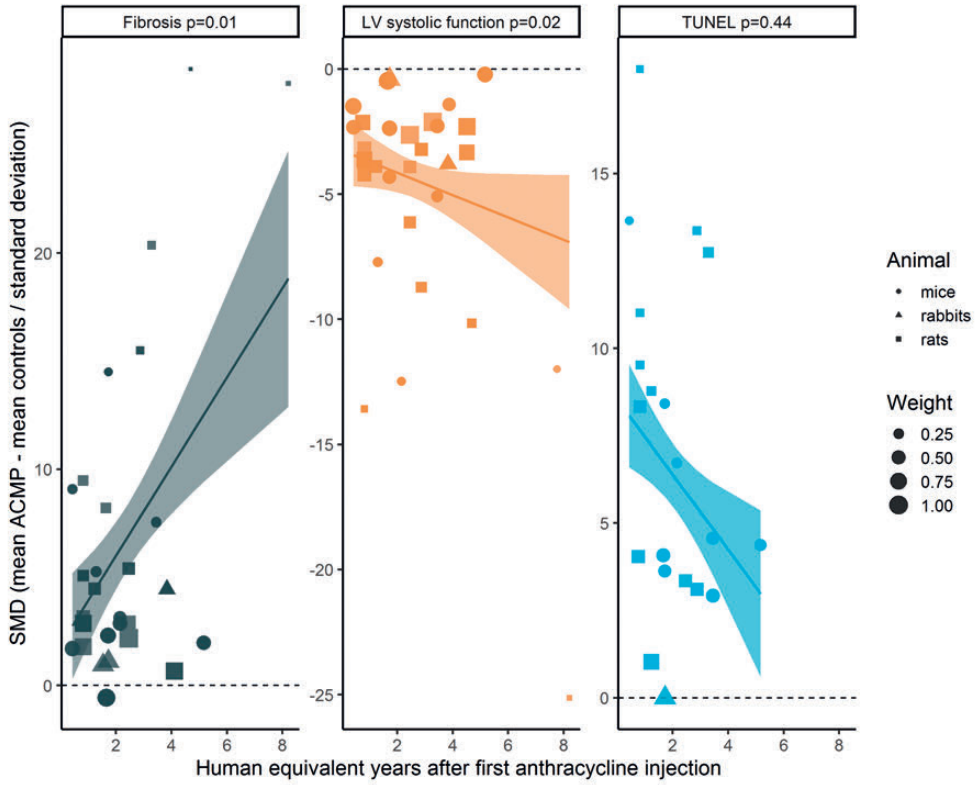


Figure 4. Relationship between interstitial fibrosis area (A), systolic function (B), apoptosis (C) and time after first anthracycline injection in individual studies in mice, rats and rabbits. Every dot represents one study where the size is proportional to the weight in the meta-regression. Lines and ribbons represent the meta-regression fit and 95% confidence interval, respectively. Interstitial fibrosis area, left ventricular (LV) systolic function (LV ejection fraction (LVEF) or fractional shortening (FS)) and apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)) were expressed as a standardized mean difference (SMD) between anthracycline-induced cardiomyopathy (ACMP) and control animals (calculated as the difference in means between ACMP animals and control animals divided by the standard deviation).

was reported in 15 of the included studies. Attrition bias might have been present in 17 studies where animal dropouts were not explained sufficiently (Table S4).

DISCUSSION

To better understand the association of ECM remodeling with anthracycline-induced cardiomyopathy development, we explored the presence and temporal patterns in protein and mRNA markers for ECM remodeling in 68 animal studies. In our meta-analysis, we demonstrate that collagens, matrix metalloproteinases MMP2 and MMP9, immune system markers and markers in the TGFß signaling and cardiac hypertrophy pathway are upregulated in anthracycline-induced cardiomyopathy whereas the AKT pro-survival pathway is downregulated. Furthermore, by using meta-regression we show temporal changes in markers for TGFß-induced fibroblast activation

(CTGF protein), cardiac hypertrophy/wall stress (BNP protein) and MMP9. In addition, with this meta-regression technique, we also markers for ECM remodeling that were associated with worse LV systolic function (BNP and MMP2 protein) and with more interstitial fibrosis (ANP protein). In the following sections, we discuss the markers identified in our systematic review per pathway. In the final section, we summarize the overall temporal ECM remodeling patterns in anthracycline-induced cardiomyopathy.

TGFß signaling and collagen synthesis

TGFß1 is the key initiator of fibroblast-myofibroblast conversion and collagen synthesis in the heart.(7) In our meta-analysis, TGFß1 and one of its signal transducers (SMAD3) were consistently upregulated two- to fourfold in the hearts of animals with anthracycline-induced cardiomyopathy from 0.8 to 4.7 human equivalent years after anthracycline administration, suggesting pro-fibrotic signaling is present at both early and late stages. A downstream target of the canonical TGFß1-SMAD pathway, CTGF, was upregulated in all studies included in our meta-analysis, with an early peak in studies performed within 1 human equivalent years after anthracycline administration.(7) A pressure overload animal model also demonstrated this early peak upregulation in CTGF in the heart, followed by a later reduced but steady upregulation.(32) In addition, the non-canonical p38 MAPK pathway was consistently upregulated in our review, which is also a critical regulator of fibrotic remodeling.(33) Fibroblast activation markers, such as osteonectin mRNA and tenascin-c mRNA were upregulated in one of the two reviewed studies in pigs.(18) Finally, as a likely result of the above described pro-fibrotic signaling, we observed upregulation of collagen type I (protein) and III (mRNA) and an increase in interstitial fibrosis area at longer follow-up durations after anthracyclines.

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are present in the normal heart in an inactive form. Activation of MMPs, especially the gelatinases MMP-2 and MMP-9, is associated with adverse remodeling and LV dilatation in heart failure patients, and precedes LV dysfunction in animal models with tachycardiomyopathy, suggesting that they are early markers of cardiomyopathy.(34) MMP2 and MMP9 are expressed and secreted by cardiac fibroblasts, cardiomyocytes, endothelial cells and immune cells.(35) During secretion a substantial fraction of MMP2, and likely also MMP9, remains intracellular where it can be activated.(36) MMP2 is activated by oxidative stress in cardiomyocytes, in part due to upregulating N-terminal truncated intracellular MMP2, which explains the acute presence and activity of MMP2 in anthracycline-induced cardiomyopathy.(37, 38) Transcription and activation of MMP2 can also be enhanced by the innate immune system, including NFkB-signaling.(39, 40) Interestingly, this innate immune system activation is also triggered by N-terminal truncated MMP2.(36) MMP2 is most well-known for proteolizing ECM proteins but is also active inside cardiomyocytes where it cleaves sarcomeric proteins.(38)

In our meta-regression, MMP2 and MMP9 were upregulated in anthracycline-induced cardiomyopathy compared to controls and MMP9 levels were higher in studies with longer follow-up

after anthracyclines. In addition, a strongly increased MMP9 mRNA expression was found in one of the included studies in pigs.(24) To localize MMPs, three studies demonstrated MMP2 activity in sarcomeres and mitochondria of cardiomyocytes and more localized MMP2 activity next to areas with fibrosis, while MMP1 activity (a collagenase) was observed mainly surrounding blood vessels and surviving cardiomyocytes. (24, 41) We did not find studies that localized MMP9, but their source is assumed to be mainly from macrophages.(28)

MMP activity should be interpreted together with the activity from their inhibitors (TIMPs).(34) In our systematic review, TIMP2 mRNA was downregulated in one study in pigs from Gyongyosi et al.(18) TIMPs (type 1, 2, 3 and 4) were not differentially expressed in our meta-analysis of 6 studies in mice, rats and rabbits.

The matricellular protein thrombospondin-2 (TSP2), mainly expressed by fibroblasts, is known to inhibit the proteolytic activity of MMP2 by binding to active MMP2.(42) In one of the studies included in our systematic review, TSP2 was upregulated early after doxorubicin-treatment of wild-type mice compared to control mice while MMP2 was not upregulated.(29) In the same study, TSP2 knock-down mice treated with doxorubicin showed enhanced MMP2 activity and ECM disruption compared to doxorubicin-treated wild-type mice.(29) This suggests that TSP2 controls MMP2 activity.

Markers for inflammation

In response to cardiomyocyte injury, the innate immune system is activated with release of pro-inflammatory cytokines and chemokines that promote immune cell infiltration and initiate pathological LV remodeling.(6) Toll-like receptors, expressed on macrophages and dendritic cells, act as sensors for doxorubicin-induced cell death (through recognition of damage-associated molecular pattern molecules (DAMPs)) and initiate release of the inflammatory cytokines (e.g., TNFα) and chemokines (e.g., C-C motif chemokine ligands).(43, 44, 45) In the setting of chronic inflammation, these pro-inflammatory cytokines can induce pathological ECM remodeling in animal models.(9) For example, in mice that selectively overexpressed TNFα, MMPs were activated in the initial stages of inflammation resulting in ECM degradation and LV dilatation, while prolonged inflammation promoted mast-cell mediated TGFß signaling and collagen synthesis.(9, 46, 47) The NF-κB protein complex, which is present in almost every cell type, is involved in the innate immune system and regulates cell survival and cytokine production.(48) NF-κB and TNFα were consistently upregulated in all studies included in our systematic review, which suggests (chronic) inflammation in anthracycline-induced cardiomyopathy. While NF-κB has a cardioprotective role during acute myocardial injury by preventing cardiomyocyte apoptosis, persistent activation of NF-κB is maladaptive by inducing production of the inflammatory cytokines TNFα, IL6 and IL1ß. (48) Whether the persistent increase in inflammation markers we observed is the result of ongoing release of DAMPs by injured cardiomyocytes or that other processes also contribute, such as the release of pro-inflammatory ECM fragments during matrix degradation by MMPs(9), is unknown.

Cardiac hypertrophy and wall stress

Natriuretic peptides are established markers for ventricular wall stress and cardiac hypertrophy and have anti-fibrotic properties.(32, 49) Not surprisingly, in our review, higher BNP levels were present in studies with longer follow-up after anthracyclines and higher BNP levels were associated with lower LV function. In addition, higher ANP levels were associated with more interstitial fibrosis, in agreement with previous studies.(49, 50)

AKT-signaling

AKT-signaling regulates cardiac hypertrophy, angiogenesis, glucose metabolism and promotes survival.(51) It has been shown that in situations with increased cardiac stress, short-term activation of AKT prevents cardiomyocyte apoptosis and promotes physiological hypertrophy, while long-term activation induces pathological hypertrophy and ECM remodeling.(52) In our review, phosphorylated AKT was downregulated in most of the included studies. However, two studies in rats included in our review reported upregulations in phosphorylated AKT.(13, 30) These seem to be exceptions, however, and overall downregulated AKT levels were seen, which suggests both impaired cardiomyocyte survival signaling in anthracycline-induced cardiomyopathy, as well as a potential failed protective effect against pathological ECM remodeling.

Limitations

Some limitations should be considered. First, heterogeneity in marker expression was significant between studies and might have been caused by differences in animal models, protein and mRNA measurement techniques and follow-up time. However, the direction of up- or downregulations were concordant for most of the markers, allowing us to draw conclusion on the direction of the association of markers with anthracycline-induced cardiomyopathy. Second, animal models are different from anthracycline-induced cardiomyopathy seen in humans as higher anthracycline doses are used in animals (15 mg/kg in rodents corresponds to ± 580 mg/m^2 in humans), latency to cardiomyopathy development is longer in humans and animal models lack exposure to aging related cardiovascular risk factors. The conversion of animal follow-up times to human equivalent years, that allowed us to compare temporal changes in marker expression across species, should mainly be seen as indicative for the effects of anthracyclines in human perspective.

Summary of temporal ECM remodeling patterns in anthracycline-induced cardiomyopathy

Based on our review and knowledge obtained from other cardiomyopathies(6, 7, 9, 53), we propose the following temporal ECM remodeling patterns in anthracycline-induced cardiomyopathy (Figure 5). We distinguish three main processes that together contribute to the observed ECM remodeling. First, in response to anthracycline-induced cardiomyocyte injury (including oxidative stress and apoptosis) and release of DAMPS, the *innate immune system* is activated with release of pro-inflammatory cytokines by macrophages and other immune cells.(9, 44) The consistent increase in expression of NFkB we observed in our review reflects a strong innate immune response

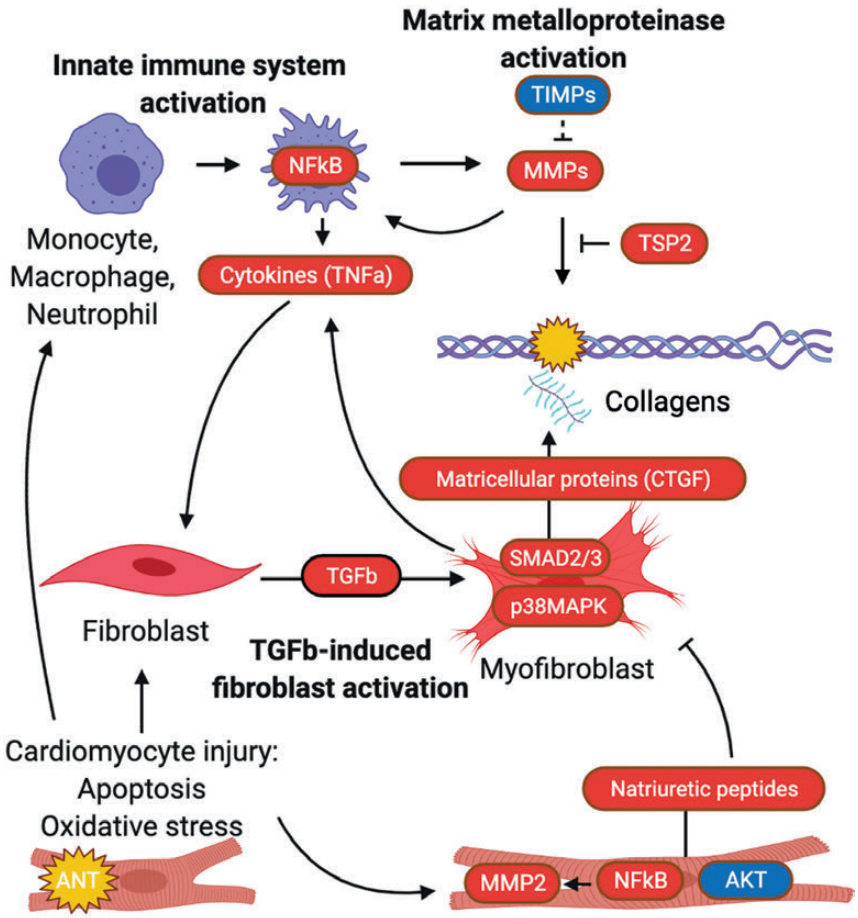


Figure 5. Summary of extracellular matrix remodeling in anthracycline-induced cardiomyopathy. Upregulated markers are in red, downregulated markers are in blue. AKT=phosphorylated protein kinase B, ANT=anthracyclines, CTGF=connective tissue growth factor, MMP=matrix metalloproteinase, NFκB=Nuclear factor-κB, p38 MAPK=p38 mitogen-activated protein kinases, SMAD=Small Mothers Against Decapentaplegic homolog, TGFb=transforming growth factor ß, TIMP=tissue inhibitor of metalloproteinase, TNF=tumor necrosis factor, TSP2=Thrombospondin 2. Created with biorender.com.

that is known to cause pathological ECM remodeling when persistently activated.(48) Similarly, we observed persistent upregulation of the pro-inflammatory cytokine TNFα, which also implicates a chronic pro-inflammatory state in anthracycline-induced cardiomyopathy.

Second, both oxidative stress and the subsequent inflammatory reaction can *activate MMPs* within cardiomyocytes, fibroblasts and immune cells. ECM fragments released during matrix degradation by activated MMPs are itself pro-inflammatory and thus result in a positive feedback loop with chronic inflammation and matrix metalloproteinase activation.(53) In this review, we observed upregulation in MMPs (MMP2 and MMP9) with increasing levels of MMP9 in studies with longer follow-up, which is inadequately counteracted by TIMPs. This shows that this pathway is

persistently activated in anthracycline-induced cardiomyopathy and that dysregulation might become more pronounced longer after anthracycline administration, possibly due to MMP9 secretion by invading immune cells.

Third, chronic inflammation as well as the release of DAMPs can trigger *TGFb1 activation* and fibroblast to myofibroblast conversion and subsequent collagen synthesis.(53) We observed upregulation of markers in the TGFb signaling pathway, such as CTGF that showed an early peak in expression and sustained upregulation in studies with longer follow-up after anthracyclines. Myofibroblasts are activated by chronic inflammation and they also secrete a number of pro-inflammatory cytokines themselves that are able to maintain the chronic inflammation that we observed in this review.(54)

In summary, the innate immune system, MMPs and the TGFb signaling pathway are tightly connected to each other and are persistently activated in animals after exposure to anthracyclines without a clear diminish over time. Together the above processes are likely to contribute to the observed ECM remodeling with accumulation of interstitial fibrosis in the hearts of animals with anthracycline-induced cardiomyopathy.

REFERENCES

1. Gatta G, Botta L, Rossi S, Aareleid T, Bielska-Lasota M, Clavel J, et al. Childhood cancer survival in Europe 1999-2007: results of EUROCARE-5--a population-based study. The Lancet Oncology. 2014;15(1):35-47.
2. Feijen EAM, Font–Gonzalez A, Van der Pal Helena JH, Kok Wouter EM, Geskus Ronald B, Ronckers Cécile M, et al. Risk and Temporal Changes of Heart Failure Among 5–Year Childhood Cancer Survivors: a DCOG–LATER Study. Journal of the American Heart Association. 2019;8(1):e009122.
3. Lenneman CG, Sawyer DB. Cardio-Oncology: An Update on Cardiotoxicity of Cancer-Related Treatment. Circulation research. 2016;118(6):1008-20.
4. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. Journal of molecular and cellular cardiology. 2012;52(6):1213-25.
5. Zhang S, Liu X, Bawa-Khalfe T, Lu L-S, Lyu YL, Liu LF, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nature medicine. 2012;18:1639.
6. Frangogiannis NG. The Extracellular Matrix in Ischemic and Nonischemic Heart Failure. Circulation research. 2019;125(1):117-46.
7. Creemers EE, Pinto YM. Molecular mechanisms that control interstitial fibrosis in the pressure-overloaded heart. Cardiovascular research. 2011;89(2):265-72.
8. Davis J, Molkentin JD. Myofibroblasts: trust your heart and let fate decide. Journal of molecular and cellular cardiology. 2014;70:9-18.
9. Mann DL. Innate immunity and the failing heart: the cytokine hypothesis revisited. Circulation research. 2015;116(7):1254-68.
10. Tanaka R, Umemura M, Narikawa M, Hikichi M, Osaw K, Fujita T, et al. Reactive fibrosis precedes doxorubicin-induced heart failure through sterile inflammation. ESC Heart Failure. 2020;7(2):588-603.
11. Lencova-Popelova O, Jirkovsky E, Mazurova Y, Lenco J, Adamcova M, Simunek T, et al. Molecular remodeling of left and right ventricular myocardium in chronic anthracycline cardiotoxicity and post-treatment follow up. PloS one. 2014;9(5):e96055.
12. Harries I, Liang K, Williams M, Berlot B, Biglino G, Lancellotti P, et al. Magnetic Resonance Imaging to Detect Cardiovascular Effects of Cancer Therapy. JACC CardioOncology. 2020;2(2):270-92.
13. Ivanova M, Dovinova I, Okruhlicova L, Tribulova N, Simoncikova P, Barte-Kova M, et al. Chronic cardiotoxicity of doxorubicin involves activation of myocardial and circulating matrix metalloproteinases in rats. Acta pharmacologica Sinica. 2012;33(4):459-69.
14. Medeiros-Lima DJM, Carvalho JJ, Tibirica E, Borges JP, Matsuura C. Time course of cardiomyopathy induced by doxorubicin in rats. Pharmacological reports : PR. 2019;71(4):583-90.
15. Tokarska-Schlattner M, Lucchinetti E, Zaugg M, Kay L, Gratia S, Guzun R, et al. Early effects of doxorubicin in perfused heart: transcriptional profiling reveals inhibition of cellular stress response genes. American journal of physiology Regulatory, integrative and comparative physiology. 2010;298(4):R1075-88.
16. Yi X, Bekeredjian R, DeFilippis NJ, Siddiquee Z, Fernandez E, Shohet RV. Transcriptional analysis of doxorubicin-induced cardiotoxicity. American journal of physiology Heart and circulatory physiology. 2006;290(3):H1098-102.
17. Vasti C, Witt H, Said M, Sorroche P, Garcia-Rivello H, Ruiz-Noppinger P, et al. Doxorubicin and NRG-1/erbB4-Deficiency Affect Gene Expression Profile: Involving Protein Homeostasis in Mouse. ISRN cardiology. 2012;2012:745185.
18. Gyongyosi M, Lukovic D, Zlabinger K, Spannbauer A, Gugerell A, Pavo N, et al. Liposomal doxorubicin attenuates cardiotoxicity via induction of interferon-related DNA damage resistance. Cardiovascular research. 2019.
19. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ (Clinical research ed). 2009;339.

20. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14:43.

21. Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, et al. Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. The Cochrane database of systematic reviews. 2019;10:Ed000142.

22. IntHout J, Ioannidis JPA, Borm GF. The Hartung-Knapp-Sidik-Jonkman method for random effects meta-analysis is straightforward and considerably outperforms the standard DerSimonian-Laird method. BMC Med Res Methodol. 2014;14(1):25.

23. Luu AZ, Chowdhury B, Al-Omran M, Teoh H, Hess DA, Verma S. Role of Endothelium in Doxorubicin-Induced Cardiomyopathy. JACC Basic to translational science. 2018;3(6):861-70.

24. Goetzenich A, Hatam N, Zernecke A, Weber C, Czarnotta T, Autschbach R, et al. Alteration of matrix metalloproteinases in selective left ventricular adriamycin-induced cardiomyopathy in the pig. The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation. 2009;28(10):1087-93.

25. Galán-Arriola C, Villena-Gutiérrez R, Higuero-Verdejo MI, Díaz-Rengifo IA, Pizarro G, López GJ, et al. Remote ischaemic preconditioning ameliorates anthracycline-induced cardiotoxicity and preserves mitochondrial integrity. Cardiovascular research. 2020;117(4):1132-43.

26. Levick SP, Soto-Pantoja DR, Bi J, Hundley WG, Widiapradja A, Manteufel EJ, et al. Doxorubicin-Induced Myocardial Fibrosis Involves the Neurokinin-1 Receptor and Direct Effects on Cardiac Fibroblasts. Heart, lung & circulation. 2018.

27. Russo M, Guida F, Paparo L, Trinchese G, Aitoro R, Avagliano C, et al. The novel butyrate derivative phenylalanine-butyramide protects from doxorubicin-induced cardiotoxicity. European journal of heart failure. 2019;21(4):519-28.

28. Johnson TA, Singla DK. PTEN inhibitor VO-OHpic attenuates inflammatory M1 macrophages and cardiac remodeling in doxorubicin-induced cardiomyopathy. American journal of physiology Heart and circulatory physiology. 2018;315(5):H1236-h49.

