Richard John Massey

Cardiac function in young survivors of allogeneic hematopoietic stem-cell transplantation

Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is a therapeutic option for various malignant and non-malignant diseases. However, patients who undergo this intensive treatment are at risk of late-onset heart disease. The prevalence and types of cardiac impairments in long-term survivors of allo-HSCT are not fully clarified. In this PhD thesis, Massey and colleagues present the results of comprehensive evaluation with echocardiography, speckle tracking echocardiography and cardio-pulmonary exercise tests in long-term survivors treated with allo-HSCT as children, adolescents and young adults. The study found left and right ventricular dysfunction and pericardial abnormalities. Treatment with anthracyclines and hypertension were the main risk factors associated with ventricular dysfunction. The adverse effects from anthracyclines were observed to be dose-dependent. Furthermore, left ventricular systolic dysfunction was found to be associated with reduced peak oxygen uptake. Overall, these findings increase understanding of heart disease after allo-HSCT, and will assist in the design of future surveillance regimes in long-term survivors of allo-HSCT.

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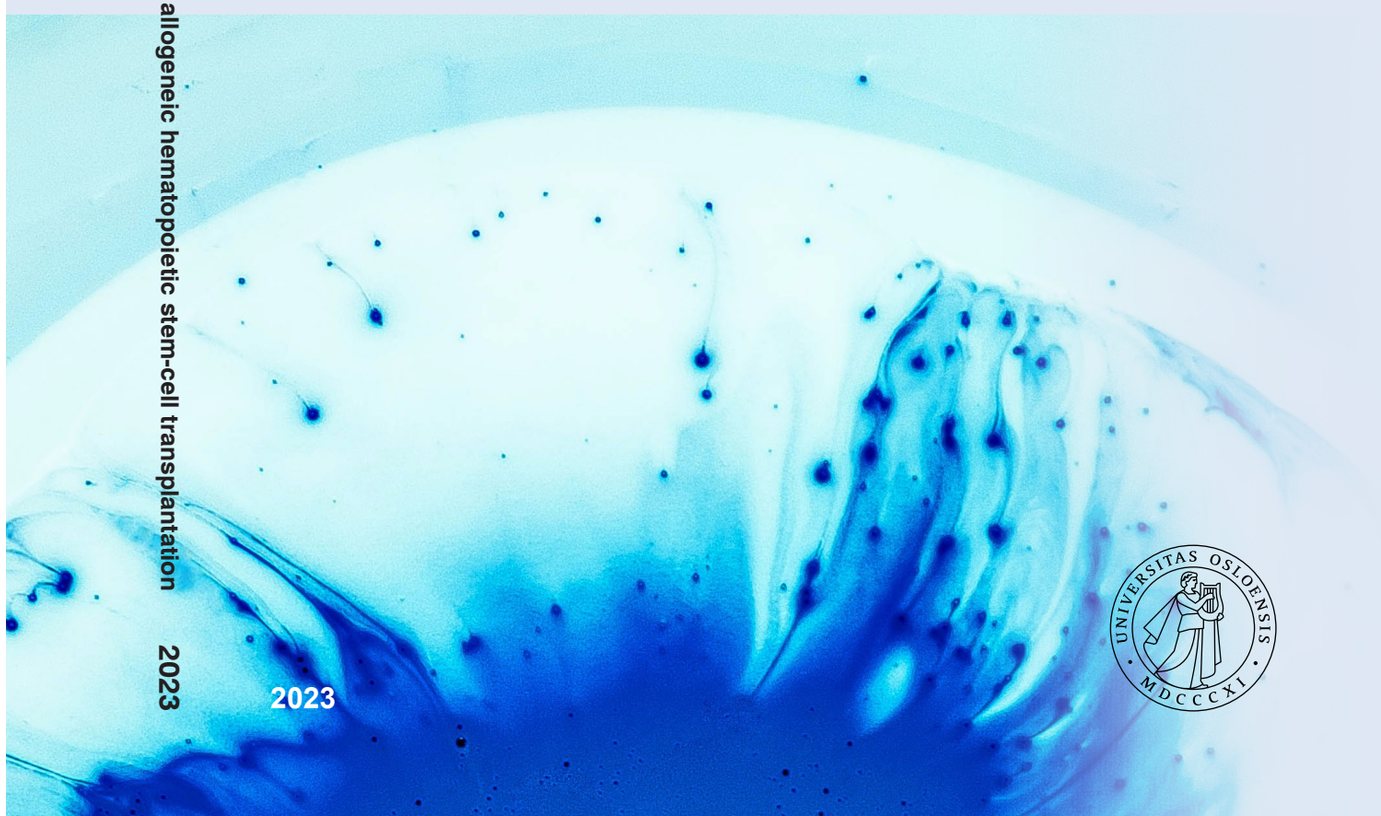