29. van Almen GC, Swinnen M, Carai P, Verhesen W, Cleutjens JP, D'Hooge J, et al. Absence of thrombospondin-2 increases cardiomyocyte damage and matrix disruption in doxorubicin-induced cardiomyopathy. Journal of molecular and cellular cardiology. 2011;51(3):318-28.

30. Bartekova M, Simoncikova P, Fogarassyova M, Ivanova M, Okruhlicova L, Tribulova N, et al. Quercetin improves postischemic recovery of heart function in doxorubicin-treated rats and prevents doxorubicin-induced matrix metalloproteinase-2 activation and apoptosis induction. International journal of molecular sciences. 2015;16(4):8168-85.

31. Zhou L, Chen L, Wang J, Deng Y. Astragalus polysaccharide improves cardiac function in doxorubicin-induced cardiomyopathy through ROS-p38 signaling. International journal of clinical and experimental medicine. 2015;8(11):21839-48.

32. Koitabashi N, Arai M, Kogure S, Niwano K, Watanabe A, Aoki Y, et al. Increased connective tissue growth factor relative to brain natriuretic peptide as a determinant of myocardial fibrosis. Hypertension (Dallas, Tex : 1979). 2007;49(5):1120-7.

33. Turner NA, Blythe NM. Cardiac Fibroblast p38 MAPK: A Critical Regulator of Myocardial Remodeling. Journal of cardiovascular development and disease. 2019;6(3):27.

34. Spinale FG, Janicki JS, Zile MR. Membrane-associated matrix proteolysis and heart failure. Circulation research. 2013;112(1):195-208.

35. Vanhoutte D, Heymans S. TIMPs and cardiac remodeling: 'Embracing the MMP-independent-side of the family'. Journal of molecular and cellular cardiology. 2010;48(3):445-53.

36. Lovett DH, Mahimkar R, Raffai RL, Cape L, Zhu BQ, Jin ZQ, et al. N-terminal truncated intracellular matrix metalloproteinase-2 induces cardiomyocyte hypertrophy, inflammation and systolic heart failure. PloS one. 2013;8(7):e68154.

37. Schulz R. Intracellular targets of matrix metalloproteinase-2 in cardiac disease:

rationale and therapeutic approaches. Annu Rev Pharmacol Toxicol. 2007;47:211-42.

38. Chan BYH, Roczkowsky A, Cho WJ, Poirier M, Sergi C, Keschrumrus V, et al. MMP inhibitors attenuate doxorubicin cardiotoxicity by preventing intracellular and extracellular matrix remodeling. Cardiovascular research. 2020.

39. Deschamps AM, Spinale FG. Pathways of matrix metalloproteinase induction in heart failure: bioactive molecules and transcriptional regulation. Cardiovascular research. 2006;69(3):666-76.

40. Siwik DA, Colucci WS. Regulation of matrix metalloproteinases by cytokines and reactive oxygen/nitrogen species in the myocardium. Heart failure reviews. 2004;9(1):43-51.

41. Adamcova M, Potacova A, Popelova O, Sterba M, Mazurova Y, Aupperle H, et al. Cardiac remodeling and MMPs on the model of chronic daunorubicin-induced cardiomyopathy in rabbits. Physiological research. 2010;59(5):831-6.

42. Schellings MWM, van Almen GC, Sage EH, Heymans S. Thrombospondins in the heart: potential functions in cardiac remodeling. Journal of cell communication and signaling. 2009;3(3-4):201-13.

43. Adamo L, Rocha-Resende C, Prabhu SD, Mann DL. Reappraising the role of inflammation in heart failure. Nat Rev Cardiol. 2020;17(5):269-85.

44. Ghigo A, Li M, Hirsch E. New signal transduction paradigms in anthracycline-induced cardiotoxicity. Biochim Biophys Acta. 2016;1863(7 Pt B):1916-25.

45. Riad A, Bien S, Gratz M, Escher F, Westermann D, Heimesaat MM, et al. Toll-like receptor-4 deficiency attenuates doxorubicin-induced cardiomyopathy in mice. European journal of heart failure. 2008;10(3):233-43.

46. Sivasubramanian N, Coker ML, Kurrelmeyer KM, MacLellan WR, DeMayo FJ, Spinale FG, et al. Left ventricular remodeling in transgenic mice with cardiac restricted overexpression of tumor necrosis factor. Circulation. 2001;104(7):826-31.

47. Zhang W, Chancey AL, Tzeng HP, Zhou Z, Lavine KJ, Gao F, et al. The development of myocardial fibrosis in transgenic mice with targeted overexpression of tumor necrosis factor requires mast cell-fibroblast interactions. Circulation. 2011;124(19):2106-16.

48. Gordon JW, Shaw JA, Kirshenbaum LA. Multiple facets of NF-κB in the heart: to be or not to NF-κB. Circulation research. 2011;108(9):1122-32.

49. Wang D, Gladysheva IP, Fan TH, Sullivan R, Houng AK, Reed GL. Atrial natriuretic peptide affects cardiac remodeling, function, heart failure, and survival in a mouse model of dilated cardiomyopathy. Hypertension (Dallas, Tex : 1979). 2014;63(3):514-9.

50. Boucek JRJ, Miracle A, Anderson M, Engelman R, Atkinson J, Dodd DA. Persistent Effects of Doxorubicin on Cardiac Gene Expression. Journal of molecular and cellular cardiology. 1999;31(8):1435-46.

51. Chaanine AH, Hajjar RJ. AKT signalling in the failing heart. European journal of heart failure. 2011;13(8):825-9.

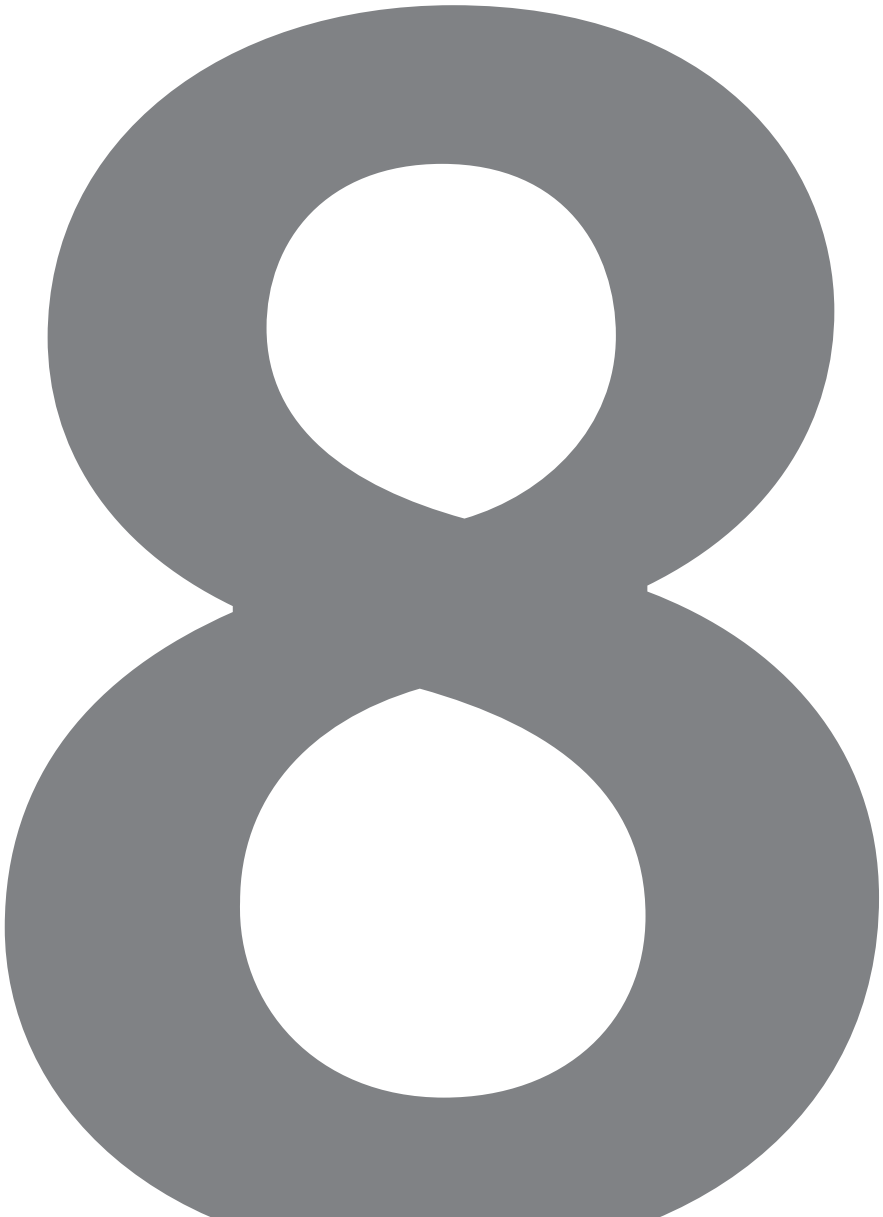
52. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. Nat Rev Mol Cell Biol. 2006;7(8):589-600.

53. Hartupee J, Mann DL. Role of inflammatory cells in fibroblast activation. Journal of molecular and cellular cardiology. 2016;93:143-8.

54. Van Linthout S, Miteva K, Tschöpe C. Crosstalk between fibroblasts and inflammatory cells. Cardiovascular research. 2014;102(2):258-69.

SUPPLEMENTAL MATERIAL

The supplemental material is available at https://doi.org/10.1007/s00109-021-02098-8.



CANDIDATE PLASMA BIOMARKERS TO DETECT ANTHRACYCLINE-RELATED CARDIOMYOPATHY IN CHILDHOOD CANCER SURVIVORS: A CASE CONTROL STUDY IN THE DUTCH CHILDHOOD CANCER SURVIVOR STUDY

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ABSTRACT

Background

Plasma biomarkers may aid in the detection of anthracycline-induced cardiomyopathy (ACMP). However, the currently available biomarkers have limited diagnostic value in long-term childhood cancer survivors (CCS). This study sought to identify diagnostic plasma biomarkers for ACMP in CCS.

Methods and results

We measured 275 plasma proteins in 28 ACMP cases with left ventricular ejection fraction (LVEF)<45%, 29 anthracycline-treated controls with LVEF≥53% matched on sex, time after cancer and anthracycline dose and 29 patients with genetically-determined dilated cardiomyopathy (DCM) with LVEF<45%. Multivariable linear regression was used to identify differentially expressed proteins. Elastic net model including clinical characteristics was used to assess discrimination of proteins diagnostic for ACMP.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) and the inflammatory markers C-C motif chemokine-ligands (CCL19, CCL20), pulmonary surfactant protein-D (PSPD) and pleiotrophin (PTN) were significantly upregulated in ACMP compared to controls. An elastic net model selected 45 proteins, including NT-proBNP, CCL19, CCL20 and PSPD, but not PTN, that discriminated ACMP cases from controls with an area under the curve (AUC) of 0.78. This model was not superior to a model including NT-proBNP and clinical characteristics (AUC=0.75, p=0.766). However, when excluding 8 ACMP cases with heart failure, the full model was superior to that including only NT-proBNP and clinical characteristics (AUC=0.75 versus AUC=0.50, p=0.022). The same 45 proteins also showed good discrimination between DCM and controls (AUC 0.89), underscoring their association with cardiomyopathy.

Conclusions

We identified three specific inflammatory proteins as candidate plasma biomarkers for ACMP in long-term CCS and demonstrated protein overlap with DCM.

INTRODUCTION

Childhood cancer survivors (CCS) treated with anthracyclines, mitoxantrone and/or chest-directed radiotherapy are at high risk for heart failure with 11.6% developing heart failure within 40 years from cancer diagnosis.(1) Due to the high risk of heart failure and the potential benefits of early detection and treatment of cardiac dysfunction, life-long echocardiographic surveillance is currently recommended.(2)

Blood biomarkers with a high sensitivity and sufficient specificity could be useful as a time-efficient and cost-effective triage test, where survivors with a normal biomarker level can safely be deferred from further workup with an echocardiogram.(3) Blood biomarkers could also be used in addition to an echocardiogram to improve its diagnostic accuracy or for prognostic reasons. Up till now, N-terminal pro B-type natriuretic peptide (NT-proBNP) and troponins have been studied but lack sufficient sensitivity to detect asymptomatic left ventricular (LV) dysfunction in long-term CCS and are therefore not recommended for surveillance purposes.(2, 4) Few studies have utilized plasma proteomics to identify additional biomarkers that might improve detection of anthracycline-related cardiomyopathy (ACMP), some of which in pediatric cancer patients in the acute phase(5) and others assessing the value of natriuretic peptides, cardiac troponin T, soluble suppression of tumourigenicity-2 (ST2) and galectin-3 carnitine, in long-term CCS(6, 7). However, most of the studies using larger scale proteomic analyses have been conducted in adult cancer patients during or shortly after anthracycline treatment.(8, 9, 10)

In this discovery case-control study in the Dutch Childhood Cancer Survivor Study (DCCSS LATER 2 CARD), we sought to identify candidate plasma proteins that would be able to discriminate ACMP cases from anthracycline-treated controls with normal left ventricular function, using a large biomarker panel consisting of markers for ventricular wall stress, oxidative stress, inflammation, cellular adhesion, apoptosis and extracellular matrix remodeling. To further support the hypothesis that the selected markers are associated with cardiomyopathy and not with a systemic effect of anthracyclines in those sensitive to them, we compared plasma levels of the proteins that we identified in ACMP with plasma levels in patients with genetically-determined dilated cardiomyopathy (DCM).

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study design and study participants

We conducted a cross-sectional case-control study as part of the DCCSS LATER 2 CARD study. The design of this cohort study has been published.(11) In short, DCCSS LATER 2 CARD is a multicenter study in 5-year CCS diagnosed with a malignancy before the age of 18 years and between 1/1/1963 and 12/31/2001 who were treated with (potentially) cardiotoxic cancer treatments. Participants visited the outpatient clinic between February 2016 and February 2020 for questionnaires, physical examination, blood sampling, electrocardiography and echocardiography. For primary analysis of this biomarker case control study, we included CCS treated with anthracyclines or mitoxantrone,

with or without concomitant chest-directed radiotherapy. CCS with congenital heart disease were excluded. The first 30 ACMP cases (defined as a LVEF<45%) included in the DCCSS LATER 2 CARD study were selected and matched with 30 anthracycline-treated controls without ACMP (defined as LVEF≥53% without ≥grade 2 diastolic dysfunction or valvular disease)(Figure 1). We chose to include these first 30 ACMP cases because 1) the inclusion for the DCCSS LATER 2 CARD was still ongoing at time of this case-control study and 2) to ensure a random selection of ACMP cases. Controls were propensity score matched to ACMP cases where the propensity score was estimated with logistic regression of case status on the covariates sex, time since cancer diagnosis and cumulative anthracycline/mitoxantrone dose (calculated as doxorubicin equivalents).(12) For secondary analysis, we included DCM patients with LVEF <45% and a pathogenic titin truncating variant or

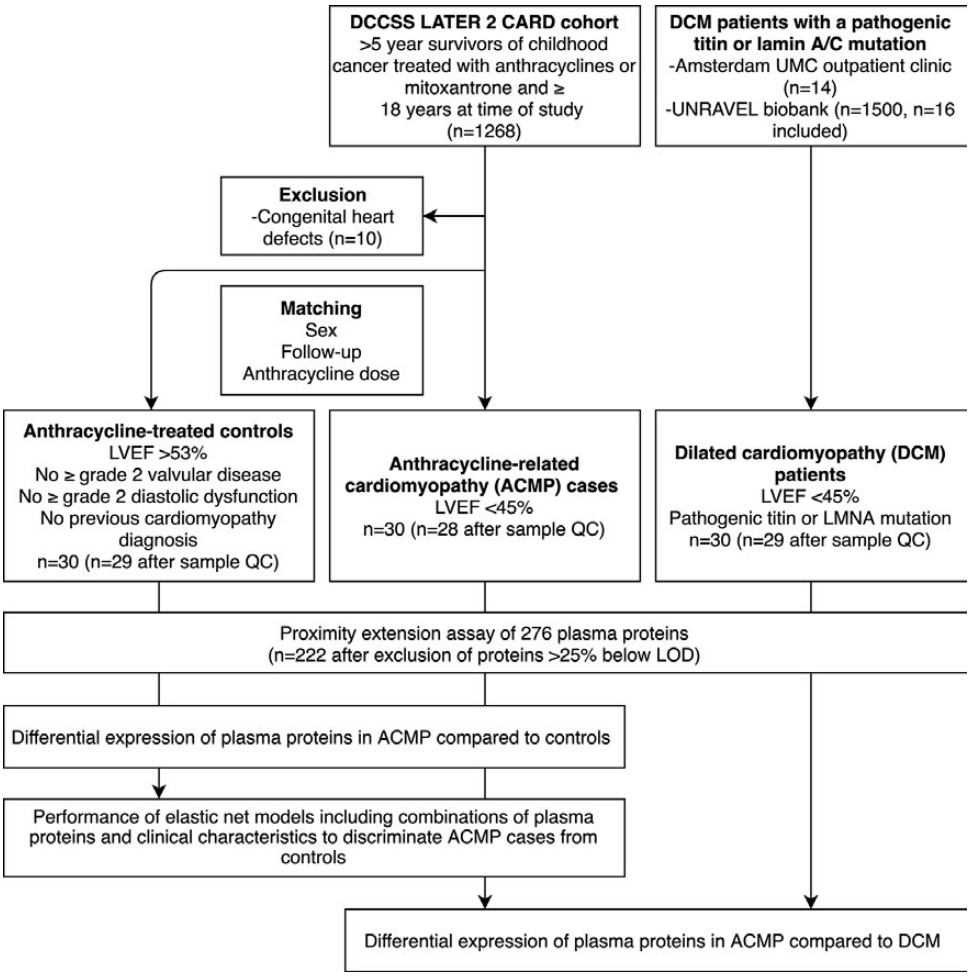

Figure 1. Study design of the LATER CARD biomarker case control study. ACMP=anthracycline cardiomyopathy, DCM=dilated cardiomyopathy, LMNA=lamin A/C, LOD=limit of detection, LVEF=left ventricular ejection fraction, QC=quality control.

a lamin A/C mutation from the Amsterdam University Medical Center and the UNRAVEL database at the University Medical Center Utrecht in the Netherlands.(13) These DCM patients were included to test whether or not the plasma proteins selected to be discriminative for ACMP would also be discriminative for DCM.

Ethics

The investigation conforms with the principles outlined in the Declaration of Helsinki. The LATER CARD study was approved by the medical ethics board of all participating centers and included blood biobanking for future analysis. The medical ethics board of the Amsterdam University Medical Center and the University Medical Center Utrecht approved the biobanking of blood samples from DCM patients. UNRAVEL follows the code of conduct and the use of data in health research and has been approved by the biobank board of the medical ethics committee of the University Medical Center Utrecht.(13) Informed consent was obtained from all participants.

Data collection

Patient and cancer treatment characteristics were obtained from the central database of the LATER study (ACMP cases and controls) and from medical records (DCM patients). Cumulative anthracycline dose was calculated as the doxorubicin equivalent dose.(12) Cardiac medication use, heart failure symptoms and modifiable cardiovascular risk factors were obtained from questionnaires (CCS) and medical records (DCM patients). In ACMP cases and controls, self-reported heart failure and cardiovascular risk factors were considered present if patients reported the use of medications for the condition. All participants underwent a physical examination at time of blood sampling to obtain body mass index (BMI) and blood pressure. Fasting citrate blood samples were obtained from participants within 6 months from the qualifying echocardiogram (86% of samples were obtained at the same day). Samples were centrifuged at 3000xg for 10 minutes, stored within one hour at -80 degrees Celsius and shipped on dry ice to the central biobank. In ACMP cases and controls, echocardiographic parameters, including biplane LVEF, were measured by a core lab blinded for clinical characteristics.(14) In DCM patients, echocardiographic parameters were obtained from medical records.

Plasma protein measurements

Plasma levels of 276 proteins were measured with a proximity extension assay in 3µL of citrate plasma per patient using the Cardiovascular III, Organ Damage and Inflammation panels from Olink Proteomics (Uppsala, Sweden). We chose these three panels because of their known association with cardiovascular disease, apoptosis, inflammation and remodeling. Panel validation data can be found at Olink.com. The proximity extension assay is based on pairs of antibodies that are linked to proximity probes. Upon binding of the antibody pair to their target protein, the probes are brought in proximity and are extended by a DNA polymerase that can subsequently be detected with real-time PCR. Protein levels are expressed as Normalized Protein Expression (NPX) values, which are relative units expressed on the log2 scale where a 1-unit higher NPX value represents a doubling

of protein concentration. Study groups were randomly distributed over the plate. Samples that did not pass Olink quality control (>0.3 NPX median deviation from the internal control) were excluded. Protein levels below the linear limit of detection (LOD) were replaced with the estimated NPX value at the non-linear part of the calibration curve if <25% was below LOD. Proteins with ≥25% below LOD were excluded (n=54 proteins). These 54 proteins were not exclusively expressed in one of the study groups. Two PCR readout failures were median imputed (macrophage-capping protein in one ACMP case and transmembrane serine protease 15 in one DCM case).

Statistical analysis

Descriptive statistics

Continuous variables were checked for normality by visual inspection using histograms and are presented as mean ± standard deviation (SD) for normally distributed variables and as median with range for skewed variables. Categorical variables are presented as numbers and percentages. Continuous variables were compared with the t-test or Wilcoxon signed rank test, where appropriate. Categorical variables were compared with the Chi-square test or the Fisher exact test (when expected counts were <5). All analyses were conducted in R version 3.6.1.

Primary analysis: ACMP cases compared to anthracycline-treated controls

Differential expression of plasma proteins in ACMP cases compared to controls was tested with multivariable linear regression models estimating log2 fold changes. Models were adjusted for sex, time since cancer diagnosis, anthracycline/mitoxantrone dose and chest-directed radiotherapy dose. P-values were corrected for multiple testing with the q-value, which can be interpreted as a false discovery rate.(15) A q-value<0.1 was considered statistically significant. In sensitivity analyses, models were adjusted for NT-proBNP levels and were restricted to ACMP cases without self-reported heart failure.

Elastic net logistic regression was used to identify a combination of plasma proteins best discriminating ACMP cases from controls. The elastic net simultaneously performs variable selection and shrinkage of coefficients of a large number of predictors and is relatively robust to collinear predictors.(16) Predictors entered in the elastic net were all plasma proteins and the clinical characteristics sex, age at cancer diagnosis, time since cancer diagnosis, anthracycline/mitoxantrone dose and chest-directed radiotherapy dose. NT-proBNP was not subjected to selection and coefficient shrinkage as we aimed to find proteins independent of NT-proBNP. Predictors were standardized to have a mean of zero and a standard deviation of one. We used a nested cross validation strategy to test performance of the elastic net on data not seen during training of the model. Matched case-control pairs were divided into a training set and test set with 10x10-fold cross-validation. The elastic net parameters (alpha and lambda) were optimized on the training set with 5-fold cross validation and the parameter combination that was within one standard error from the optimal area under the receiver operating characteristic curve (AUC) was chosen. Median model performance over the cross validation folds was evaluated on the test set with the AUC, and with sensitivity and specificity at the threshold maximizing the sum of sensitivity and specificity.

Proteins selected in ≥40% of the cross validation folds were considered important. Performance of the elastic net model including all proteins and clinical characteristics was compared to an elastic net model including only NT-proBNP and clinical characteristics. AUCs were compared and 95% confidence intervals were calculated with the Wilcoxon signed rank test. In additional analysis in asymptomatic CCS, elastic net models were also fitted in ACMP cases without heart failure and their matched controls only.

Secondary analysis: ACMP cases compared to DCM patients

Differential expression of plasma proteins in ACMP cases compared to DCM patients was tested with multivariable linear regression models adjusted for sex, age at blood sample and LVEF. A q-value <0.1 was considered statistically significant. The group of proteins discriminating ACMP cases from controls were tested for their ability to also discriminate DCM patients from controls with the elastic net using the same modeling steps as described for the primary analysis.

RESULTS

Patient characteristics

After exclusion of 4 samples that did not pass quality control, we included 28 ACMP cases, 29 matched anthracycline-treated controls and 29 DCM patients in this study (Figure 1). Characteristics of the participants are outlined in Table 1. ACMP cases and controls were successfully matched with respect to sex (46.4 and 48.3% male, respectively, p=1.0), time since cancer diagnosis (median 25.4 years and 29.4 years, respectively, p=0.107) and cumulative anthracycline dose (median 360.0 mg/m^2 and 300.0 mg/m^2, respectively, p=0.626). As compared to ACMP cases, DCM patients were older (median 37.6 and 56.0 years, respectively; p<0.001) and were more frequently male (46.4% and 82.8%, p=0.006). Mean LVEF in ACMP cases was 40.6%±5.8%, versus 58.1%±3.2% in controls and was lowest in DCM patients (37.0%±7.5%). Cardiac medications were used by all of the DCM patients, by 16 (57.1%) of the ACMP cases and by 3 (10.3%) of the controls. Heart failure was reported by 8 (28.6%) of the ACMP cases and 22 (75.9%) of the DCM patients. Hypertension, diabetes and dyslipidemia were reported by a minority of CCS and DCM patients. Characteristics of ACMP cases without heart failure compared to matched controls are presented in Table S1.

Primary analysis: ACMP cases compared to anthracycline treated controls

Differential expression of plasma proteins in ACMP cases compared to controls

In multivariable linear regression analyses adjusted for sex, time since cancer diagnosis, anthracycline and chest-directed radiotherapy dose, plasma levels of NT-proBNP, C-C motif chemokine 19 (CCL19), pleiotrophin (PTN), C-C motif chemokine (CCL20), and pulmonary surfactant protein D (PSPD) were significantly higher in ACMP cases compared to controls (q-value <0.1, Figure 2, Table S2). When we additionally adjusted for NT-proBNP levels, CCL19, CCL20, PSPD and PTN remained significantly upregulated (Table S3). When we performed the analysis in 20 ACMP cases without heart failure (reflecting a surveillance population) and their matched controls, NT-proBNP was not significantly upregulated (p=0.231), while the other 4 proteins remained significantly upregulated (Table S3).

Table 1. Characteristics of the anthracycline-related cardiomyopathy cases (ACMP), anthracycline-treated controls and dilated cardiomyopathy (DCM) patients.

Characteristic	Controls (n=29)	ACMP (n=28)	DCM (n=29)	P-value ACMP-controls	P-value DCM-ACMP
Male sex	14 (48.3)	13 (46.4)	24 (82.8)	1	0.006
Age at cancer diagnosis	7.97 [4.03, 11.82]	8.30 [3.52, 13.11]	NA	0.936	NA
Age at blood sampling	43.30 [34.71, 46.97]	37.63 [30.26, 45.30]	56.00 [39.00, 64.00]	0.271	<0.001
Time since cancer diagnosis	29.44 [24.13, 32.33]	25.35 [18.85, 30.21]	NA	0.107	NA
Primary cancer diagnosis			NA	0.671	
Leukemias	8 (27.6)	5 (17.9)	NA		
Lymphomas	11 (37.9)	10 (35.7)	NA		
Neuroblastoma	0 (0.0)	1 (3.6)	NA		
Renal tumors	3 (10.3)	2 (7.1)	NA		
Bone tumors	3 (10.3)	7 (25.0)	NA		
Soft tissue sarcomas	3 (10.3)	3 (10.7)	NA		
Germ cell tumors	1 (3.4)	0 (0.0)	NA		
Anthracyclines	27 (93.1)	23 (82.1)	NA	0.253	NA
Anthracyclines cumulative dose*, mg/m2	300.00 [216.00, 400.00]	360.00 [169.00, 462.50]	NA	0.626	NA
Mitoxantrone	7 (24.1)	7 (25.0)	NA	1	NA
Mitoxantrone dose, mg/m2	50.00 [40.00, 102.00]	120.00 [50.00, 121.00]	NA	0.299	NA
Chest RT	2 (20.0)	3 (20.0)	NA	0.670	NA
Chest RT cumulative dose, Gray	20.00 [20.00, 20.00]	25.00 [19.50, 37.50]	NA	0.554	NA
DCM causing mutation				NA	NA
Titin	NA	NA	23 (79.3)	NA	NA
Lamine A/C	NA	NA	6 (21.7)	NA	NA
Heart failure	0 (0.0)	8 (28.6)	22 (75.9)	0.006	0.001
Cardiac medication(s)	3 (10.3)	16 (57.1)	29 (100)	0.001	<0.001
Hypercholesterolemia	1 (3.4)	2 (7.1)	9 (31.0)	0.611	0.051
Diabetes	0 (0.0)	0 (0.0)	2 (6.9)	NA	0.491
Hypertension	1 (3.4)	2 (7.1)	6 (20.7)	0.611	0.253
Systolic blood pressure, mmHg	125.5 (16.1)	117.1 (19.8)	114.9 (18.4)	0.086	0.669

Table 1. (continued)

Characteristic	Controls (n=29)	ACMP (n=28)	DCM (n=29)	P-value ACMP-controls	P-value DCM-ACMP
Diastolic blood pressure, mmHg	78.8 (10.1)	72.9 (15.5)	72.7 (13.3)	0.093	0.962
Heart rate, bpm	69.2 (14.4)	71.2 (12.6)	69.3 (8.3)	0.582	0.509
BMI, kg/m2	25.1 (4.6)	25.0 (4.9)	26.3 (4.2)	0.926	0.301
Biplane LVEF, %	58.1 (3.2)	40.6 (5.8)	37.0 (7.5)	<0.001	0.045
LVIDd, cm	4.6 (0.6)	5.2 (0.7)	6.2 (0.8)	0.003	<0.001

Categorical values are presented as number (%). Continuous values are presented as mean ± standard deviation (sd) or as median [inter-quartile range]. *Doxorubicin equivalents (Daunorubicin*0.6 + epirubicin*0.8 + idarubicin*3). Abbreviations: BMI=Body mass index, LVEF=left ventricular ejection fraction, LVIDd=left ventricular end diastolic diameter, chest RT=chest-directed radiotherapy, NA=not applicable.

Biomarkers which have previously been shown to have diagnostic or prognostic value in heart failure patients, including soluble suppression of tumourigenicity-2 (sST2), galectin-3, troponin I, tumor necrosis factor (TNF), interleukin-6, osteopontin and tumor necrosis factor receptor superfamily member 6 (FAS), were not differentially expressed in ACMP cases compared to controls (all q-values >0.1, Table S2).

Discriminative plasma proteins identified with elastic net

The elastic net model trained on all proteins and clinical characteristics selected 45 proteins in >40% of the elastic net cross validation folds, which indicates they are potentially important in discriminating ACMP cases from controls (Figure 2, Table S2). Next to NT-proBNP which was not subjected to selection, this panel mainly consisted of inflammatory markers such as CCL19, CCL20,

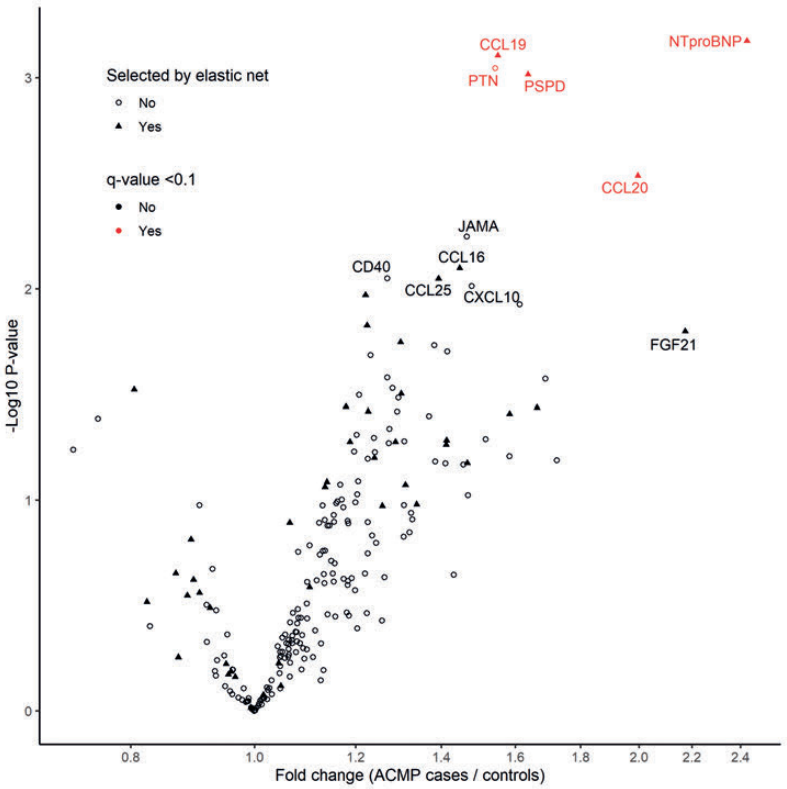

Figure 2. Volcano plot showing fold changes (x-axis) and p-values (y-axis) of 222 plasma proteins in anthracycline related cardiomyopathy (ACMP) compared to matched anthracycline-treated controls. Fold changes and p-values were estimated with multivariable linear regression analysis adjusted for sex, time since cancer diagnosis, anthracycline and chest-directed radiotherapy dose. Significantly upregulated proteins (q-value <0.1) are shown in red. Proteins selected by the elastic net in >40% of the cross validation folds are shown as a triangle. Abbreviations: CCL=C-C motif chemokine ligand, CD40=Cluster of differentiation 40, CXCL10=C-X-C motif chemokine ligand 10, FGF21=Fibroblast growth factor 21, JAMA=Junctional adhesion molecule A, NT-proBNP=N-terminal pro-B-type natriuretic peptide, PSPD=Pulmonary surfactant protein D, PTN=pleiotrophin.

CCL25 and PSPD, and adhesion molecules such as chitinase 3 like 1 (CHI3L1), P selectin (SELP), EPH receptor B4 (EPHB4) and intracellular adhesion molecule 2 (ICAM2). All proteins that were significantly upregulated in the multivariable linear regression analysis were also selected by the elastic net, except for PTN which was selected in 18% of the folds, suggesting PTN does not contribute much to the discrimination when combined with other proteins.

Performance of the elastic net in discriminating ACMP cases from controls

The elastic net model trained on all proteins and clinical characteristics had a cross-validated AUC of 0.78, a sensitivity of 87% and a specificity of 78% (Table 2). Discrimination of this model was slightly but not significantly higher compared to an elastic net model trained on NT-proBNP and clinical characteristics only (AUC=0.75, p=0.766). To better reflect a surveillance population of asymptomatic CCS, we repeated the analysis in 20 ACMP cases without self-reported heart failure and their matched controls (n=21). In this analysis, discrimination of the elastic net model trained on all proteins and clinical characteristics retained its discriminative value better as compared to the elastic net trained on NT-proBNP and clinical characteristics only (AUC 0.75 versus AUC 0.50, p=0.022)(Table 2). Importantly, CCL19, CCL20 and PSPD were also selected by the elastic net in >40% of the cross-validation folds in this analysis in ACMP cases without heart failure.

Secondary analysis: Plasma protein expression in ACMP cases compared to DCM patients

In multivariable linear regression analyses adjusted for sex, age and LVEF, none of the 5 upregulated proteins in ACMP cases compared to controls (NT-proBNP, CCL19, CCL20, PSPD, PTN) were differentially expressed in ACMP cases compared to genetically-determined DCM patients

Table 2. Cross validated performance measures of elastic net models, including clinical characteristics and plasma proteins, to discriminate anthracycline-related cardiomyopathy cases from anthracycline-treated controls. Performance measures are reported for elastic net models fitted in all participants and in asymptomatic cases without heart failure.

Performance measure	NT-proBNP + clinical characteristics*	All proteins + clinical characteristics*	Wilcoxon test p-value
Main analysis in all participants (n=57)			
AUC (95% CI)	0.75 (0.71-0.80)	0.78 (0.72-0.83)	0.766
Sensitivity (95% CI)	86% (0.82-0.90)	87% (0.83-0.91)	-
Specificity (95% CI)	78% (0.82-0.90)	78% (0.72-0.84)	-
Analysis in asymptomatic cases without heart failure (n=41)			
AUC (95% CI)	0.50 (0.50-0.62)	0.75 (0.63-0.75)	0.022
Sensitivity (95% CI)	89% (0.84-0.93)	90% (0.86-0.94)	-
Specificity (95% CI)	61% (0.54-0.68)	69% (0.62-0.76)	-

*Clinical characteristics included sex, age at cancer diagnosis, time since cancer diagnosis, anthracycline/mitoxantrone dose (doxorubicin equivalents) and chest-directed radiotherapy dose. Sensitivities and specificities are reported at the threshold maximizing the sum of sensitivity and specificity. Abbreviations: CI=confidence interval.

(Table S2). Similar results were obtained when not adjusting the multivariable linear regression analysis for differences in LVEF between ACMP and DCM (NT-proBNP, CCL19, CCL20, PSPD and PTN all had p>0.05). In the elastic net model, the 45 discriminative proteins for ACMP including NT-proBNP were also highly discriminative for DCM compared to controls (elastic net AUC 0.89), and the AUC remained high when excluding NT-proBNP from this protein panel (elastic net AUC 0.86).

DISCUSSION

In this discovery case-control study, we identified three inflammatory proteins CCL19, CCL20 and PSPD as candidate plasma biomarkers for detection of ACMP in long-term CCS, independently of clinical characteristics such as anthracycline dose, independently of NT-proBNP levels and independently of the presence of heart failure. Supporting the role of these proteins in detecting ACMP is their similarly increased presence in patients with genetically determined DCM. As our sample population is small, we regard the results as a promising finding that awaits confirmation in a larger cohort.

Previous studies in survivors of breast cancer treated with anthracyclines have also reported an association of inflammatory biomarkers with decreased left ventricular function.(10, 17) In one of the studies, at a mean of 11±5.5 years after treatment with anthracyclines and/or radiotherapy, 11 plasma proteins related to cardiovascular disease were associated with decreasing LVEFs that were still in the normal range (median LVEF of 58%, inter quartile range 55-60%).(10) We confirm upregulation of one of these proteins, the inflammatory adipokine and chemokine RARRES2, which in our study showed an association with ACMP in CCS that did not surpass the multiple testing threshold in our study (Table S2). In another study in breast cancer patients with more severely depressed LVEF (i.e. ≤40%, n=5) compared to anthracycline-treated controls (n=10), a transcriptomics analysis demonstrated differential expression in genes related to lymphocyte activation and B cell receptor signaling(17), which is interesting in relation to our study since CCL19 and CCL20 are chemotactic for T and B cells. In accordance with previous studies in CCS and breast cancer survivors, we show that galectin-3, ST2, IL6, TNF and troponin I are not differentially expressed in ACMP cases compared to controls.(4, 6, 10) This finding is interesting since these biomarkers have been shown to be predictive of heart failure in the general population but may be related to other etiologies of heart failure.(18, 19, 20)

Inclusion of a third group of patients with genetically-determined DCM in secondary analysis allowed us to study potential overlap in biomarker profile in ACMP as compared to DCM. Interestingly, we did not find significant differences between ACMP cases and DCM patients in plasma levels of the proteins upregulated in ACMP and the majority of proteins identified with elastic net. This overlap in upregulation strengthens the hypothesis that these proteins are associated with cardiomyopathy and do not reflect a systemic sensitivity for anthracyclines.

Despite the association of CCL19, CCL20 and PSPD with cardiomyopathy in our study, the cellular source(s) contributing to the elevated plasma levels remain uncertain. CCL19 and CCL20 are chemokines secreted by immune cells and cardiac fibroblasts in the heart under the influence

of pro-inflammatory cytokines, but also by peripheral immune cells residing in lymph nodes.(21) PSPD is an innate immune pattern recognition collection expressed in the myocardium, but also in the lung and the vascular endothelium.(22) In addition, previous studies in heart failure patients have demonstrated discrepancies between plasma and myocardial protein levels of other inflammatory proteins, such as galectin-3, GDF-15, TNF and IL-6.(23, 24) It is therefore likely that the elevated plasma levels found in our study in ACMP and DCM are to a large extent produced by extracardiac sources such as peripheral immune cells or vascular endothelial cells.

As for clinical utility, it is promising that the biomarker panel had a high sensitivity, while maintaining sufficient specificity to limit false positives, even in those patients in whom NT-proBNP could not discriminate between ACMP and controls. However, clinical utility of the identified plasma biomarker levels for the diagnosis of LV dysfunction may be better assessed in larger cohorts and will be the subject of ongoing research.

Limitations

One may question the generalizability of our results to a surveillance population because we defined cardiomyopathy as a LVEF<45% and because 8 patients already had symptoms of heart failure. However, the LVEF thresholds of <45% for ACMP and ≥53% for controls made it possible to make a clear distinction between ACMP and controls, which was of importance in this discovery study. We also replicated the results in ACMP cases without heart failure. The patients with DCM were not matched to the ACMP patients. However, we adjusted the analyses for differences in sex, age and LVEF. This study should be seen as exploratory, with the purpose to select promising biomarker candidates to further study for their diagnostic value to detect asymptomatic cardiomyopathy in the DCCSS LATER 2 CARD cohort.(11)

CONCLUSION

We identified the chemokine ligands CCL19 and CCL20 and the innate immune system marker PSPD as candidate diagnostic plasma biomarkers for anthracycline-related cardiomyopathy in long-term CCS. By demonstrating overlap in expression of these biomarkers with those found in patients with genetically-determined DCM the hypothesis is strengthened that these protein markers are related to cardiac dysfunction. Further exploration and validation of the findings in a larger cohort is still needed.

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REFERENCES

1. Feijen E, Font-Gonzalez A, Van der Pal HJH, Kok WEM, Geskus RB, Ronckers CM, et al. Risk and Temporal Changes of Heart Failure Among 5-Year Childhood Cancer Survivors: a DCOG-LATER Study. Journal of the American Heart Association. 2019;8(1):e009122.
2. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2015;16(3):e123-36.
3. Bossuyt PM, Irwig L, Craig J, Glasziou P. Comparative accuracy: assessing new tests against existing diagnostic pathways. BMJ (Clinical research ed). 2006;332(7549):1089-92.
4. Leerink JM, Verkleij SJ, Feijen EAM, Mavinkurve-Groothuis AMC, Pourier MS, Ylänen K, et al. Biomarkers to diagnose ventricular dysfunction in childhood cancer survivors: a systematic review. Heart (British Cardiac Society). 2019;105(3):210.
5. Toro-Salazar OH, Lee JH, Zellars KN, Perreault PE, Mason KC, Wang Z, et al. Use of integrated imaging and serum biomarker profiles to identify subclinical dysfunction in pediatric cancer patients treated with anthracyclines. Cardio-Oncology. 2018;4(1):4.
6. Armenian SH, Gelehrter SK, Vase T, Venkatramani R, Landier W, Wilson KD, et al. Screening for cardiac dysfunction in anthracycline-exposed childhood cancer survivors. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(24):6314-23.
7. Armenian SH, Gelehrter SK, Vase T, Venkatramani R, Landier W, Wilson KD, et al. Carnitine and cardiac dysfunction in childhood cancer survivors treated with anthracyclines. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2014;23(6):1109-14.
8. Beer LA, Kossenkov AV, Liu Q, Luning Prak E, Domchek S, Speicher DW, et al. Baseline Immunoglobulin E Levels as a Marker of Doxorubicin- and Trastuzumab-Associated Cardiac Dysfunction. Circulation research. 2016;119(10):1135-44.
9. Ky B, Putt M, Sawaya H, French B, Januzzi JL, Sebag IA, et al. Early Increases in Multiple Biomarkers Predict Subsequent Cardiotoxicity in Patients With Breast Cancer Treated With Doxorubicin, Taxanes, and Trastuzumab. Journal of the American College of Cardiology. 2014;63(8):809-16.
10. Tromp J, Boerman LM, Sama IE, Maass S, Maduro JH, Hummel YM, et al. Long-term survivors of early breast cancer treated with chemotherapy are characterized by a pro-inflammatory biomarker profile compared to matched controls. European journal of heart failure. 2020;22(7):1239-46.
11. Leerink JM, Feijen E, van der Pal HJH, Kok WEM, Mavinkurve-Groothuis AMC, Kapusta L, et al. Diagnostic tools for early detection of cardiac dysfunction in childhood cancer survivors: Methodological aspects of the Dutch late effects after childhood cancer (LATER) cardiology study. Am Heart J. 2020;219:89-98.
12. Feijen EAM, Leisenring WM, Stratton KL, Ness KK, van der Pal HJH, van Dalen EC, et al. Derivation of Anthracycline and Anthraquinone Equivalence Ratios to Doxorubicin for Late-Onset Cardiotoxicity. JAMA oncology. 2019;5(6):864-71.
13. Sammani A, Jansen M, Linschoten M, Bagheri A, de Jonge N, Kirkels H, et al. UNRAVEL: big data analytics research data platform to improve care of patients with cardiomyopathies using routine electronic health records and standardised biobanking. Neth Heart J. 2019;27(9):426-34.
14. Merkx R, Leerink JM, Feijen E, Kremer LCM, de Baat EC, Bellersen L, et al. Echocardiography protocol for early detection of cardiac dysfunction in childhood cancer survivors in the multicenter DCCSS LATER 2 CARD study: Design, feasibility, and reproducibility. Echocardiography. 2021.
15. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(16):9440-5.

16. Zou H, Hastie T. Regularization and Variable Selection via the Elastic Net. Journal of the Royal Statistical Society Series B (Statistical Methodology). 2005;67(2):301-20.

17. Wan GX, Ji LH, Xia WB, Cheng L, Zhang YG. Bioinformatics identification of potential candidate blood indicators for doxorubicin-induced heart failure. Experimental and therapeutic medicine. 2018;16(3):2534-44.

18. de Boer RA, Nayor M, deFilippi CR, Enserro D, Bhambhani V, Kizer JR, et al. Association of Cardiovascular Biomarkers With Incident Heart Failure With Preserved and Reduced Ejection Fraction. JAMA Cardiol. 2018;3(3):215-24.

19. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, et al. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. Journal of the American College of Cardiology. 2012;60(14):1249-56.

20. Vasan RS, Sullivan LM, Roubenoff R, Dinarello CA, Harris T, Benjamin EJ, et al. Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: the Framingham Heart Study. Circulation. 2003;107(11):1486-91.

21. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu Rev Immunol. 2014;32:659-702.

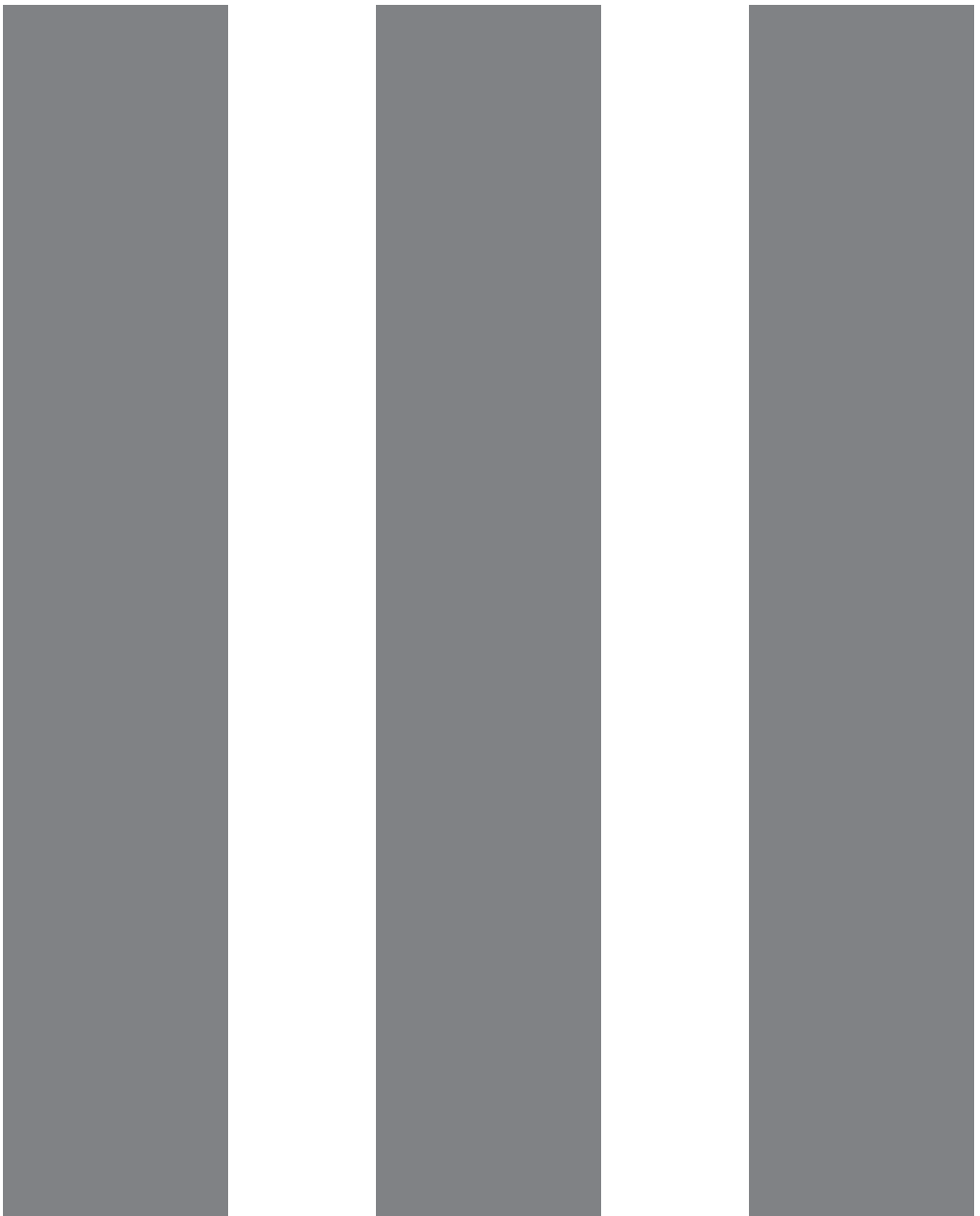
22. Sorensen GL. Surfactant Protein D in Respiratory and Non-Respiratory Diseases. Frontiers in Medicine. 2018;5(18).

23. Petretta M, Condorelli GL, Spinelli L, Scopacasa F, de Caterina M, Leosco D, et al. Circulating levels of cytokines and their site of production in patients with mild to severe chronic heart failure. Am Heart J. 2000;140(6):E28.

24. Du W, Piek A, Schouten EM, van de Kolk CWA, Mueller C, Mebazaa A, et al. Plasma levels of heart failure biomarkers are primarily a reflection of extracardiac production. Theranostics. 2018;8(15):4155-69.

SUPPLEMENTAL MATERIAL

The supplemental material is available at https://doi.org/10.1161/jaha.121.025935.



SURVEILLANCE FOR CARDIAC DYSFUNCTION IN CHILDHOOD CANCER SURVIVORS

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SYSTEMATIC REVIEW AND UPDATED CARDIOMYOPATHY SURVEILLANCE RECOMMENDATIONS FOR SURVIVORS OF CHILDHOOD, ADOLESCENT, AND YOUNG ADULT CANCER FROM THE INTERNATIONAL LATE EFFECTS OF CHILDHOOD CANCER GUIDELINE HARMONIZATION GROUP

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SUMMARY

Survivors of childhood, adolescent, and young adult cancer, previously treated with anthracycline chemotherapy (including mitoxantrone) or radiotherapy in which the heart was exposed, are at increased risk of cardiomyopathy. Symptomatic cardiomyopathy is typically preceded by a series of gradually progressive, asymptomatic changes in structure and function of the heart that can be ameliorated with treatment, prompting specialist organisations to endorse guidelines on cardiac surveillance in at-risk survivors of cancer. In 2015, the International Late Effects of Childhood Cancer Guideline Harmonization Group compiled these guidelines into a uniform set of recommendations applicable to a broad spectrum of clinical environments with varying resource availabilities. Since then, additional studies have provided insight into dose thresholds associated with a risk of asymptomatic and symptomatic cardiomyopathy, have characterised risk over time, and have established the cost-effectiveness of different surveillance strategies. This systematic Review and guideline provides updated recommendations based on the evidence published up to September, 2020.

INTRODUCTION

Survivors of childhood, adolescent, and young adult (CAYA) cancer who have been previously exposed to anthracycline chemotherapy or the anthraquinone mitoxantrone (both of which are hereafter referred to as anthracycline), or radiotherapy in which the heart was exposed, are at increased risk for heart failure. Survivors exposed to doxorubicin-equivalent anthracycline doses of less than 250 mg/m² are at an approximately three-times increased risk of heart failure, and survivors exposed to at least 250 mg/m² are at an approximately nine-times increased risk of heart failure, compared with unexposed survivors.(1–5) Notably, survivors are seven times more likely to die from heart failure than their siblings.(6) Heart failure is typically preceded by a series of progressive asymptomatic changes in structure and function of the heart that occur over time. Timely identification of survivors at risk of heart failure is essential to expedite treatment and improve prognosis.(3,7) Asymptomatic changes (eg, impaired global longitudinal strain, reduction in left-ventricular ejection fraction [LVEF], or the presence of late gadolinium enhancement of the left ventricle) can be detected by echocardiography or cardiac magnetic resonance (CMR) imaging. Since the development of the first International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) cardiomyopathy guideline in 2015,(3) several reports have refined dose thresholds associated with cardiomyopathy risk, investigated longitudinal cohorts to identify risk over time, and established the costeffectiveness of different surveillance strategies. In this Review, we assessed and graded the quality of studies included in the previous IGHG guideline and those published since, summarised available evidence, and formulated recommendations supported by these data.

METHODS

The IGHG cardiac guideline development panel included a working group of 34 international experts. Panellists were selected on the basis of expertise in paediatric and radiotherapy oncology, cancer survivorship, cardiology, epidemiology, and guideline methodology (appendix pp 2–3).

Scope and definitions

The objective of the current guidelines are to provide tailored cardiomyopathy surveillance recommendations for health-care providers and survivors of CAYA cancer who were younger than 25 years at diagnosis, are at least 2 years from the completion of cancer therapy, and were previously treated with anthracycline chemotherapy or radiotherapy in which the heart was exposed (hereafter referred to as chest-directed radiotherapy).

Heart failure was defined according to the European Society of Cardiology and American Heart Association guidelines (appendix pp 4–5). Asymptomatic left- ventricular systolic or diastolic dysfunction (ALVD) was defined according to the American Society of Echocardiography and the Echocardiography and the European Association Cardiovascular Imaging recommendations (appendix pp 5–6).

Systematic literature review

We performed a systematic literature review according to previously published IGHG methods (figure 1).(3,8) The expert panel developed clinical queries regarding anthracycline-related and radiotherapy-related cardio- myopathy to evaluate: (1) who needs cardiomyopathy surveillance; (2) what surveillance modality should be used; (3) at what frequency should cardio- myopathy surveillance be performed; and (4) what should be done when abnormalities are identified (appendix pp 6–10).

Search strategy and selection criteria

We used MEDLINE (via PubMed) to update the previous systematic literature search, including studies published between Jan 1, 2013, and Sept 30, 2020. Specific search terms can be found in appendix (pp 11–16). Manuscripts and references were reviewed by two or more expert panel members to ensure key studies were not overlooked. Detailed study inclusion criteria are provided in the appendix (p 17). Eligible populations included cohorts of survivors of childhood, adolescent, and young adult cancer of whom at least 75% had been diagnosed with cancer at younger than 25 years and at least 50% had been followed up 2 years or more after the completion of therapy, with all participants having completed cancer treatment. Eligible cancer treatment exposures included anthracyclines (including mitoxantrone), chest-directed radiotherapy, and novel cancer treatments (appendix pp 4–5). Primary outcomes included heart failure and asymptomatic left ventricular systolic or diastolic dysfunction (ALVD). For study eligibility, we accepted all definitions of heart failure and ALVD and all study designs except case reports and case series. The minimum sample size for inclusion was 100 for studies reporting risk factors and risk over time and 20 for studies reporting the other clinical questions (eg, what surveillance modality should be used and what should be done when abnormalities are identified).

Levels of evidence and strength of recommendations

We used previously standardised data-extraction templates in the summarising of the evidence for newly identified studies.(8) The quality of previously published(3) and newly identified evidence was graded with the established Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) methods (appendix pp 18–23).(9–11) We graded the quality of the total body of evidence as high quality (ie, additional research is unlikely to change the confidence in the estimate of effect), moderate quality (ie, additional research is likely to have an important effect on the confidence in the estimate of effect and might change the estimate), low quality (ie, additional research is very likely to have an important effect on the confidence in the estimate of effect and is likely to change the estimate), or very low quality (ie, any estimate of effect is very uncertain). The level of the evidence decreased for study limitations, inconsistency of results between studies, indirectness of the study populations or outcomes, imprecision of the effect estimates, or the presence of publication bias. The level of the evidence increased if effect sizes were large or there was evidence for a dose-response relationship or plausible confounding. Two authors, MJE and JML, did the GRADE assessments and discrepancies were resolved by an additional author as needed. If no

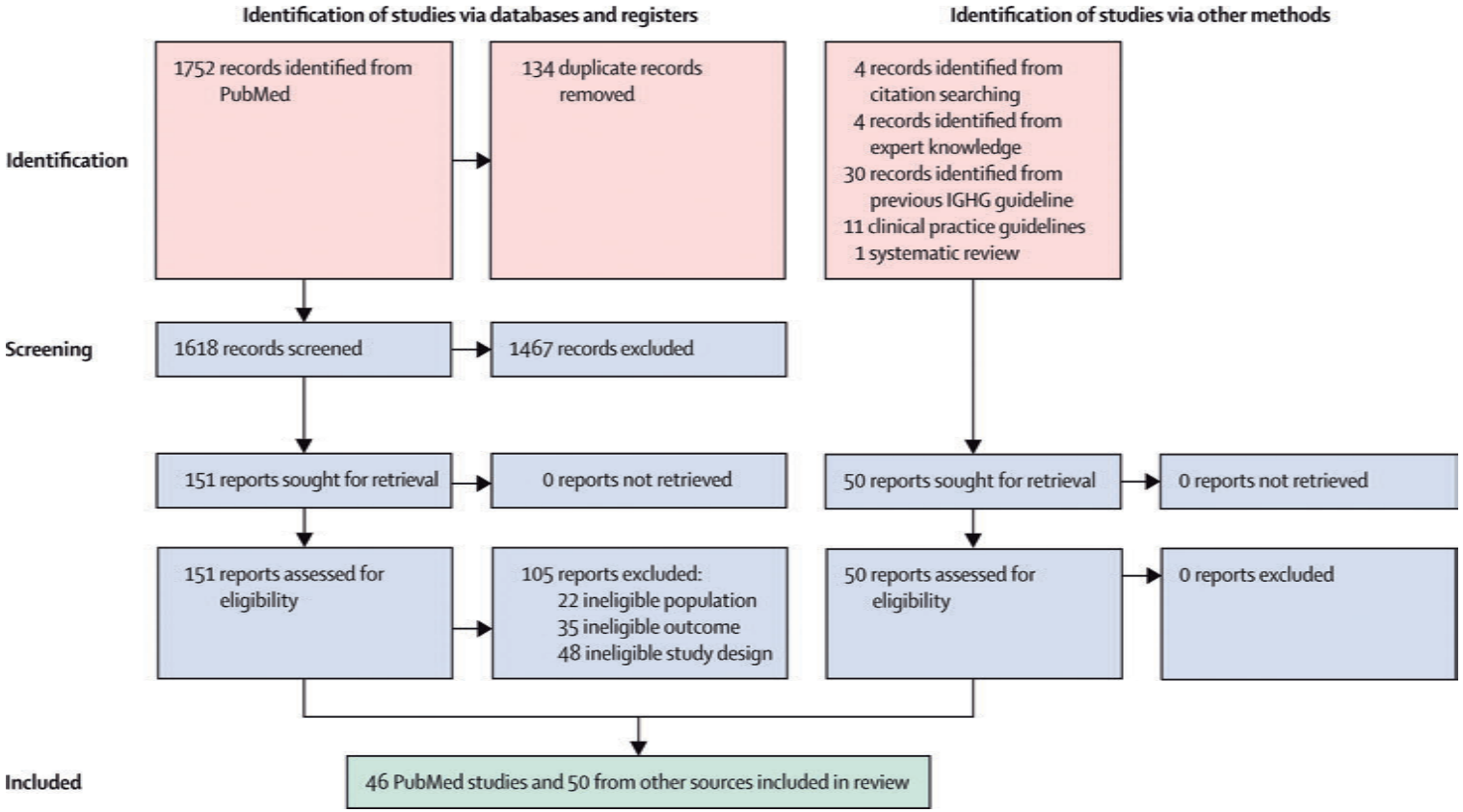

Figure 1. PRISMA flow diagram. IGHG=International Late Effects of Childhood Cancer Guideline Harmonization Group. PRISMA=Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

evidence was identified, relevant information was extrapolated from clinical practice guidelines in the general population. For genetic variants associated with anthracycline- induced cardiotoxicity, quality of evidence was adopted from the Canadian Pharmacogenomics Network for Drug Safety and the pharmacogenetics database (appendix pp 23–24).

Translating evidence into recommendations

Systematically collated evidence was translated into clinical recommendations according to the GRADE evidence-to-decision framework.(12) Surveillance recommendations were formulated following consideration of the quality of evidence, magnitude of effect, costs versus benefits of surveillance, ability to effectively intervene when reduced cardiac function was detected, and generalisability across various health-care systems with varying resource availability. As in the previous guidelines,(3) survivors at an increased risk for heart failure of less than 1·5 times compared with unexposed survivors were considered to be at low risk, 1·5–4 times were considered to be at moderate risk, and 4 times or higher were considered to be at high risk.

RESULTS

Our updated search yielded 1618 reports for title and abstract review, of which 46 met the inclusion criteria and underwent data abstraction. Also included were four studies identified from citation searching, four from expert knowledge, 11 clinical practice guidelines, and one systematic review. An additional 30 studies from the previous report were reviewed and quality was graded to harmonise the quality assessment. We identified high- quality studies supporting changes to the previous guidelines with respect to radiation dose thresholds associated with cardiomyopathy, surveillance intensity, and the need for surveillance in the low-risk group (figure 2). The appendix includes studies from the updated literature search (appendix pp 24–35), evidence tables (appendix pp 36–189), summary of findings (appendix pp 189–308), and the conclusions of evidence (appendix pp 309–18).

Treatment-related risk factors for cardiomyopathy

High cumulative anthracycline and chest-directed radio-therapy doses were associated with an increased risk of cardiomyopathy in survivors of CAYA cancer. We identified high-quality evidence that survivors of CAYA cancer exposed to doxorubicin-equivalent(1,13) cumulative anthracycline doses of at least 250 mg/m2 were at more than a five times increased risk of heart failure compared with patients who received none.(1,2,5,13–20) Similarly, the risk of ALVD, as measured by left-ventricular ejection fraction, was 2·7 to 3·8 times higher in patients treated with 100–300 mg/m2 and more than four times higher in patients treated with over 300 mg/m2 of anthracyclines compared with patients who received none.(21–23) In contrast, patients treated with cumulative anthracycline doses of less than 100 mg/m2 were not at a significantly increased risk of heart failure or ALVD compared with those who received none.(14,16,17,21,23)

We identified novel, moderate-quality evidence suggesting differential potency associated with anthra- cycline derivatives with respect to heart failure,(13,19) whereas previous estimations were

2015	2022
General recommendation	
Survivors treated with anthracyclines or chest radiation (RT) or both and their healthcare providers should be aware of the risk of cardiomyopathy	CAYA cancer survivors treated with anthracyclines, chest RT, or both (high-quality evidence), and their health care providers should be aware of the risk of cardiomyopathy (strong recommendation).
Who needs cardiomyopathy surveillance?	
Anthracyclines and/or mitoxantrone (as doxorubicin equivalent dose) alone	
Cardiomyopathy surveillance is recommended for survivors treated with high dose (≥250 mg/m^2) anthracyclines.	Cardiomyopathy surveillance is recommended for CAYA cancer survivors treated with high dose (≥250 mg/m^2) anthracyclines (high-quality evidence, strong recommendation)
Cardiomyopathy surveillance is reasonable for survivors treated with moderate dose (≥100 to <250 mg/m^2) anthracyclines.	Cardiomyopathy surveillance is reasonable for CAYA cancer survivors treated with moderate dose (≥100 to <250 mg/m^2) anthracyclines (high-quality evidence, moderate recommendation).
Cardiomyopathy surveillance may be reasonable for survivors treated with low dose (<100 mg/m^2) anthracyclines.	**Cardiomyopathy surveillance is not recommended for CAYA cancer survivors treated with low dose (<100 mg/m^2) anthracyclines (high-quality evidence, strong recommendation).**
Chest-directed radiotherapy alone	
Cardiomyopathy surveillance is recommended for survivors treated with high dose (≥35 Gy) chest RT.	Cardiomyopathy surveillance is recommended for CAYA cancer survivors treated with high dose **(≥30 Gy)** chest RT (high-quality evidence, strong recommendation).
Cardiomyopathy surveillance may be reasonable for survivors treated with moderate dose (≥15 to <35 Gy) chest RT.	Cardiomyopathy surveillance is reasonable for CAYA cancer survivors treated with moderate dose **(≥15 to <30 Gy)** chest RT (high-quality evidence, moderate recommendation).
No recommendation can be formulated for cardiomyopathy surveillance for survivors treated with low dose (<15 Gy) chest RT with conventional fractionation.	**Cardiomyopathy surveillance is not recommended for CAYA cancer survivors treated with low dose (<15 Gy) chest RT with conventional fractionation (high-quality evidence, strong recommendation).**
Anthracyclines and chest-directed radiotherapy	
Cardiomyopathy surveillance is recommended for survivors treated with moderate to high dose anthracyclines (≥100 mg/m^2) and moderate to high dose chest RT (≥15 Gy).	Cardiomyopathy surveillance is recommended for CAYA cancer survivors treated with moderate to high dose anthracyclines (≥100 mg/m^2) and moderate to high dose chest RT (≥15 Gy) (high-quality evidence, strong recommendation).

Figure 2. Cardiomyopathy surveillance recommendations formulated in 2015 versus 2022. Green=strong recommendation to do; Orange=moderate recommendation to do; Red=recommendation not to do. 2D=two-dimensional. 3D=three-dimensional. ACE=angiotensin converting enzyme. ARB=angiotensin receptor blocker. CAYA=childhood, adolescent, and young adult. CMR=cardiac magnetic resonance. *Left-ventricular systolic and diastolic dysfunction as defined by the America Society of Echocardiography and the European Association of Cardiovascular Imaging (appendix pp 4–5).

2015	2022
Dexrazoxane	
-	No recommendation can be formulated to change cardiomyopathy surveillance in CAYA cancer survivors who received dexrazoxane cardioprotection with anthracycline administration (low-quality evidence).
Pregnancy	
Cardiomyopathy surveillance is reasonable before pregnancy or in the first trimester for all female survivors treated with anthracyclines or chest RT.	Cardiomyopathy surveillance is reasonable before pregnancy or in the first trimester for moderate and high-risk female CAYA cancer survivors treated with anthracyclines or chest RT (moderate-quality evidence, moderate recommendation).
No recommendations can be formulated for the frequency of ongoing surveillance in pregnant survivors who have normal left ventricular (LV) systolic function immediately before or during the first trimester of pregnancy.	Continuing cardiomyopathy surveillance is reasonable during pregnancy for survivors of CAYA cancer treated with any dose of anthracyclines or chest-directed radiotherapy who had a history of previous left- ventricular systolic dysfunction that has resolved, even in the presence of a normal baseline ejection fraction, in the first trimester (moderate-quality evidence, moderate recommendation)
Genetic variants	
-	No recommendation can be formulated for cardiomyopathy surveillance in CAYA cancer survivors carrying a genetic variant that increases or decreases the risk of developing cardiomyopathy (low-quality evidence).
What surveillance modality should be used?	
Echocardiography is recommended as the primary cardiomyopathy surveillance modality for assessment of LV systolic function in survivors treated with anthracyclines or chest RT.	LV ejection fraction measured with 2D or 3D echocardiography is recommended as the primary cardiomyopathy surveillance modality for assessment of LV systolic function in CAYA cancer survivors treated with anthracyclines or chest RT (moderate-quality evidence, strong recommendation).
Radionuclide angiography or CMR may be reasonable for cardiomyopathy surveillance in at-risk survivors for whom echocardiography is not technically feasible or optimal. Assessment of cardiac blood biomarkers (e.g., natriuretic peptides) in conjunction with imaging studies may be reasonable in instances where symptomatic cardiomyopathy is strongly suspected or in individuals who have borderline cardiac function during primary surveillance.	Cardiac magnetic resonance imaging may be reasonable for cardiomyopathy surveillance in at-risk CAYA cancer survivors for whom echocardiography is not technically feasible or optimal (expert opinion, moderate recommendation). Assessment of cardiac blood biomarkers (e.g., natriuretic peptides) in conjunction with imaging studies may be reasonable in instances where symptomatic cardiomyopathy is strongly suspected or in CAYA cancer survivors who have borderline cardiac function during primary surveillance (expert opinion, moderate recommendation).

Figure 2. Cardiomyopathy surveillance recommendations formulated in 2015 versus 2022. (continued)

2015	2022
Assessment of cardiac blood biomarkers (e.g., natriuretic peptides and troponins) is not recommended as the only strategy for cardiomyopathy surveillance in at-risk survivors.	Assessment of cardiac blood biomarkers (e.g., natriuretic peptides and troponins) is not recommended as the only strategy for cardiomyopathy surveillance in at-risk CAYA cancer survivors (low- to moderate-quality evidence, strong recommendation).
At what frequency should cardiomyopathy surveillance be performed?	
High-risk CAYA cancer survivors	
Cardiomyopathy surveillance is recommended for high-risk survivors to begin no later than 2-years after completion of cardiotoxic therapy, repeated at 5-years after diagnosis and continued every 5 years thereafter.	Cardiomyopathy surveillance is recommended for high-risk CAYA cancer survivors to begin no later than 2 years after completion of cardiotoxic therapy and continued **every 2 years thereafter** (moderate-quality evidence, strong recommendation).
More frequent cardiomyopathy surveillance is reasonable for high-risk survivors.	-
Lifelong cardiomyopathy surveillance may be reasonable for high-risk survivors.	Lifelong cardiomyopathy surveillance is reasonable for high-risk CAYA cancer survivors (expert opinion, moderate recommendation).
Moderate-risk CAYA cancer survivors	
Cardiomyopathy surveillance is reasonable for moderate- and low-risk survivors to begin no later than 2 years after completion of cardiotoxic therapy, repeated at 5-years after diagnosis, and continue every 5 years thereafter.	Cardiomyopathy surveillance is reasonable for **moderate-risk** CAYA cancer survivors to begin no later than 2 years after completion of cardiotoxic therapy, repeated at 5 years after diagnosis, and continue every 5 years thereafter (low-quality evidence, moderate recommendation).
More frequent cardiomyopathy surveillance may be reasonable for moderate- and low-risk survivors.	-
Lifelong cardiomyopathy surveillance may be reasonable for moderate- and low-risk survivors.	Lifelong cardiomyopathy surveillance is reasonable for moderate-risk CAYA cancer survivors (expert opinion, moderate recommendation).
Low-risk CAYA cancer survivors	
Cardiomyopathy surveillance is reasonable for moderate and low-risk survivors to begin no later than 2 years after completion of cardiotoxic therapy, repeated at 5 years after diagnosis and continue every 5 years thereafter.	Cardiomyopathy surveillance is not recommended in **low-risk** CAYA cancer survivors **(**moderate-quality evidence).
What should be done when abnormalities are identified?	
Cardiology consultation is recommended for survivors with asymptomatic cardiomyopathy following treatment with anthracyclines or chest RT.	Cardiology consultation is recommended for CAYA cancer survivors with **asymptomatic LV systolic or diastolic dysfunction**** following treatment with anthracyclines or chest RT (expert opinion, strong recommendations).
-	Treatment with heart failure medications (ACE inhibitors, ARBs, beta-blockers) is recommended in CAYA cancer survivors with asymptomatic LV ejection fraction <40%, according to guidelines from the general population (low- to high-quality evidence in the general population, strong recommendation).

Figure 2. Cardiomyopathy surveillance recommendations formulated in 2015 versus 2022. (continued)

2015	2022
	No recommendations can be formulated about treatment with heart failure medications in CAYA cancer survivors with asymptomatic borderline (LV ejection fraction between 40% and the lower limit of normal) cardiac function (no studies in CAYA cancer survivors, no evidence in the general population).
Advice regarding physical activity and modifiable cardiovascular risk factors	
Cardiology consultation is recommended for survivors with asymptomatic cardiomyopathy to define limits and precautions for exercise.	Cardiology consultation is recommended for CAYA cancer survivors with asymptomatic cardiomyopathy to define limits and precautions for exercise (expert opinion, strong recommendation).
Cardiology consultation may be reasonable for high-risk survivors who plan to participate in high intensity exercise to define limits and precautions for physical activity.	Cardiology consultation is reasonable for high-risk CAYA cancer survivors who plan to participate in high intensity exercise to define limits and precautions for physical activity (expert opinion, moderate recommendation).
Screening for modifiable cardiovascular risk factors (hypertension, diabetes, dyslipidemia, and obesity) is recommended for all survivors treated with anthracyclines or chest RT so that necessary interventions can be initiated to help avert the risk of symptomatic cardiomyopathy.	Screening for and management of modifiable cardiovascular risk factors (hypertension, diabetes, dyslipidemia, obesity, smoking, alcohol intake) is recommended for all CAYA cancer survivors treated with anthracyclines or chest RT to help avert the risk of symptomatic cardiomyopathy (evidence-based guidelines, strong recommendation).

Figure 2. Cardiomyopathy surveillance recommendations formulated in 2015 versus 2022. (continued)

derived from associations with haematopoietic toxicity. In the first of these pooled cohort studies, investigators reported a 50% reduction in the risk of heart failure with daunorubicin compared with doxorubicin (hazard ratio [HR] 0·45, 95% CI 0·23–0·73).(13) A follow-up report expanded the initial study population and identified an approximately 10:1 potency of mitoxantrone compared with doxorubicin (HR 10·5, 95% CI 6·2–19·1), a substantial increase from the widely accepted 4:1 ratio.(19)

Survivors of CAYA cancer who have been exposed to chest-directed radiotherapy were also at increased risk for cardiomyopathy. High-quality evidence supports an increase in heart failure of 1·6 to 6·1 times among survivors exposed to chest doses ranging from 15–34 Gy,(1,2,5,14–16,18–20) whereas in studies that exclusively evaluated the risk in individuals exposed to doses of 30–35 Gy or higher, the risk ranged from 3·5 to 19·7 times that observed in unexposed individuals.(1,2,5,14–16,18–20) Three studies specifically investigated a dose threshold of at least 30 Gy and found a 6·7 to 20·6 times increased risk of heart failure compared with unexposed individuals,(15,18,20) suggesting a need to revise the previous threshold of more than 35 Gy to define individuals at high risk(3) to more than 30 Gy. In contrast, those exposed to chest doses less than 15 Gy without anthracyclines were not at a significantly increased risk of heart failure compared with those who received none.(2,5,14–16,18,20,24)

Several studies with high-quality evidence reported a higher risk of heart failure in individuals who had received both anthracyclines and chest-directed radiotherapy compared with individuals who had received either treatment alone.(1,4,15,18,19,24,25) However, no study investigated this

association with respect to asymptomatic cardiomyopathy. We identified no study that reported the risk of cardiomyopathy with novel therapies (eg, checkpoint inhibitors and tyrosine kinase inhibitors; appendix pp 4–5) in survivors of CAYA cancer.

Health-care providers have coadministered dexrazoxane and anthracycline chemotherapy to potentially mitigate the risk of cardiomyopathy. A 2022 meta-analysis and IGHG guideline found low-quality evidence for a lowered risk of heart failure in anthracycline-treated children (pooled relative risk 0·20, 95% CI 0·01–4·19) and adults (0·22, 0·11–0·43) who received dexrazoxane.(26) However, there are no data on the effect of dexrazoxane use on surveillance practices in survivors of CAYA cancer, highlighting a crucial need for studies quantifying its effect on risk stratification.

Other risk factors for cardiomyopathy

We found moderate-quality evidence of an increased risk of heart failure in female survivors of CAYA cancer compared with male survivors.(1–5,13–25,27–94) The increased risk of ALVD was not statistically significant in female survivors compared with male survivors in most studies reviewed; however, most studies did not use the currently recommended sex-specific definitions of ALVD,(94) which might have contributed to this finding (appendix pp 4–5). We found low-quality evidence for an association between a younger age at diagnosis and an increased risk of heart failure, but no significant association with ALVD.(1–5,13–25,27–94)

Our search yielded a range of studies that reported on the association between traditional cardiovascular risk factors and the risk of anthracycline-associated or radiotherapy-associated cardiomyopathy. Most notably, high-quality and moderate-quality evidence supported the association between hypertension and heart failure(5,14,16,25,27,28,95) and ALVD.(21,29,30) Armstrong and colleagues(95) showed a multiplicative interaction between hypertension and both anthracyclines and chest-directed radiotherapy, highlighting the need to remain vigilant for hypertension in survivors otherwise at risk for heart failure related to previous treatments for cancer.

Regarding the risk of peri-pregnancy ALVD or heart failure, we identified a meta-analysis of six studies of moderate-quality evidence that suggested that pregnant survivors of CAYA cancer without a previous diagnosis of chemotherapy-related cardiac dysfunction were at low risk of developing ALVD or heart failure during pregnancy (incidence 0·24%, 95% CI 0–0·81%), whereas the risk was substantial in those with a previous diagnosis of chemotherapy-related cardiac dysfunction (28%, 15–44%).(31)

We identified one systematic review and four additional studies investigating 38 genetic variants possibly associated with risk modification of ALVD and heart failure in survivors of CAYA cancer treated with anthracyclines (appendix pp 243–52). Of these 38 variants, only RARG rs222977432,(90) and UGT1A6 rs1786378333,(34,90) had moderate- quality evidence to support an association with an increased risk of ALVD or heart failure in anthracycline-exposed survivors of CAYA cancer (appendix pp 309–18).

Diagnostic value of surveillance modalities for ALVD

Due to the relatively low cost and widespread availability, two-dimensional (2D) echocardiography has been the preferred ALVD surveillance modality in survivors of CAYA cancer. The emergence of CMR and serum bio- markers prompted us to review the evidence regarding the use of these modalities for cardiomyopathy surveillance assessments in survivors of CAYA cancer. Although CMR is known to have less inter-observer and intra-observer variability than echocardiography, we found moderate- quality evidence supporting reasonable agreement between CMR and motion-mode echocardiography (mean difference in absolute LVEF ranging from 3·1% to 5·5% lower by CMR), 2D echocardiography (mean difference in absolute LVEF ranging from 1·8% to 5·4% lower by CMR), and three-dimensional (3D) echocardiography (mean difference in absolute LVEF ranging from 1·1% higher to 7·7% lower by CMR).(35–37) No studies on the survivors of CAYA cancer directly compared 2D to 3D echocardiography. However, studies from the general population suggest that although 2D LVEF had higher variance than 3D LVEF, both echocardiographic modalities had low average bias compared with CMR-measured LVEF (2D LVEF 0·1%; 3D LVEF 0·0%).(38,94)

Several studies have investigated associations between serum biomarkers and asymptomatic cardiomyopathy in survivors of CAYA cancer. In general, biomarkers showed relatively low sensitivity and high specificity in survivors of CAYA cancer. Numerous studies reported low sensitivity (8–32% excluding a single study in which the abnormal threshold was not defined) and high specificity (81–100%) for N-terminal pro-brain natriuretic peptide compared with either echocardiography or CMR for detection of asymptomatic cardiomyopathy.(36,39–48) We identified only one study, of low-quality evidence, that reported moderate sensitivity (63%) of atrial or brain natriuretic peptide for asymptomatic cardiomyopathy in survivors of CAYA cancer.(50) Moderate-quality evidence suggested that troponin T and troponin I have low sensitivity (0–50%) for asymptomatic cardiomyopathy in survivors of CAYA cancer.(39,40,42–44,47–49)

Cost-effectiveness of cardiomyopathy surveillance

We identified three studies that investigated the cost- effectiveness of echocardiography for cardiomyopathy surveillance using variably defined groups at risk of cardiomyopathy.(51–53) These studies, of moderate-quality evidence, showed that surveillance was cost-effective in survivors of CAYA cancer who are at high risk for heart failure but suggested that surveillance performed at any frequency in survivors at low risk was either of little benefit or not cost-effective. A study published in 2020 specifically evaluated the effectiveness of performing surveillance echocardiograms according to the 2015 IGHG-defined, risk-stratified groups of survivors who have been exposed to anthracyclines or chest-directed radiotherapy.(53) The study found that the most cost-effective strategies were 2-year interval echocardiography in survivors at high risk (ie, survivors who received cumulative anthracyclines ≥250 mg/m2, chest-directed radiotherapy ≥35 Gy, or a combination of cumulative anthracyclines ≥100 mg/m2 and chest-directed radiotherapy ≥15 Gy) and no surveillance in survivors at low risk (i.e., survivors who received cumulative anthracyclines 1–99 mg/m2 or chest-directed radiotherapy <15 Gy). Both findings remained consistent despite testing a wide range of values for model input parameters (e.g., echocardiogram sensitivity or specificity and treatment

effectiveness). However, when evaluating the same model input variables in individuals at moderate risk (ie, survivors who received cumulative anthracyclines 100 to <250 mg/m2 or chest-directed radiotherapy 15 to <35 Gy), the findings were less consistent.(53) Thus, there is only low-quality evidence describing the cost-effectiveness of the surveillance of survivors at moderate risk of CAYA cancer every 5 years.

Latency to onset of cardiomyopathy

Few studies reported on the latency to onset of ALVD or heart failure after anthracycline or chest-directed radiotherapy exposure. Although moderate-quality evidence exists reporting the latency in survivors exposed to both treatment modalities, the ranges were wide (0·1–35·7 years for ALVD and 1–42 years for heart failure), making interpretation of the results difficult.(4,54–60) Moderate-quality evidence supports an increasing cumulative incidence of heart failure over time in individuals exposed to higher doses of anthracyclines,(2,5,14–16,18,20,28,33,51–53,61,62) whereas very low-quality evidence suggests a plateau in risk of ALVD over time in survivors of CAYA cancer treated with doses of less than 250 mg/m^2.(52,55,58,63)

Interventions to prevent heart failure in survivors of CAYA cancer

Our search identified one systematic review,(97) which included only one randomized controlled trial, evaluating the effect of pharmacological interventions for ALVD identified on surveillance echocardiograms. Silber and colleagues(64) conducted a double-blind, randomized, controlled, clinical trial comparing enalapril to placebo in asymptomatic anthracycline-exposed survivors of CAYA cancer who had one or more cardiac abnormalities identified at any time during or after cancer treatment (i.e., fractional shortening ≤29%, 10% decrease in fractional shortening compared with before treatment, gated nuclear angiography LVEF ≤55%, decrease in LVEF with exercise, 10% decrease in LVEF over time, maximal cardiac index ≤7·4 L/min per m2, and QTc interval ≥440 ms). The study found no differences in maximal cardiac index during exercise testing after 1 year of treatment (i.e., the primary outcome). As a secondary outcome, the study reported a sustained rate of improvement in left-ventricular end-systolic wall stress in the enalapril group (-8·59 g/cm^2) compared with the placebo group (1·85g/cm^2; p=0·033). Notably, compared with individuals who received the placebo, those who received enalapril reported significantly higher rates of dizziness or hypotension (22% enalapril vs 3% placebo; p=0·0003) and fatigue (10% enalapril vs 0% placebo; p=0·013).

Given the little high-quality evidence published to inform decision making in survivors of CAYA cancer, we turned to clinical-practice guidelines in the general population in which we found moderate-quality to high- quality evidence supporting the use of angiotensin converting enzyme (ACE) inhibitors(65–70) and low-quality evidence supporting the use of βblockers for preventing heart failure in asymptomatic individuals with an LVEF of 40% or less.(68) We found no evidence in the guidelines supporting the effective use of pharmacological intervention in individuals within the general population with asymptomatic cardiomyopathy and a mildly reduced (40–49%) or preserved (≥50%) LVEF.

We found no studies regarding the effectiveness of physical activity, lifestyle interventions, and treatment of traditional cardiovascular risk factors to prevent cardio- myopathy in survivors of CAYA cancer with normal left-ventricular function. However, in the general population, the treatment of hypertension, diabetes, lipid disorders, and obesity, maintaining physical activity, and reducing smoking and alcohol intake are effective in preventing symptomatic cardiomyopathy in individuals with normal left-ventricular function (evidence from general-population clinical-practice guidelines ranging from low to high quality).(65–73,93) In the general population, ACE inhibitors or angiotensin II receptor blockers are effective to prevent heart failure in people with normal left-ventricular function and coronary artery disease, atherosclerotic vascular disease, diabetes, or hypertension,(65,66,68,69) whereas SGLT2 inhibitors are effective to prevent heart failure in people with diabetes at a high risk for cardiovascular disease.(93)

Potential benefits of cardiomyopathy surveillance

When considering the benefits of surveillance, it is important to consider the ability to intervene on findings that are atypical. Although direct evidence of the benefits of intervention in survivors of cancer is scarce, multiple modelling studies have shown extended survival in survivors of CAYA cancer who participated in surveillance programmes when assuming similar treatment efficacy to that observed in the general population with asymptomatic cardiomyopathy.(51–53) For example, Ehrhardt and colleagues(53) reported a 4–11% reduction in the cumulative lifetime risk of heart failure depending on the surveillance strategy used. Such data support a potential benefit in life-years gained via early intervention for asymptomatic cardiomyopathy identified on surveillance echocardiograms in survivors of CAYA cancer.

Potential harms of cardiomyopathy surveillance

Although surveillance echocardiograms are safe and essentially without adverse side-effects, they carry a risk of yielding false-positive findings and overdetection of asymptomatic conditions.(98) False-positive results might lead to unnecessary follow-up testing, cardiology consultation, pharmacological intervention, or even stress and anxiety. Although false-positive results have negative effects at the patient level, a 2020 modelling study that accounted for costs, including time off work associated with false-positive test results, found that surveillance remained cost-effective at the population level.(53)

Evidence-to-decision framework and recommendations

Who needs cardiomyopathy surveillance?

Overall, the balance between the benefits and harms of cardiomyopathy surveillance are dependent on the risk of heart failure (figures 2, 3). In survivors at high risk of CAYA cancer treated with at least 250 mg/m2 of anthracyclines, at least 30 Gy of chest-directed radiotherapy, or a combination of both, the benefits clearly outweigh the harms. Therefore, we strongly recommend cardiomyopathy surveillance in this risk group. We also recommend cardiomyopathy surveillance in survivors at moderate risk of CAYA cancer treated with 100–249 mg/m2 of anthracyclines or 15–29 Gy of

	Echocardiography (3D or 2D LVEF*)	CMR	Blood biomarkers
High-risk (anthracyclines ≥250 mg/m2, chest RT ≥30 Gy, or a combination of anthracyclines ≥100 mg/m2, and chest RT ≥15 Gy)	⊕ High-risk of HF (>3·5-fold) ⊕ Widely available, cheap, and cost-effective at 2-year intervals ⊖ Reasonable agreement with CMR†	⊕ High-risk of HF (>3·5-fold) ⊕ High reproducibility, cost-effective at 5-year intervals ⊖ Costs, availability, waiting times, interpretability by practitioners, and burden for survivors	⊖ Poor diagnostic value of biomarkers
Moderate-risk (anthracyclines 100-249 mg/m2, or chest-RT 15-29 Gy, no combined treatment)	⊕ Risk of HF >1·6 fold ⊕ Widely available, cheap, cost-effective at 5-year intervals ⊖ Reasonable agreement with CMR†	⊕ Risk of HF >1·6 fold ⊕ High reproducibility, cost-effective at 10-year intervals ⊖ Costs, availability, waiting times, interpretability by practitioners, and burden for survivors	⊖ Poor diagnostic value of biomarkers
Low-risk (anthracyclines <100 mg/m2 and chest-RT <15 Gy)	⊖ No increased risk of HF	⊖ No increased risk of HF	⊖ No increased risk of HF

Balance of benefits (⊕) and harms (⊖)				
Undesirable consequences clearly outweigh desirable consequences in most settings	Undesirable consequences probably outweigh desirable consequences in most settings	The balance between desirable and undesirable consequences is closely balanced or uncertain	Desirable consequences probably outweigh undesirable consequences in most settings	Desirable consequences clearly outweigh undesirable consequences in most settings

Figure 3. Overall balance of benefits and harms of primary surveillance by risk group and modality. 2D=two-dimensional. 3D=three-dimensional. LVEF=left-ventricular ejection fraction. CMR=cardiac magnetic resonance imaging. *2D in centres without experience with 3D or insufficient image quality. †Cost-effectiveness studies weighted for misclassification of echocardiography compared with CMR.

chest-directed radio- therapy (moderate recommendation). Conversely, we identified evidence suggesting that survivors at low risk treated with less than 100 mg/m2 of anthracyclines and less than 15 Gy of chest-directed radiotherapy were not at a clinically significant increase of risk of heart failure compared with unexposed survivors and that surveillance was not cost-effective in survivors at low risk. Therefore, we no longer recommend universal surveillance for the low-risk group (strong recommendation).

We identified moderate-quality evidence supporting changes to the previously estimated relative isoequivalencies of anthracycline and anthraquinone derivatives and recommend their use in clinical and research practice (appendix p 340). These changes will have important implications on risk-group assignment because cumulative anthracycline dose is a key factor in risk-group and subsequent surveillance-group stratification.

The identified evidence highlights the importance of baseline cardiac function with respect to peripregnancy cardiomyopathy.(31) Accordingly, cardiomyopathy surveillance is reasonable to identify the evidence of reduced cardiac function before pregnancy or in the first trimester for survivors at moderate risk and high risk of CAYA cancer (moderate-quality evidence; moderate recommendation). Additionally, it is reasonable to continue cardiomyopathy surveillance during pregnancy in survivors of CAYA cancer with a history of left-ventricular dysfunction that has resolved at the time of peripregnancy surveillance, regardless of risk group.

Although we identified two genetic variants from moderate-quality evidence that increase the risk of cardiomyopathy in survivors of CAYA cancer treated with anthracyclines, whether these variants would reclassify survivors to a higher risk group than when defined by the dose of anthracycline and chest-directed radiotherapy is currently unclear. Their effect on the latency to onset of cardiomyopathy is also unclear. Therefore, no recommendation could be formulated for survivors of CAYA cancer who carry a genetic variant.

What surveillance modality should be used?

Because of its wide availability, low costs, and reasonable agreement with CMR, we recommend 2D echocardiography, or 3D echocardiography if available, as the primary surveillance modality for assessment of LVEF in survivors of CAYA cancer, with normal values based on those reported in the general population.(94) CMR, although currently limited by availability, cost, and expertise required for interpretation, is a reasonable consideration in situations in which echocardiography is not feasible or technically inadequate. Similarly, the use of cardiac blood biomarkers is not recommended as the only surveillance modality in at-risk survivors of CAYA cancer and might be considered as a supplementary evaluation in those with borderline cardiac function during primary surveillance.

At what frequency and for how long should cardiomyopathy surveillance be performed?

We recommend surveillance echocardiograms at 2-year intervals for survivors at high risk and at 5-year intervals for survivors at moderate risk. We no longer recommend screening survivors

at low risk. Given that the highest-quality evidence available supports an increased risk of heart failure that does not plateau over time, we continue to recommend lifelong surveillance for survivors at intermediate risk and survivors at high risk at intervals supported by cost-effectiveness studies (table 1).

What should be done when abnormalities are identified?

The decision to intervene for asymptomatic cardiomyopathy remains challenging and is complicated by scarce clear evidence to guide decision making, nuances of detection methods, and the age of individuals in question. Given these complexities, we strongly recommend referral to a cardiologist for additional evaluation of survivors of CAYA cancer with asymptomatic cardiomyopathy and for consideration of treatment with heart failure medications in those with an LVEF of less than 40%. We also strongly recommend screening for and the management of modifiable risk factors to mitigate the risk of cardiovascular disease.

DISCUSSION

We have systematically identified and summarised changes to existing evidence generated since the publication of our 2015 guidelines, graded the quality of these data, and provided evidence-supported and consensus-based, contemporary recommendations regarding cardiomyopathy surveillance in survivors of CAYA cancer to prevent heart failure. We identified novel studies informing anthracycline isoequivalency, threshold doses for radiotherapy-associated cardiomyopathy, and cost-effectiveness of asymptomatic cardiomyopathy surveillance in this rapidly growing and at-risk population. Our findings and recommendations provide much needed support to survivorship and community care providers seeking guidance for cardiac monitoring in survivors of CAYA cancer.

Most notably, studies conducted in the past decade have suggested that surveillance echocardiograms in variably defined survivors at low risk might be low-yield and not cost-effective. (51–53,57) The most recent of these studies(53) found that surveillance in survivors at low risk according to previously defined IGHG risk-stratified groups,(3) despite inputting a wide range of model input variables for which less robust estimates exist (eg, effectiveness of treatment once asymptomatic cardiomyopathy is identified), was ineffective at reducing cases of heart failure. (53) Collectively, these studies have called into question previous surveillance practices. When considering our recommendations within the context of a value-based screening framework,(98) the panel felt that routine surveillance was no longer warranted in the low-risk group. Supporting

Table 1. Risk groups and surveillance recommendations

	Anthracycline (mg/m^2)	Chest radiation (Gy)	Anthracycline (mg/m^2) + chest radiation (Gy)	Is screening recommended?	At what interval?
High risk	≥250	≥30	≥100 and ≥15	Yes	2-year
Moderate risk	100 to <250	15 to <30	–	Maybe	5-year
Low risk	>0 to <100	>0 to <15	–	No	No screening

evidence for these recommendations estimated the risk of heart failure in survivors with existing comorbidities (e.g., hypertension or genetic variants associated with anthracycline- induced cardiomyopathy) and thus provided reasonable population-level estimates for cost-effectiveness in the context of existing comorbidities. However, we recognise that accumulation of combined risk factors can enhance risk at the individual patient level and might merit individualised cardiomyopathy surveillance in some patients in clinical practice. In these situations, health- care providers should weigh these factors into shared decision making for individual patients. For example, as genetic testing becomes more readily available, the presence of variants associated with anthracycline- associated cardiomyopathy might affect future surveillance recommendations for individual survivors, thus might merit greater awareness moving forward.

Although 2D echocardiography represents the standard approach to cardiomyopathy surveillance in both the general population and in survivors of CAYA cancer, we were interested in identifying data regarding the use of CMR. We identified no study on survivors of CAYA cancer that reported on the effect of advanced detection with CMR on the subsequent development of heart failure. Although the heightened sensitivity and precision of CMR offers promise, data regarding the benefit of this enhanced sensitivity, including cost-effectiveness evaluations, are absent for survivors of CAYA cancer. Thus, we were only able to recommend CMR as an adjunct diagnostic evaluation in individuals for whom there remains a high concern for ALVD in the absence of definitive changes in LVEF on an echocardiogram or when image quality is suboptimal.

There was scant literature to inform several key clinical questions that should be the focus of future investigations (figure 4). Few studies examined the conditional effect of interval echocardiography results on future cardio- myopathy risk.(59,74,75,92) Leerink and colleagues(74) evaluated the effect of LVEF at entry into long-term follow-up on the 10-year risk of developing an LVEF of less than 40% and found that a normal baseline LVEF was highly predictive of normal cardiac function at 10 years. Although the study's relatively small sample size and limited follow-up prohibit adoption of this approach into the current guidelines, it represents a crucial first step towards future implementation of conditional surveillance approaches for ALVD in survivors of CAYA cancer.

We found little and low-quality evidence to guide therapeutic intervention when abnormalities are identified on surveillance echocardiograms. Our recommendations regarding the use of ACE inhibitors and β blockers in individuals with an asymptomatic LVEF of less than 40% are thus derived from general-population guidelines. There are no data on survivors of CAYA cancer or the general population to support the treatment of individuals with asymptomatic, mildly reduced LVEF (i.e., 40–49%) in the absence of comorbidities. Therefore, no recommendations could be made for this group. However, we do recommend consultation with a cardiologist for anthracycline-exposed or chest-directed radiotherapy-exposed survivors of CAYA cancer who have asymptomatic, mildly reduced LVEF or diastolic dysfunction for additional testing, follow-up, and consideration of pharmacological intervention. The paucity of literature to inform decisions regarding pharmacological intervention highlights the need for randomised trials to inform this essential knowledge gap in survivors of CAYA cancer. We also found no evidence to inform asymptomatic cardiomyopathy surveillance in survivors treated with novel cancer treatments or for the modification of surveillance approaches in those who received concurrent cardioprotective

- Change in RT-related cardiomyopathy risk by treatment era owing to advances in RT administration techniques
- Long-term (>5 years) efficacy of the cardioprotectant dexrazoxane for cardiomyopathy risk reduction and surveillance frequency
- Prognostic use of (change in) intermediate echocardiographic indices of left ventricular systolic and diastolic function (i.e., abnormal wall stress, decreased thickness-to-dimension ratio, elevated myocardial performance index, abnormal E/A ratio, global longitudinal strain), CMR, blood biomarkers and/or ECG on future cardiomyopathy risk and surveillance personalization in asymptomatic survivors
- Prognostic use of decrease in left ventricular ejection fraction and shortening fraction, as detected by cardiac MRI or radionuclide angiography on subsequent cardiomyopathy risk in asymptomatic survivors
- Prognostic use of increase in cardiac troponins or natriuretic peptides during anthracycline or chest RT administration on long-term (>5 years) cardiomyopathy risk
- Lifetime risk of cardiomyopathy in very long-term survivors (>40 years after treatment)
- Rate of deterioration of cardiac function over time
- Assessment of potential harms associated with excessive surveillance and resultant false-positive findings
- Role of pharmacological interventions to reduce cardiomyopathy risk in asymptomatic survivors with normal cardiac function
- Long-term use of pharmacological interventions in symptomatic survivors with abnormal cardiac function
- Benefits of interventions to reduce modifiable risk factors such as smoking, obesity, hypertension, diabetes, or dyslipidaemia, in survivors of childhood cancer at risk of cardiomyopathy
- Role of genetic susceptibility on subsequent cardiomyopathy risk and surveillance frequency in survivors treated with anthracyclines or chest RT
- Risk of cardiomyopathy in CAYA cancer survivors treated with emerging cancer treatments
- Prognostic use of echocardiography (i.e., LVEF, GLS, diastolic function), CMR, blood biomarkers and/or ECG obtained during cancer treatment on risk for subsequent cardiomyopathy to individualize surveillance frequency
- Effectiveness of pharmacological interventions to reduce cardiomyopathy risk in asymptomatic CAYA cancer survivors with mildly reduced (LVEF 40-50%) or normal LV function
- Accuracy of a combined diagnostic approach using serum natriuretic peptide (ANP, BNP, NT-pro-BNP) and imaging characteristics (e.g., asymptomatic echocardiogram findings) to predict cardiomyopathy in survivors of childhood cancer treated with anthracyclines or RT

Figure 4. Gaps in knowledge regarding cardiomyopathy surveillance in survivors of CAYA cancer. ANP=atrial natriuretic peptide. BNP=brain natriuretic peptide. CAYA=childhood, adolescent, and young adult. CMR=cardiac magnetic resonance. ECG=electrocardiogram. GLS=global longitudinal strain. LVEF=left-ventricular ejection fraction. NT-pro-BNP=N-terminal pro-brain natriuretic peptide.

treatment with dexrazoxane. These findings highlight crucial areas for future research to inform subsequent surveillance guidelines.

These guidelines are strengthened by their evidence-based approach and the systematic and transparent process used for grading the quality of evidence and strength of our recommendations. However, in some instances, little high-quality evidence precluded the formulation of strong recommendations. In these areas, we turned to general-population guidelines. We believe this approach, combined with a study team comprised of diverse clinical backgrounds and health-care expertise, has increased the likelihood that our recommendations are generalisable to a broad group of providers.

In conclusion, these guidelines identified novel data and summarise recommendations regarding changes to anthracycline-related and chest radiotherapy-related surveillance strategies for asymptomatic cardiomyopathy in survivors of CAYA cancer.

REFERENCES

1. Feijen EAML, Font-Gonzalez A, Van der Pal HJH, et al. Risk and temporal changes of heart failure among 5-year childhood cancer survivors: a DCOG-LATER study. J Am Heart Assoc 2019; 8: e009122.
2. Mulrooney DA, Yeazel MW, Kawashima T, et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. BMJ 2009; 339: b4606.
3. Armenian SH, Hudson MM, Mulder RL, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. Lancet Oncol 2015; 16: e123–36.
4. van der Pal HJ, van Dalen EC, van Delden E, et al. High risk of symptomatic cardiac events in childhood cancer survivors. J Clin Oncol 2012; 30: 1429–37.
5. Mulrooney DA, Hyun G, Ness KK, et al. Major cardiac events for adult survivors of childhood cancer diagnosed between 1970 and 1999: report from the Childhood Cancer Survivor Study cohort. BMJ 2020; 368: l6794.
6. Armstrong GT, Liu Q, Yasui Y, et al. Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. J Clin Oncol 2009; 27: 2328–38.
7. Yusuf S, Pitt B, Davis CE, Hood WB Jr, Cohn JN. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. N Engl J Med 1992; 327: 685–91.
8. Kremer LC, Mulder RL, Oeffinger KC, et al. A worldwide collaboration to harmonize guidelines for the long-term follow-up of childhood and young adult cancer survivors: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. Pediatr Blood Cancer 2013; 60: 543–49.
9. Atkins D, Best D, Briss PA, et al. Grading quality of evidence and strength of recommendations. BMJ 2004; 328: 1490.
10. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008; 336: 924–26.
11. Guyatt GH, Oxman AD, Schünemann HJ, Tugwell P, Knottnerus A. GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology. J Clin Epidemiol 2011; 64: 380–82.
12. Alonso-Coello P, Oxman AD, Moberg J, et al. GRADE evidence to decision (EtD) frameworks: a systematic and transparent approach to making well informed healthcare choices. 2: clinical practice guidelines. BMJ 2016; 353: i2089.
13. Feijen EA, Leisenring WM, Stratton KL, et al. Equivalence ratio for daunorubicin to doxorubicin in relation to late heart failure in survivors of childhood cancer. J Clin Oncol 2015; 33: 3774–80.
14. Chen Y, Chow EJ, Oeffinger KC, et al. Traditional cardiovascular risk factors and individual prediction of cardiovascular events in childhood cancer survivors. J Natl Cancer Inst 2020; 112: 256–65.
15. Bates JE, Howell RM, Liu Q, et al. Therapy-related cardiac risk in childhood cancer survivors: an analysis of the childhood cancer survivor study. J Clin Oncol 2019; 37: 1090–101.
16. Chow EJ, Chen Y, Kremer LC, et al. Individual prediction of heart failure among childhood cancer survivors. J Clin Oncol 2015; 33: 394–402.
17. Blanco JG, Sun CL, Landier W, et al. Anthracycline-related cardiomyopathy after childhood cancer: role of polymorphisms in carbonyl reductase genes—a report from the Children's Oncology Group. J Clin Oncol 2012; 30: 1415–21.
18. Mansouri I, Allodji RS, Hill C, et al. The role of irradiated heart and left ventricular volumes in heart failure occurrence after childhood cancer. Eur J Heart Fail 2019; 21: 509–18.
19. Feijen EAM, Leisenring WM, Stratton KL, et al. Derivation of anthracycline and anthraquinone equivalence ratios to doxorubicin for late-onset cardiotoxicity. JAMA Oncol 2019; 5: 864–71.
20. Dietz AC, Seidel K, Leisenring WM, et al. Solid organ transplantation after treatment for childhood cancer: a retrospective cohort analysis from the Childhood Cancer Survivor Study. Lancet Oncol 2019; 20: 1420–31.
21. Armstrong GT, Joshi VM, Ness KK, et al. Comprehensive echocardiographic detection

of treatment-related cardiac dysfunction in adult survivors of childhood cancer: results from the St Jude Lifetime Cohort Study. J Am Coll Cardiol 2015; 65: 2511–22.

22. van der Pal HJ, van Dalen EC, Hauptmann M, et al. Cardiac function in 5-year survivors of childhood cancer: a long-term follow-up study. Arch Intern Med 2010; 170: 1247–55.
23. Armenian SH, Gelehrter SK, Vase T, et al. Screening for cardiac dysfunction in anthracycline-exposed childhood cancer survivors. Clin Cancer Res 2014; 20: 6314–23.
24. Pein F, Sakiroglu O, Dahan M, et al. Cardiac abnormalities 15 years and more after adriamycin therapy in 229 childhood survivors of a solid tumour at the Institut Gustave Roussy. Br J Cancer 2004; 91: 37–44.
25. Aleman BM, van den Belt-Dusebout AW, De Bruin ML, et al. Late cardiotoxicity after treatment for Hodgkin lymphoma. Blood 2007; 109: 1878–86.
26. de Baat EC, van Dalen EC, Mulder RL, et al. Primary cardioprotection with dexrazoxane in patients with childhood cancer who are expected to receive anthracyclines: recommendations from the International Late Effects of Childhood Cancer Guideline Harmonization Group. Lancet Child Adolesc Health 2022; 6: 885–94.
27. Armenian SH, Sun CL, Shannon T, et al. Incidence and predictors of congestive heart failure after autologous hematopoietic cell transplantation. Blood 2011; 118: 6023–29.
28. Khanna A, Pequeno P, Gupta S, et al. Increased risk of all cardiovascular disease subtypes among childhood cancer survivors: population-based matched cohort study. Circulation 2019; 140: 1041–43.
29. Nolan MT, Marwick TH, Plana JC, et al. Effect of traditional heart failure risk factors on myocardial dysfunction in adult survivors of childhood cancer. JACC Cardiovasc Imaging 2018; 11: 1202–03.
30. Mulrooney DA, Armstrong GT, Huang S, et al. Cardiac outcomes in adult survivors of childhood cancer exposed to cardiotoxic therapy: a cross-sectional study. Ann Intern Med 2016; 164: 93–101.
31. Nolan M, Oikonomou EK, Silversides CK, et al. Impact of cancer therapy-related cardiac dysfunction on risk of heart failure in pregnancy. JACC Cardiooncol 2020; 2: 153–62.
32. Aminkeng F, Bhavsar AP, Visscher H, et al. A coding variant in RARG confers susceptibility to anthracycline-induced cardiotoxicity in childhood cancer. Nat Genet 2015; 47: 1079–84.
33. Visscher H, Ross CJ, Rassekh SR, et al. Pharmacogenomic prediction of anthracycline-induced cardiotoxicity in children. J Clin Oncol 2012; 30: 1422–28.
34. Visscher H, Ross CJ, Rassekh SR, et al. Validation of variants in SLC28A3 and UGT1A6 as genetic markers predictive of anthracyclineinduced cardiotoxicity in children. Pediatr Blood Cancer 2013; 60: 1375–81.
35. Ylänen K, Eerola A, Vettenranta K, Poutanen T. Three-dimensional echocardiography and cardiac magnetic resonance imaging in the screening of long-term survivors of childhood cancer after cardiotoxic therapy. Am J Cardiol 2014; 113: 1886–92.
36. Shah SS, McClellan W, Knowlton JQ, et al. Medium-term assessment of cardiac function in pediatric cancer survivors. Comparison of different echocardiographic methods, cardiac MRI and cardiac biomarker testing in adolescent cancer survivors. Echocardiography 2017; 34: 250–56.
37. Armstrong GT, Plana JC, Zhang N, et al. Screening adult survivors of childhood cancer for cardiomyopathy: comparison of echocardiography and cardiac magnetic resonance imaging. J Clin Oncol 2012; 30: 2876–84.
38. Dorosz JL, Lezotte DC, Weitzenkamp DA, Allen LA, Salcedo EE. Performance of 3-dimensional echocardiography in measuring left ventricular volumes and ejection fraction: a systematic review and meta-analysis. J Am Coll Cardiol 2012; 59: 1799–808.
39. Corella Aznar EG, Ayerza Casas A, Jiménez Montañés L, Calvo Escribano MAC, Labarta Aizpún JI, Samper Villagrasa P. Use of speckle tracking in the evaluation of late subclinical myocardial damage in survivors of childhood acute leukaemia. Int J Cardiovasc Imaging 2018; 34: 1373–81.

40. Rathe M, Carlsen NL, Oxhøj H, Nielsen G. Long-term cardiac follow-up of children treated with anthracycline doses of 300 mg/m² or less for acute lymphoblastic leukemia. Pediatr Blood Cancer 2010; 54: 444–48.

41. Leerink JM, Verkleij SJ, Feijen EAM, et al. Biomarkers to diagnose ventricular dysfunction in childhood cancer survivors: a systematic review. Heart 2019; 105: 210–16.

42. Ylänen K, Poutanen T, Savukoski T, Eerola A, Vettenranta K. Cardiac biomarkers indicate a need for sensitive cardiac imaging among long-term childhood cancer survivors exposed to anthracyclines. Acta Paediatr 2015; 104: 313–19.

43. Pourier MS, Kapusta L, van Gennip A, et al. Values of high sensitive troponin T in long-term survivors of childhood cancer treated with anthracyclines. Clin Chim Acta 2015; 441: 29–32.

44. Sherief LM, Kamal AG, Khalek EA, Kamal NM, Soliman AA, Esh AM. Biomarkers and early detection of late onset anthracyclineinduced cardiotoxicity in children. Hematology 2012; 17: 151–56.

45. Mladosievicova B, Urbanova D, Radvanska E, Slavkovsky P, Simkova I. Role of NT-proBNP in detection of myocardial damage in childhood leukemia survivors treated with and without anthracyclines. J Exp Clin Cancer Res 2012; 31: 86.

46. Krawczuk-Rybak M, Dakowicz L, Hryniewicz A, Maksymiuk A, Zelazowska-Rutkowska B, Wysocka J. Cardiac function in survivors of acute lymphoblastic leukaemia and Hodgkin's lymphoma. J Paediatr Child Health 2011; 47: 455–59.

47. Mavinkurve-Groothuis AM, Groot-Loonen J, Bellersen L, et al. Abnormal NT-pro-BNP levels in asymptomatic long-term survivors of childhood cancer treated with anthracyclines. Pediatr Blood Cancer 2009; 52: 631–36.

48. Dixon SB, Howell CR, Lu L, et al. Cardiac biomarkers and association with subsequent cardiomyopathy and mortality among adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort. Cancer 2021; 127: 458–66.

49. Soker M, Kervancioglu M. Plasma concentrations of NT-pro-BNP and cardiac troponin-I in relation to doxorubicin-induced cardiomyopathy and cardiac function in childhood malignancy. Saudi Med J 2005; 26: 1197–202.

50. Hayakawa H, Komada Y, Hirayama M, Hori H, Ito M, Sakurai M. Plasma levels of natriuretic peptides in relation to doxorubicininduced cardiotoxicity and cardiac function in children with cancer. Med Pediatr Oncol 2001; 37: 4–9.

51. Yeh JM, Nohria A, Diller L. Routine echocardiography screening for asymptomatic left ventricular dysfunction in childhood cancer survivors: a model-based estimation of the clinical and economic effects. Ann Intern Med 2014; 160: 661–71.

52. Wong FL, Bhatia S, Landier W, et al. Cost-effectiveness of the Children's Oncology Group long-term follow-up screening guidelines for childhood cancer survivors at risk for treatmentrelated heart failure. Ann Intern Med 2014; 160: 672–83.

53. Ehrhardt MJ, Ward ZJ, Liu Q, et al. Cost-effectiveness of the International Late Effects of Childhood Cancer Guideline Harmonization Group screening guidelines to prevent heart failure in survivors of childhood cancer. J Clin Oncol 2020; 38: 3851–62.

54. van Dalen EC, van der Pal HJ, Kok WE, Caron HN, Kremer LC. Clinical heart failure in a cohort of children treated with anthracyclines: a long-term follow-up study. Eur J Cancer 2006; 42: 3191–98.

55. Border WL, Sachdeva R, Stratton KL, et al. Longitudinal changes in echocardiographic parameters of cardiac function in pediatric cancer survivors. JACC Cardiooncol 2020; 2: 26–37.

56. Markman TM, Ruble K, Loeb D, et al. Electrophysiological effects of anthracyclines in adult survivors of pediatric malignancy. Pediatr Blood Cancer 2017; 64: e26556.

57. Spewak MB, Williamson RS, Mertens AC, Border WL, Meacham LR, Wasilewski-Masker KJ. Yield of screening echocardiograms during pediatric follow-up in survivors treated with anthracyclines and cardiotoxic radiation. Pediatr Blood Cancer 2017; 64: e26367.

58. Ramjaun A, AlDuhaiby E, Ahmed S, et al. Echocardiographic detection of cardiac dysfunction in childhood cancer survivors: how long is screening required? Pediatr Blood Cancer 2015; 62: 2197–203.

59. Abosoudah I, Greenberg ML, Ness KK, Benson L, Nathan PC. Echocardiographic surveillance for asymptomatic late-onset anthracycline cardiomyopathy in childhood cancer survivors. Pediatr Blood Cancer 2011; 57: 467–72.

60. Creutzig U, Diekamp S, Zimmermann M, Reinhardt D. Longitudinal evaluation of early and late anthracycline cardiotoxicity in children with AML. Pediatr Blood Cancer 2007; 48: 651–62.

61. Chellapandian D, Pole JD, Nathan PC, Sung L. Congestive heart failure among children with acute leukemia: a population-based matched cohort study. Leuk Lymphoma 2019; 60: 385–94.

62. Getz KD, Sung L, Ky B, et al. Occurrence of treatment-related cardiotoxicity and its impact on outcomes among children treated in the AAML0531 clinical trial: a report from the Children's Oncology Group. J Clin Oncol 2019; 37: 12–21.

63. Pourier MS, Mavinkurve-Groothuis AMC, Dull MM, et al. Myocardial 2D strain during long-term (>5 years) follow-up of childhood survivors of acute lymphoblastic leukemia treated with anthracyclines. Am J Cardiol 2020; 127: 163–68.

64. Silber JH, Cnaan A, Clark BJ, et al. Enalapril to prevent cardiac function decline in long-term survivors of pediatric cancer exposed to anthracyclines. J Clin Oncol 2004; 22: 820–28.

65. Ezekowitz JA, O'Meara E, McDonald MA, et al. 2017 comprehensive update of the Canadian Cardiovascular Society guidelines for the management of heart failure. Can J Cardiol 2017; 33: 1342–433.

66. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016; 37: 2129–200.

67. Kirk R, Dipchand AI, Rosenthal DN, et al. The International Society for Heart and Lung Transplantation Guidelines for the management of pediatric heart failure: executive summary. [corrected]. J Heart Lung Transplant 2014; 33: 888–909.

68. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Circulation 2013; 128: 1810–52.

69. Tsutsui H, Isobe M, Ito H, et al. JCS 2017/JHFS 2017 guideline on diagnosis and treatment of acute and chronic heart failure—digest version. Circ J 2019; 83: 2084–184.

70. Healthcare Improvement Scotland. Management of chronic heart failure: a national clinical guideline. Edinburgh: Scottish Intercollegiate Guidelines Network (SIGN); 2016.

71. Ministry of Health Malaysia. Clinical practice guidelines: management of heart failure 2019, 4th edn. Kuala Lumpur: National Heart Association of Malaysia, 2019.

72. O'Meara E, McDonald M, Chan M, et al. CCS/CHFS heart failure guidelines: clinical trial update on functional mitral regurgitation, SGLT2 inhibitors, ARNI in HFpEF, and tafamidis in amyloidosis. Can J Cardiol 2020; 36: 159–69.

73. Yancy CW, Jessup M, Bozkurt B, et al. 2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. Circulation 2017; 136: e137–61.

74. Leerink JM, van der Pal HJH, Kremer LCM, et al. Refining the 10-year prediction of left ventricular systolic dysfunction in longterm survivors of childhood cancer. JACC Cardiooncol 2021; 3: 62–72.

75. Temming P, Qureshi A, Hardt J, et al. Prevalence and predictors of anthracycline cardiotoxicity in children treated for acute myeloid leukaemia: retrospective cohort study in a single centre in the United Kingdom. Pediatr Blood Cancer 2011; 56: 625–30.

76. Green DM, Grigoriev YA, Nan B, et al. Congestive heart failure after treatment for Wilms' tumor: a report from the National Wilms' Tumor Study group. J Clin Oncol 2001; 19: 1926–34.

77. Kremer LC, van Dalen EC, Offringa M, Voûte PA. Frequency and risk factors of anthracycline-induced clinical heart failure in children: a systematic review. Ann Oncol 2002; 13: 503–12.

78. Hudson MM, Rai SN, Nunez C, et al. Noninvasive evaluation of late anthracycline cardiac toxicity in childhood cancer survivors. J Clin Oncol 2007; 25: 3635–43.

79. Paulides M, Kremers A, Stöhr W, et al. Prospective longitudinal evaluation of doxorubicin-induced cardiomyopathy in sarcoma patients: a report of the late effects surveillance system (LESS). Pediatr Blood Cancer 2006; 46: 489–95.

80. Sorensen K, Levitt GA, Bull C, Dorup I, Sullivan ID. Late anthracycline cardiotoxicity after childhood cancer: a prospective longitudinal study. Cancer 2003; 97: 1991–98.

81. Kremer LC, van der Pal HJ, Offringa M, van Dalen EC, Voûte PA. Frequency and risk factors of subclinical cardiotoxicity after anthracycline therapy in children: a systematic review. Ann Oncol 2002; 13: 819–29.

82. Slieker MG, Fackoury C, Slorach C, et al. Echocardiographic assessment of cardiac function in pediatric survivors of anthracyclinetreated childhood cancer. Circ Cardiovasc Imaging 2019; 12: e008869.

83. Christiansen JR, Massey R, Dalen H, et al. Utility of global longitudinal strain by echocardiography to detect left ventricular dysfunction in long-term adult survivors of childhood lymphoma and acute lymphoblastic leukemia. Am J Cardiol 2016; 118: 446–52.

84. Yu AF, Raikhelkar J, Zabor EC, et al. Two-dimensional speckle tracking echocardiography detects subclinical left ventricular systolic dysfunction among adult survivors of childhood, adolescent, and young adult cancer. BioMed Res Int 2016; 2016: 9363951.

85. Christiansen JR, Hamre H, Massey R, et al. Left ventricular function in long-term survivors of childhood lymphoma. Am J Cardiol 2014; 114: 483–90.

86. Brouwer CA, Postma A, Vonk JM, et al. Systolic and diastolic dysfunction in long-term adult survivors of childhood cancer. Eur J Cancer 2011; 47: 2453–62.

87. Guldner L, Haddy N, Pein F, et al. Radiation dose and long term risk of cardiac pathology following radiotherapy and anthracyclin for a childhood cancer. Radiother Oncol 2006; 81: 47–56.

88. van der Pal HJ, van Dalen EC, Kremer LC, Bakker PJ, van Leeuwen FE. Risk of morbidity and mortality from cardiovascular disease following radiotherapy for childhood cancer: a systematic review. Cancer Treat Rev 2005; 31: 173–85.

89. Niska JR, Thorpe CS, Allen SM, et al. Radiation and the heart: systematic review of dosimetry and cardiac endpoints. Expert Rev Cardiovasc Ther 2018; 16: 931–50.

90. Aminkeng F, Ross CJ, Rassekh SR, et al. Recommendations for genetic testing to reduce the incidence of anthracycline-induced cardiotoxicity. Br J Clin Pharmacol 2016; 82: 683–95.

91. Kismet E, Varan A, Ayabakan C, Alehan D, Portakal O, Büyükpamukçu M. Serum troponin T levels and echocardiographic evaluation in children treated with doxorubicin. Pediatr Blood Cancer 2004; 42: 220–24.

92. Lipshultz SE, Lipsitz SR, Sallan SE, et al. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. J Clin Oncol 2005; 23: 2629–36.

93. McDonagh TA, Metra M, Adamo M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2021; 42: 3599–726.

94. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015; 16: 233–70.

95. Armstrong GT, Oeffinger KC, Chen Y, et al. Modifiable risk factors and major cardiac events among adult survivors of childhood cancer. J Clin Oncol 2013; 31: 3673–80.

96. Leerink JM, Verkleij SJ, Feijen EAM, et al. Biomarkers to diagnose ventricular dysfunction in childhood cancer survivors: a systematic review. Heart 2019; 105: 210–16.

97. Cheuk DK, Sieswerda E, van Dalen EC, Postma A, Kremer LCM. Medical interventions for treating anthracycline-induced symptomatic and asymptomatic cardiotoxicity during and after treatment for childhood cancer. Cochrane Database Syst Rev 2016; 2016: CD008011.

98. Harris RP, Wilt TJ, Qaseem A. A value framework for cancer screening: advice for high-value care from the American College of Physicians. Ann Intern Med 2015; 162: 712–17.

SUPPLEMENTAL MATERIAL

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APPENDIX

10

SUMMARY AND DISCUSSION

SUMMARY

Cardiac disease is a major concern among the growing group of childhood cancer survivors exposed to cardiotoxic cancer treatments. In part I, **chapter 2**, we reviewed the current state of knowledge on cardiac disease in survivors of childhood cancer, discussing risk factors, risk prediction models, prevention, surveillance, and management of cardiac dysfunction.

The goal of surveillance is to prevent heart failure by timely detecting abnormalities in cardiac function before heart failure symptoms occur in order to start treatment early and prevent deterioration of cardiac function and development of heart failure. Currently, echocardiographic surveillance frequency (ranging from every 2 to 5 years) is guided by cumulative exposures to anthracyclines and chest-directed radiotherapy.(1, 2) In **chapter 3** we demonstrated that risk stratification for development of a therapeutically relevant decreased left ventricular ejection fraction (LVEF) below 40% within 10 years can be improved by adding the result of an initial surveillance echocardiogram to a prediction model including anthracycline and chest-directed radiotherapy dose. Overall, the 10-year cumulative incidence of LVEF <40% was 3.7%. Addition of the initial surveillance LVEF to the prediction model was most useful for the identification of a low-risk group, representing 75% of survivors, in whom less frequent surveillance might be appropriate. In addition, survivors with an initial midrange LVEF of 40-49% (14% of the cohort) had an incidence of 11% of a LVEF <40% within 10 years compared to 2.6% in survivors with a preserved LVEF. These findings indicate that developing cardiomyopathy in the late phase after cardiotoxic treatment may be seen as a continuing deterioration of left ventricular function, and that it is worthwhile to include signs of a developing cardiomyopathy to the existing clinical prediction models.(3, 4, 5)

Part II of this thesis focuses on blood biomarkers for the diagnosis of cardiac dysfunction in childhood cancer survivors. The use of blood biomarkers for long-term cardiac surveillance is attractive due to their relative ease of measurement, low costs and time efficiency in comparison to echocardiography, but data on their diagnostic use was limited before this thesis. In **chapter 4**, we systematically reviewed the literature on the diagnostic accuracy of blood biomarkers for the detection of various definitions of LV dysfunction in >1-year survivors of childhood cancer exposed to cardiotoxic cancer treatments. Among the 5 included studies, cardiac troponins (troponin T and I) were rarely abnormal (lowest cutoff 10 ng/L) even in those with LV dysfunction and therefore were not useful for the diagnosis of LV dysfunction. Abnormal N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels were encountered more frequently but had insufficient diagnostic accuracy in 5 studies for either rule-in or rule-out of asymptomatic LV dysfunction. A study from Dixon et al. in 1213 long-term childhood cancer survivors, which was published after this systematic review was conducted, found similar results with cardiac troponin T being rarely abnormal (not high sensitive) and age and sex defined abnormal NT-proBNP levels having poor sensitivity (≤31% for all comparisons) and only moderate specificity for identifying survivors with new onset of an abnormal LVEF < 53%, global longitudinal strain, or diastolic dysfunction (75%, 77%, and 76%, respectively). (6) However, due to the small range of NT-proBNP cutoffs studied (age and sex defined and 63-125 ng/L) and the development of highly sensitive cardiac troponin T (Hs-cTnT) assays providing lower detection limits and more precision at lower concentrations, we further studied both of these

biomarkers for their diagnostic accuracy in detecting cardiomyopathy in the Dutch Childhood Cancer Survivor Study, cardiology sub-study (DCCSS LATER CARD). The design of DCCSS LATER CARD is outlined in **chapter 5**. The DCCSS LATER CARD is a prospectively designed, cross-sectional multicenter study evaluating diagnostic tools (echocardiography, electrocardiography, and blood biomarkers) for the detection of cardiac dysfunction in >5-year survivors of childhood cancer.

In **chapter 6**, we studied NT-proBNP and Hs-cTnT for their diagnostic accuracy to detect LV dysfunction in the DCCSS LATER CARD cohort. We found age and sex defined abnormal NT-proBNP levels in 22.1% of the survivors, compared to 5.4% in sibling controls. Even though we used a highly sensitive assay, abnormal Hs-cTnT levels >10 ng/L were uncommon in survivors (5.9%) and not significantly different from siblings (5.0%).(6) There were no cut-off plasma concentrations of NT-proBNP or Hs-cTnT that accurately ruled-in or ruled-out any definition of LV dysfunction (LVEF<45%, LVEF<50% and LVEF<52% in males and LVEF <54% in females). NT-proBNP or Hs-cTnT as single biomarkers should therefore not be used for cardiac surveillance in long-term survivors of childhood cancer. In clinical practice, decisions are rarely based on the results of a single diagnostic test because patient related clinical risk factors can increase or decrease the pre-test probability of the disease. Indeed, we found that a multivariable model combining both cardiac biomarkers with clinical risk factors significantly improved diagnostic accuracy and enabled rule-out of LV dysfunction (LVEF <50%) with high sensitivity and negative predictive value in 18.5% of the survivors in our cohort. As the updated cardiomyopathy surveillance guidelines (chapter 9 of this thesis) no longer recommends surveillance in low-risk survivors, we showed in a secondary analysis that this diagnostic model was also able to rule-out LVEF<50% in 13.1% of moderate- and high-risk survivors. Thus, if confirmed by a validation study, NT-proBNP and Hs-cTnT may be clinically useful when combined with clinical risk factors to reduce the number of unnecessary surveillance echocardiograms. Rule-in of LV dysfunction was not possible with this multivariable model due to limited specificity and positive predictive value in our cohort.

The findings in the previous chapters underline the need to find novel blood biomarkers marking other and potentially earlier disease processes in the development of cardiomyopathy due to anthracyclines. Therefore, in **chapter 7**, we systematically reviewed 68 animal studies to unravel the role and time course of extracellular matrix remodeling in anthracycline cardiomyopathy. Extracellular matrix remodeling and fibroblasts play a crucial role in several types of cardiomyopathies in response to pathological stimuli, such as ischemia, pressure overload, volume overload, inflammation and toxins, such as anthracyclines.(7) While cardiomyocyte death occurs early after anthracycline treatment, extracellular matrix remodeling is especially important in the progression from cardiomyocyte injury to late-onset cardiotoxicity. Indeed, more interstitial fibrosis was found in animal studies performed longer after anthracycline administration accompanying worse LV systolic function. Collagens, matrix metalloproteinases, inflammation markers (such as tumor necrosis factor, interleukin 1b and 6, galectin-3) and transforming growth factor ß signaling markers were upregulated in the hearts of animals with anthracycline cardiomyopathy. Based on the findings in this review and findings in other cardiomyopathies, we recognize three major steps in extracellular matrix remodeling in anthracycline-induced cardiomyopathy: 1) activation of the innate immune system in response to cardiomyocyte injury, 2) activation of matrix metalloproteinases by the innate

immune system that remodel the extracellular matrix, and 3) transforming growth factor ß-induced fibroblast to myofibroblast conversion causing interstitial fibrosis. This review provides a rationale to investigate extracellular matrix remodeling and inflammatory markers in anthracycline-related cardiomyopathy, which we did in chapter 8.

In **chapter 8**, we searched for novel candidate blood biomarkers for anthracycline-related cardiomyopathy using a matched case-control design within the DCCSS LATER study cohort. From a panel of 278 plasma proteins marking different pathophysiological processes, we identified 3 specific inflammatory plasma proteins upregulated in cases with anthracycline cardiomyopathy, independently of NT-proBNP and clinical characteristics: C-C motif chemokine ligand 19 (CCL19) and 20 (CCL20), and pulmonary surfactant protein-D (PSPD). We found these markers to be similarly upregulated in patients with dilated cardiomyopathy that was related to a genetic mutation, which suggest that they mark a more common pathway of heart failure progression. Dysregulation of the immune system is also reported in previous studies in heart failure due of various etiology.(8) Interestingly, other inflammatory markers known to be upregulated in heart failure(8), such as tumor necrosis factor, galectin-3, soluble suppression of tumourigenicity-2 (ST2), interleukin 1b and 6, were not upregulated in our study in anthracycline cardiomyopathy. Although we did not measure heart specific expression of these novel identified plasma proteins, this study suggests dysregulation of specific parts of the immune system in chronic anthracycline-related cardiomyopathy that will be further explored in future studies.

Part III focuses on the ongoing improvements for cardiac surveillance in childhood cancer survivors. In **chapter 9**, we present the updated cardiomyopathy surveillance guideline from the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) from 2023. In this updated guideline, we again systematically reviewed the literature and graded the level of evidence on the need for surveillance, surveillance modalities, surveillance frequency and management of asymptomatic cardiac dysfunction. The main change compared to the previous guideline(9) is the recommendation to no longer screen low-risk survivors treated with a doxorubicin equivalent dose of <100 mg/m^2 and a chest-directed radiotherapy dose of <15 Gray as heart failure risk is low and surveillance was found not cost-effective in simulation studies.(10, 11, 12) Following the previous guideline, the updated guideline recommends to use echocardiography as the primary surveillance modality due to its wide availability and low costs, with surveillance intervals of 2 to 5 years in moderate and high-risk survivors, respectively. The guideline recommends against the use of cardiac blood biomarkers (e.g., natriuretic peptides and troponins) as the only strategy for cardiomyopathy surveillance. The results of chapter 6 strengthen this recommendation against the use of blood biomarkers only. However, if our multivariable diagnostic model including cardiac biomarkers and clinical risk factors developed in chapter 6 can be validated in an independent cohort, biomarker-based surveillance might be a cost-effective surveillance strategy to consider in future updates of the guideline. The guideline further recommends cardiology referral in survivors with asymptomatic LV dysfunction and treatment is advised in those with LVEF<40% following heart failure guidelines in the general population(3, 4, 5) as evidence on treatment in (childhood) cancer survivors was very limited.

STRENGTHS AND LIMITATIONS

Results and conclusions of this thesis are supported by the use of a large and well characterized cohort of long-term childhood cancer survivors. In particular, it enabled multivariable modeling of blood biomarkers in combination with clinical characteristics.

Another strength of this thesis is the exploration of a novel concept to improve cardiac surveillance in childhood cancer survivors. The prediction model presented in chapter 3 is the first to include information from an initial surveillance echocardiogram - next to cancer treatment exposures - to improve risk stratification for a therapeutically meaningful decline in LVEF below 40% within 10 years. This is also the first prediction model that predicts an asymptomatic disease state (LVEF<40%) that typically precedes heart failure and can be treated to prevent heart failure. Although the low number of events in our study underlines the need for confirmation of our findings in a larger cohort, we believe that such a conditional approach, where risk predictions are updated as survivors age based on echocardiography or other biomarkers, is a promising one that should be further explored.

The main limitation of this thesis is that follow-up after blood biomarker sampling was not yet available in the Dutch Childhood Cancer Survivors study. This implies that it was not yet possible to study whether blood biomarkers might be early indicators of heart failure (i.e., earlier than LV function becomes abnormal). Follow-up studies are currently being conducted to answer this question. Second, we did not study heart specific expression of the inflammatory biomarkers for anthracycline-related cardiomyopathy identified in chapter 8. Third, we focused on predicting (chapter 3) or diagnosing (part II) LV dysfunction, as the main preceding event before development of heart failure. However, other cardiotoxic events, such as valvular disease and ischemic heart disease, can also cause heart failure symptoms and are also frequently encountered in childhood cancer survivors.(13, 14)

Implications for long-term survivorship care

The findings of this thesis have important clinical implications for the long-term surveillance of childhood cancer survivors. Natriuretic peptides and cardiac troponins should not be relied upon as sole diagnostic tests for detecting asymptomatic left ventricular (LV) dysfunction due to their limited sensitivity and negative predictive value that is needed for reliable and safe rule-out. Instead, a multivariable model that incorporates natriuretic peptides, cardiac troponins, and clinical risk factors is more useful to rule-out of asymptomatic LV dysfunction (LVEF<50%) and decrease the number of unnecessary surveillance echocardiograms. However, external validation of this finding is still necessary before it can be adopted into clinical practice. Also, as shown in chapter 9 of this thesis, long-term cardiac surveillance is no longer recommended for low-risk survivors who were treated with an anthracycline dose less than 100 mg/m2 or chest-directed radiotherapy less than 15 Gray. Implementation of this guideline will decrease the number of unnecessary surveillance echocardiograms by 23% in the Dutch LATER cohort and by approximately 40% in the Childhood Cancer Survivor Study.(10)

Future perspectives for research

Our recommendations for future studies are as follows. First, the diagnostic performance of the multivariable diagnostic model for LV dysfunction developed in chapter 6 should be externally validated in an independent cohort. We showed that multivariable models combining clinical risk factors (i.e., baseline risk factors) with blood biomarkers measured during follow-up provides superior diagnostic accuracy compared to univariable approaches. Hence, we believe that multivariable modeling is the way forward. Our diagnostic model could potentially be further improved with additional baseline risk factors (e.g., genetics) and factors measured during follow-up (e.g., the inflammatory biomarkers identified in chapter 8, electrocardiographic parameters). Machine learning algorithms may potentially further improve diagnostic performance because they are able to model complex interactions and non-linear effects that may be missed with traditional models. Eventually, if such a biomarker-based rule-out strategy can be externally validated, clinical usefulness largely depends on whether it is cost-effective in preventing heart failure compared to an "echocardiography in all" strategy. This could be addressed in a simulation study given the long follow-up required to conduct a randomized controlled trial.

Second, in this thesis we focused on the diagnostic use of blood biomarkers but they may also be useful for prognostication. Indeed, NT-proBNP has been shown to be associated with future cardiomyopathy (LVEF <50%) in childhood cancer survivors.(6) Future studies may address the prognostic use of (changes in) blood biomarkers next to baseline risk factors (i.e., cancer treatment exposures and genetics) for refining risk estimation at cancer treatment completion and during scheduled surveillance visits during follow-up. This may help to guide surveillance frequency in childhood cancer survivors at risk for heart failure (Figure 1).

Third, the biological role and cellular sources of the 3 inflammatory biomarkers identified in anthracycline-related cardiomyopathy in this thesis should be elucidated in preclinical and cohort studies. The immune system has been implicated in heart failure due to various causes, including

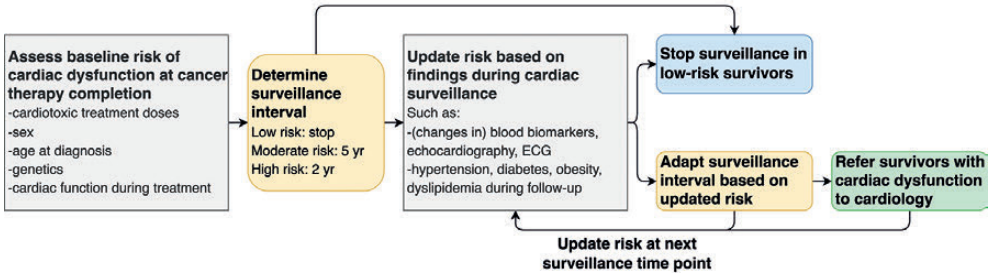

Figure 1. Framework for individualized cardiac surveillance in childhood cancer survivors treated with cardiotoxic cancer treatments. Risk of cardiac dysfunction is first estimated using baseline risk factors known at cancer therapy completion and subsequently updated using cardiac function parameters and risk factors obtained during follow-up. Abbreviations: ECG=electrocardiography, yr=years.Risk of cardiac dysfunction is first estimated using baseline risk factors known at cancer therapy completion and subsequently updated using cardiac function parameters and risk factors obtained during follow-up. Abbreviations: ECG=electrocardiography, yr=years.

anthracycline cardiotoxicity, but beneficial immunomodulating therapies have not yet been found possibly due to a lack of a clear or proven target.(8)

Fourth and finally, the benefit of asymptomatic cardiomyopathy surveillance depends on whether treatment in early (asymptomatic) stages of heart failure is effective. In the absence of data in childhood cancer survivors, cost-effectiveness studies that informed the current cardiac surveillance recommendations presented in this thesis used treatment effectiveness of pharmacological treatment in the general population with asymptomatic LVEF at or below 40%.(10, 11, 12, 15, 16) There is currently no data in childhood cancer survivors showing that treatment of a milder severity of LV dysfunction (or guided by a biomarker level) is effective. Future research in childhood cancer survivors should 1) find out what represents an early enough sign of cardiomyopathy that warrants treatment and 2) subsequently conduct a randomized controlled trial to test the effectiveness of treatment to prevent heart failure, or to prevent intermediate outcomes (such as deterioration in LVEF or changes in blood biomarker levels) given the long follow-up required before heart failure occurs.

REFERENCES

1. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2015;16(3):e123-36.
2. Ehrhardt MJ, Leerink JM, Mulder RL, Mavinkurve-Groothuis A, Kok W, Nohria A, et al. Systematic review and updated recommendations for cardiomyopathy surveillance for survivors of childhood, adolescent, and young adult cancer from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2023.
3. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European Heart Journal. 2016;37(27):2129-200.
4. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) With the special contribution of the Heart Failure Association (HFA) of the ESC. European Heart Journal. 2021.
5. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Jr., Colvin MM, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. Circulation. 2017;136(6):e137-e61.
6. Dixon SB, Howell CR, Lu L, Plana JC, Joshi VM, Luepker RV, et al. Cardiac biomarkers and association with subsequent cardiomyopathy and mortality among adult survivors of childhood cancer: A report from the St. Jude Lifetime Cohort. Cancer. 2021;127(3):458-66.
7. Frangogiannis NG. The Extracellular Matrix in Ischemic and Nonischemic Heart Failure. Circulation Research. 2019;125(1):117-46.
8. Adamo L, Rocha-Resende C, Prabhu SD, Mann DL. Reappraising the role of inflammation in heart failure. Nat Rev Cardiol. 2020;17(5):269-85.
9. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2015;16(3):e123-36.
10. Ehrhardt MJ, Ward ZJ, Liu Q, Chaudhry A, Nohria A, Border W, et al. Cost-Effectiveness of the International Late Effects of Childhood Cancer Guideline Harmonization Group Screening Guidelines to Prevent Heart Failure in Survivors of Childhood Cancer. J Clin Oncol. 2020:Jco2000418.
11. Yeh JM, Nohria A, Diller L. Routine echocardiography screening for asymptomatic left ventricular dysfunction in childhood cancer survivors: a model-based estimation of the clinical and economic effects. Annals of internal medicine. 2014;160(10):661-71.
12. Wong FL, Bhatia S, Landier W, Francisco L, Leisenring W, Hudson MM, et al. Cost-effectiveness of the children's oncology group long-term follow-up screening guidelines for childhood cancer survivors at risk for treatment-related heart failure. Ann Intern Med. 2014;160(10):672-83.
13. van der Pal HJ, van Dalen EC, van Delden E, van Dijk IW, Kok WE, Geskus RB, et al. High risk of symptomatic cardiac events in childhood cancer survivors. J Clin Oncol. 2012;30(13):1429-37.
14. Feijen EAM, van Dalen EC, van der Pal HJH, Reulen RC, Winter DL, Keuhni CE, et al. Increased risk of cardiac ischaemia in a pan-European cohort of 36 205 childhood cancer survivors: a PanCareSurFup study. Heart. 2021;107(1):33-40.

15. Exner DV, Dries DL, Waclawiw MA, Shelton B, Domanski MJ. Beta-adrenergic blocking agent use and mortality in patients with asymptomatic and symptomatic left ventricular systolic dysfunction: a post hoc analysis of the Studies of Left Ventricular Dysfunction. J Am Coll Cardiol. 1999;33(4):916-23.

16. Yusuf S, Pitt B, Davis CE, Hood WB, Jr., Cohn JN. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The New England journal of medicine. 1992;327(10):685-91.

11

NEDERLANDSE SAMENVATTING

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De vijfjaarsoverleving van kinderen met kanker is sterk toegenomen de laatste decennia en bedraagt op dit moment meer dan 80%.(1) Helaas krijgen veel overlevenden van kinderkanker gedurende hun leven te maken met late effecten van de behandeling.(2) Hartfalen is een belangrijk probleem in overlevenden van kinderkanker die blootgesteld zijn aan cardiotoxische middelen zoals anthracyclines, mitoxantrone en radiotherapie op de hart. Bijna 11% van de kinderen behandeld met deze middelen krijgt hartfalen binnen 40 jaar na kanker.(3) Ook hebben overlevenden een 7 keer zo hoog risico op sterfte door aandoeningen van het hart ten opzichte van hun broers of zussen.(4) **Hoofdstuk 2** van dit proefschrift geeft een uitgebreid overzicht van de huidige literatuur over hartfalen in overlevenden van kinderkanker. Onder andere risicofactoren, risicovoorspellingsmodellen, preventie, lange-termijn follow-up (surveillance) en behandeling worden besproken.

Hartfalen wordt vaak voorafgegaan door asymptomatische afwijkingen van het hart. Door deze afwijkingen van het hart vroeg op te sporen, voordat er klachten ontstaan, kunnen we hartfalen mogelijk voorkomen door behandeling vroeg te starten. Daarom krijgen op dit moment alle overlevenden van kinderkanker die in het verleden behandeld zijn met cardiotoxische middelen elke 2 tot 5 jaar een echo van het hart.(5) Risicostratificatie wordt op dit moment gedaan op basis van anthracycline dosis en radiotherapie dosis terwijl iemand met een laag-normale cardiale functie tijdens follow-up waarschijnlijk een hoger risico heeft dan iemand met een normale functie van het hart. In **hoofdstuk 3** lieten we zien dat een laag-normale linkerventrikel ejectiefractie - gemeten met een echocardiogram gemiddeld 17 jaar na kanker - sterk voorspellend is voor een belangrijke daling in de linkerventrikel ejectiefractie onder de 40% in de daaropvolgende 10 jaar. Het toevoegen van de linkerventrikel ejectiefractie aan een predictiemodel met anthracycline dosis en radiotherapie dosis bleek met name nuttig voor het identificeren van een laag-risicogroep waarin waarschijnlijk minder frequente follow-up nodig is. Alhoewel de resultaten nog gevalideerd moeten worden in een groter cohort, is dit de eerste studie die laat zien dat risicostratificatie verbeterd kan worden door follow-up echo gegevens mee te nemen in de risicoafweging.

Bloed biomarkers kunnen een kosteneffectieve en tijdsefficiënte methode zijn om asymptomatische cardiale dysfunctie op te sporen in overlevenden van kinderkanker. Deel II van dit proefschrift gaat hierover. **Hoofdstuk 4** is een systematische review van 8 studies waaruit blijkt dat natriuretische peptiden en cardiale troponines niet geschikt zijn voor het diagnosticeren van asymptomatische linkerventrikel dysfunctie (gemeten met echocardiografie) in overlevenden van kinderkanker. Althans, niet met de afkapwaardes die gebruikt werden in deze 8 studies (NT-proBNP 63-125 ng/L, troponine T 10-14 ng/L). We besloten deze bloed biomarkers verder te onderzoeken in de Dutch Childhood Cancer Survivor Study (DCCSS LATER CARD). DCCSS LATER CARD is een prospectieve, cross-sectionele multicenter studie naar methoden (echocardiografie, elektrocardiografie en bloed biomarkers) voor het diagnosticeren van cardiale dysfunctie in >5-jaar overlevenden van kinderkanker. De studieopzet is beschreven in **hoofdstuk 5**.

In **hoofdstuk 6** onderzochten we NT-proBNP (een natriuretische peptide) en cardiaal troponine T voor het diagnosticeren van linkerventrikel dysfunctie in de DCCSS LATER CARD studie. We vonden een afwijkende NT-proBNP waarde voor leeftijd en geslacht in 22.1% van de overlevenden van

kinderkanker, terwijl maar 5.4% van hun broers en zussen een afwijkende NT-proBNP waarde had. Ondanks het gebruik van een zogenaamde "high sensitive" test vonden we afwijkende troponine T waardes (>10 ng/L) in een minderheid van de overlevenden (5.9%) en kwamen afwijkende troponine T waardes niet vaker voor dan in hun broers en zussen (5,0%). Verder toonden we aan dat NT-proBNP en troponine T alleen niet geschikt zijn om linkerventrikel dysfunctie betrouwbaar aan te tonen of uit te sluiten. Echter, niet alleen bloed biomarkers maar ook patiënt kenmerken bepalen in belangrijke mate het risico op de aanwezigheid van linkerventrikel dysfunctie. Daarom ontwikkelde we in hoofdstuk 6 ook een diagnostisch model dat NT-proBNP en troponine T combineert met klinische kenmerken (zoals geslacht, leeftijd, dosis anthracycline en dosis radiotherapie). Dit model verbeterde de diagnostische accuraatheid aanzienlijk en maakte het mogelijk om linkerventrikel dysfunctie uit te sluiten in 18.5% van de overlevenden van kinderkanker in ons cohort met een hoge mate van betrouwbaarheid (dat wil zeggen een hoge sensitiviteit en hoge negatieve voorspellende waarde). Als deze bevingen gevalideerd kunnen worden in een ander cohort (externe validatie) dan zou dit diagnostische model gebruikt kunnen worden om het aantal onnodige follow-up echo's te verminderen, wat een aanzienlijke kostenbesparing zou betekenen.

Hoofdstuk 7 is een systematische review van 68 dierstudies waarin we de rol en het tijdsverloop van extracellulaire matrix remodellering in anthracycline cardiomyopathie bestudeerde. Extracellulaire matrix remodellering speelt een belangrijke rol in het ontstaan van cardiomyopathieën ten gevolge van pathologische stimuli zoals ischemie, hypertensie, volumeoverbelasting, inflammatie en ook toxines zoals anthracyclines. De geïncludeerde studies vonden in hartweefsel van dieren met anthracycline cardiomyopathie meer collageen, matrix metalloproteïnasen, inflammatie markers (zoals tumor necrosis factor, interleukine 1ß en 6, galectin-3) en markers betrokken in het "transforming growth factor ß-pathway". Op basis van de bevindingen in deze review onderscheiden we drie stappen in extracellulaire matrix remodellering in anthracycline cardiomyopathie: 1) activering van het aangeboren immuunsysteem als reactie op anthracycline geïnduceerde schade aan het myocard, 2) activering van matrix metalloproteïnasen door het aangeboren immuunsysteem die vervolgens de extracellulaire matrix remodelleren, en 3) transformatie van fibroblasten naar myofibroblasten - onder de invloed van "transforming growth factor ß" - die grote hoeveelheden collageen produceren een daarmee interstitiële fibrose in het hart veroorzaken. Deze bevindingen suggereren dat biomarkers voor inflammatie en extracellulaire matrix remodellering mogelijk bruikbaar kunnen zijn voor het diagnosticeren van anthracycline cardiomyopathie in mensen. Dit hebben we onderzocht in het volgende hoofdstuk.

Hoofdstuk 8 is een case-controle studie waarin we opzoek zijn gegaan naar nieuwe bloed biomarkers voor antracycline cardiomyopathie in overlevenden van kinderkanker. Van de 278 gemeten biomarkers vonden we 3 specifieke inflammatie biomarkers die in een significant hogere concentratie aanwezig waren in bloed van patiënten met anthracycline cardiomyopathie vergeleken met controles, onafhankelijk van anthracycline dosis en NT-proBNP. Een van deze gevonden biomarkers betrof het zogenaamde pulmonary surfactant proteïne-D (PSPD), een "pattern recognition receptor" die onder andere betrokken is in de herkenning van "schade geassocieerde moleculaire patronen (DAMPS)βvan beschadigde cellen en daarmee een belangrijke rol heeft in het initiëren van het aangeboren immuunsysteem als reactie op schade.(6) De andere twee biomarkers

betroffen chemokines (CCL19 en CCL20) die chemotactisch zijn voor dendritische cellen en specifieke lymfocyten en belangrijk zijn voor het initiëren van het adaptieve immuunsysteem door het faciliteren van antigeen presentatie en transport van lymfocyten door het lichaam.(7) Deze 3 biomarkers vonden we in gelijke mate in patiënten met gedilateerde cardiomyopathie ten gevolge van een genetische mutatie in het titine of lamine A/C gen wat suggereert dat deze biomarkers in het algemeen betrokken zijn bij cardiomyopathie en niet specifiek zijn voor de effecten van anthracyclines. Interessant genoeg vonden we inflammatie biomarkers die eerder beschreven zijn in hartfalen (zoals tumor necrosis factor, galectin-3, soluble interleukin 1 receptor-like 1, interleukin 1b en 6) niet in onze studie in antracycline cardiomyopathie. De betekenis van deze nieuw ontdekte biomarkers in de pathofysiologie en (vroeg) detectie van anthracycline cardiomyopathie is nog onduidelijk. Hier zal nog verder onderzoek naar gedaan worden in de DCCSS LATER CARD studie.

Deel III (**hoofdstuk 9**) van dit proefschrift gaat over de cardiale follow-up na kinderkanker en bevat de vernieuwde richtlijn van de International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG). In deze richtlijn staan 4 vragen centraal: 1) bij wie is cardiale follow-up noodzakelijk? 2) welke modaliteit moet daarvoor gebruikt worden? 3) hoe vaak is cardiale follow-up nodig? en 4) wat moet er gedaan worden als er cardiale afwijkingen gevonden worden? Wat betreft vraag 1 is de vernieuwde aanbeveling dat cardiale follow-up niet nodig is overlevenden van kinderkanker behandeld met een anthracycline dosis <100 mg/m2 en een radiotherapie dosis op het hart <15 Gray omdat cardiale follow-up in deze groep niet kosteneffectief is. Wat betreft vraag 2 en 3 wordt echocardiografie aanbevolen elke 2 tot 5 jaar vanwege de klinische beschikbaarheid en relatief lage kosten ten opzichte van MRI. Vanwege de matige sensitiviteit wordt het gebruik van cardiale biomarkers alleen (zoals natriuretische peptiden en troponines) als enige strategie voor cardiale follow-up niet aanbevolen. De resultaten uit hoofdstuk 6 ondersteunen deze aanbeveling om biomarkers niet alleen te gebruiken. Echter, als ons diagnostische model beschreven in hoofdstuk 6 (gebruik makende van onder andere NT-proBNP and troponin T) extern gevalideerd kan worden dan zou gebruik van dit model een kosteneffectieve methode zijn om het aantal echo's te verminderen. Wat betreft vraag 4 is de aanbeveling om patiënten met asymptomatische linkerventrikel dysfunctie te verwijzen naar een cardioloog (ejectiefractie <52% in mannen en <54% in vrouwen). In overlevenden van (kinder)kanker zijn er nauwelijks gerandomiseerde studies verricht naar de effectiviteit van behandeling van asymptomatische linkerventrikel dysfunctie. (8) De aanbeveling voor behandeling is daarom gebaseerd op richtlijnen voor hartfalen in de algemene populatie waarin behandeling wordt aanbevolen in patiënten met een linkerventrikel ejectiefractie <40%.(9, 10, 11)

LITERATUUR

1. Gatta G, Botta L, Rossi S, Aareleid T, Bielska-Lasota M, Clavel J, et al. Childhood cancer survival in Europe 1999-2007: results of EUROCARE-5--a population-based study. The Lancet Oncology. 2014;15(1):35-47.
2. Geenen LW, Baggen VJM, van den Bosch AE, Eindhoven JA, Cuypers J, Witsenburg M, et al. Prognostic value of soluble ST2 in adults with congenital heart disease. Heart. 2019;105(13):999-1006.
3. Feijen EAM, Font-Gonzalez A, Van der Pal Helena JH, Kok Wouter EM, Geskus Ronald B, Ronckers Cécile M, et al. Risk and Temporal Changes of Heart Failure Among 5-Year Childhood Cancer Survivors: a DCOG-LATER Study. Journal of the American Heart Association. 2019;8(1):e009122.
4. Armstrong GT, Liu Q, Yasui Y, Neglia JP, Leisenring W, Robison LL, et al. Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. J Clin Oncol. 2009;27(14):2328-38.
5. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2015;16(3):e123-36.
6. Sorensen GL. Surfactant Protein D in Respiratory and Non-Respiratory Diseases. Frontiers in Medicine. 2018;5(18).
7. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu Rev Immunol. 2014;32:659-702.
8. Silber JH, Cnaan A, Clark BJ, Paridon SM, Chin AJ, Rychik J, et al. Enalapril to prevent cardiac function decline in long-term survivors of pediatric cancer exposed to anthracyclines. J Clin Oncol. 2004;22(5):820-8.
9. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European Heart Journal. 2016;37(27):2129-200.
10. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) With the special contribution of the Heart Failure Association (HFA) of the ESC. European Heart Journal. 2021.
11. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Jr., Colvin MM, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. Circulation. 2017;136(6):e137-e61.

LIST OF PUBLICATIONS

1. Leerink JM, Verkleij SJ, Feijen EAM, Mavinkurve-Groothuis AMC, Pourier MS, Ylänen K, et al. Biomarkers to diagnose ventricular dysfunction in childhood cancer survivors: a systematic review. Heart. 2018.

2. Leerink JM, Pinto YM. Towards evidence-based management of patients treated with cardiotoxic chemotherapy: A collaborative effort of cardiologists and oncologists. Neth Heart J. 2018;26(11):519-20.

3. Leerink JM, Feijen E, van der Pal HJH, Kok WEM, Mavinkurve-Groothuis AMC, Kapusta L, et al. Diagnostic tools for early detection of cardiac dysfunction in childhood cancer survivors: Methodological aspects of the Dutch late effects after childhood cancer (LATER) cardiology study. Am Heart J. 2020;219:89-98.

4. Leerink JM, de Baat EC, Feijen EAM, Bellersen L, van Dalen EC, Grotenhuis HB, et al. Cardiac Disease in Childhood Cancer Survivors: Risk Prediction, Prevention, and Surveillance: JACC CardioOncology State-of-the-Art Review. JACC CardioOncol. 2020;2(3):363-78.

5. EC de Baat JL, RIJ Merkx, EAM Feijen, HJ van der Pal, L Kapusta, JJ Loonen, WE Kok, LC Kremer, AMC Mavinkurve-Groothuis. Hartschade als een laat effect van de behandeling van kinderkanker. Nederlands Tijdschrift voor Oncologie. 2020;17(3):94-9.

6. Merkx R, Leerink JM, Feijen ELAM, Kremer LCM, De Baat EC, Bellersen L, et al. Echocardiography protocol for early detection of cardiac dysfunction in childhood cancer survivors in the multicenter DCCSS LATER 2 CARD study: Design, feasibility, and reproducibility. Echocardiography. 2021.

7. Merkx R, Leerink JM, De Baat EC, Feijen EAM, Kok WEM, Mavinkurve-Groothuis AMC, et al. Asymptomatic systolic dysfunction on contemporary echocardiography in anthracycline-treated long-term childhood cancer survivors: a systematic review. Journal of Cancer Survivorship. 2021.

8. Leerink JM, van der Pal HJH, Kremer LCM, Feijen EAM, Meregalli PG, Pourier MS, et al. Refining the 10-Year Prediction of Left Ventricular Systolic Dysfunction in Long-Term Survivors of Childhood Cancer. JACC CardioOncol. 2021;3(1):62-72.

9. Leerink JM, van de Ruit M, Feijen EAM, Kremer LCM, Mavinkurve-Groothuis AMC, Pinto YM, et al. Extracellular matrix remodeling in animal models of anthracycline-induced cardiomyopathy: a meta-analysis. J Mol Med (Berl). 2021;99(9):1195-207.

10. Vehmeijer JT, Koyak Z, Leerink JM, Zwinderman AH, Harris L, Peinado R, et al. Identification of patients at risk of sudden cardiac death in congenital heart disease: The PRospEctiVE study on implaNTable cardIOverter defibrillator therapy and suddeN cardiac death in Adults with Congenital Heart Disease (PREVENTION-ACHD). Heart Rhythm. 2021;18(5):785-92.

11. Merkx R, Feijen E, Leerink JM, de Baat EC, Bellersen L, van Dalen EC, et al. Cardiac function in childhood cancer survivors treated with vincristine: Echocardiographic results from the DCCSS LATER 2 CARD study. Int J Cardiol. 2022;369:69-76.

12. de Baat EC, van Dalen EC, Mulder RL, Hudson MM, Ehrhardt MJ, Engels FK, et al. Primary cardioprotection with dexrazoxane in patients with childhood cancer who are expected to receive anthracyclines: recommendations from the International Late Effects of Childhood Cancer Guideline Harmonization Group. Lancet Child Adolesc Health. 2022.

13. Leerink JM, Feijen EAM, Moerland PD, de Baat EC, Merkx R, van der Pal HJH, et al. Candidate Plasma Biomarkers to Detect Anthracycline-Related Cardiomyopathy in Childhood Cancer Survivors: A Case Control Study in the Dutch Childhood Cancer Survivor Study. J Am Heart Assoc. 2022;11(14):e025935.

14. van Dalen EC, Leerink JM, Kremer LCM, Feijen EAM. Risk Prediction Models for Myocardial Dysfunction and Heart Failure in Patients with Current or Prior Cancer. Curr Oncol Rep. 2023.

15. Ehrhardt MJ, Leerink JM, Mulder RL, Mavinkurve-Groothuis A, Kok W, Nohria A, et al. Systematic review and updated recommendations for cardiomyopathy surveillance for survivors of childhood, adolescent, and young adult cancer from the International Late Effects of Childhood Cancer Guideline Harmonization Group. The Lancet Oncology. 2023.

PORTFOLIO

	Year(s)	EC*
Courses		
AMC Practical biostatistics	2018	1.1
AMC Advanced topics in biostatistics	2018	2.1
AMC Clin. Epid. Systematic reviews	2018	0.7
AMC Clin. Epid. Observational epid.	2018	0.6
AMC Clin. Epid. Randomized clinical trials	2018	0.6
AMC Clin. Epid. Evaluation of medical tests	2018	0.9
AMC Computing in R	2018	0.4
AMC Genetic epidemiology	2021	1.1
AMC BROK	2017	1.0
AMC E-science (big data)	2020	0.6
AMC bioinformatics	2020	1.1
NIHES Advanced clinical trials	2020	1.9
NIHES Survival analysis for clinicians	2020	1.9
NIHES Repeated measurements	2021	1.7
VU Klinische predictie modellen	2019	2.0
VU Longitudinale data-analyse	2019	3.0
VU Mediatie analyse	2021	2.0
VU Missing values	2020	2.0
VU Klinimetrie	2020	3.0
Coursera (Utrecht University): Clinical epidemiology	2019	0.5
Elevate/Utrecht: Advanced diagnostic research	2019	1.5
Coursera (North Carolina at Chapel Hill): Epidemiology	2020	0.5
Coursera (John Hopkins): Regression Models	2020	0.7
Presentations at scientific conferences		
Heart Failure Society of America Scientific Meeting, Nashville (poster)	2018	0.8
Dutch Heart Foundation, Papendal (poster)	2018	0.2
Dutch Heart Foundation Early Recognition, Utrecht (poster)	2018	0.2
Heart Failure Society of America Scientific Meeting, Philadelphia (oral poster)	2019	1.0
North American Symposium on Late Complications After Childhood Cancer, Atlanta (poster)	2019	0.8
European Society of Cardiology Heart Failure Conference, Digital (2 posters)	2020	1.2
European Society of Cardiology, Digital (posters, oral poster)	2020	2.0
International Symposium on Late Complications After Childhood Cancer, Utrecht (oral)	2022	2.0
Teaching		
Supervision of 1 bachelor and 1 master student	2019-2021	3.0
Other		
Editorial consultant, JACC CardioOncology	2021-present	2.0
Peer review (Nature Medicine, British Medical Journal, European Heart Journal, JACC CardioOncology, Circulation Heart Failure, Cancer, ESC heart failure, American Heart Journal)	2017-2022	3.0

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ABOUT THE AUTHOR

Jan Leerink was born in Utrecht on the 6th of January 1992 as part of a triplet to Bas Leerink and Janine de Keijser. The first years of his life he grew up in Utrecht and Curacao where his younger brother Casper was born. At the age of three, the family returned to the Netherlands, where he enjoyed the rest of his childhood mostly in Schalkhaar and Brummen. After graduating from high school at the Vrije School de Berkel in Zutphen in 2010, he went to study medicine at the University of Amsterdam. Next to medical school, he spent countless hours in a rowing boat as a competitive lightweight rower at the student rowing club Skøll in Amsteram, where he met most of his friends and his girlfriend Jessica. During internships, he realized that he wanted to pursue a career as a cardiologist. After graduating medical school in 2016, he worked for 8 months as a medical doctor not in training at the Cardiology ward of the Academic Medical Center in Amsterdam. In October 2017, he started his PhD project at the Department of Cardiology of the Academic Medical Center in Amsterdam and the Princess Máxima Center for Pediatric Oncology in Utrecht, under supervision of prof. dr. L.C.M. Kremer, prof. dr. Y.M. Pinto, dr. W.E.M. Kok and dr. E.A.M. Feijen, which resulted in this thesis. He is currently completing a masterβs degree in Epidemiology. He started his cardiology training in October 2022 at the Amsterdam UMC - Academic Medical Center under supervision of dr. Marije Vis and dr. Daniëlle Robbers-Visser. Jan currently lives in Amsterdam, together with his girlfriend Jessica.

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