Cardiac function in young survivors of allogeneic hematopoietic stem-cell transplantation.

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1.0 Acknowledgements

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I have thoroughly enjoyed my PhD. It was good to return to university, although I was dishearten to see how the digital era has altered the learning vibe during seminars. However, it has been an incredible learning experience and has greatly expanded my appreciation for research. This PhD thesis hasn't been without some challenges; starting with the reoccurring frustration with data access and fire-walls, losing posters at congress, learning statistics ('it's never a failure, it's just data'), author blindness, corona, an inbox filled with invitations from dubious journals and apprehension when pressing the submit button.

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2.0 List of papers

I **Left ventricular systolic function in long-term survivors of allogeneic hematopoietic stem cell transplantation.**
Richard J Massey MSc., Phoi Phoi Diep MD, Ellen Ruud MD, PhD, Marta Marie Burman MD, Anette Borger Kvaslerud MD, Lorentz Brinch MD, PhD, Svend Aakhus MD, PhD, Lars L Gullestad MD, PhD, Jan Otto Beitnes MD, PhD.

Published in the Journal of the American College of Cardiology (JACC) – Cardio-Oncology, 2020. DOI:10.1016/j.jaccao.2020.06.011.

II **Impaired right ventricular function in long-term survivors of allogeneic hematopoietic stem-cell transplantation.**
Richard J Massey MSc., Phoi Phoi Diep MD, Marta Marie Burman MD, Anette Borger Kvaslerud MD, Lorentz Brinch MD, PhD, Svend Aakhus MD, PhD, Lars L Gullestad MD, PhD, Ellen Ruud MD, PhD, Jan Otto Beitnes MD, PhD.

Published in the Journal of Open Heart, 2021. DOI:10.1136/openhrt-2021-001768

III **Reduced exercise capacity is associated with left ventricular systolic dysfunction in long-term survivors of allogeneic hematopoietic stem-cell transplantation.**
Richard John Massey MSc., Ole Henrik Myrdal MD, Phoi Phoi Diep MD, Marta Maria Burman MD, Lorentz Brinch MD, PhD, Lars Lysgaard Gullestad MD, PhD, Ellen Ruud MD, PhD, Svend Aakhus MD, PhD, Jan Otto Beitnes MD, PhD.

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3.0 Selected Abbreviations

Allo-HSCT: Allogeneic hematopoietic stem-cell transplantation,

BMI: Body mass index,

BOS: Bronchiolitis obliterans syndrome,

CPET: Cardio-pulmonary exercise test,

CAYA: Children, adolescents and young adults,

DBP: Diastolic blood pressure,

E/e': Ratio of early-diastolic velocity (MV_E) and myocardial velocity (e'),

FEV_1: Forced expiratory volume in one second,

GLS: Global longitudinal strain,

GVHD: Graft-versus-host disease (acute: aGVHD, chronic: cGVHD),

LV: Left ventricular,

LVEF: Left ventricular ejection fraction,

LVSD: Left ventricular systolic dysfunction,

MAPSE: Mitral annular plane systolic excursion

MV: Mitral valve

NT-proBNP: N-terminal pro-b-type natriuretic peptide,

NYHA: New York Heart Association,

RV: Right ventricular,

RVFWS: Right ventricular free-wall strain,

RVSD: Right ventricular systolic dysfunction,

SBP: Systolic blood pressure,

STE: Speckle tracking echocardiography,

TAPSE: Tricuspid annulus plane systolic excursion,

TVI: Tissue velocity imaging,

VO_{2peak}: Peak oxygen uptake.

4.0 Summary

Allogeneic hematopoietic stem-cell transplantation (HSCT) is a potential curative therapy for a variety of malignant or non-malignant diseases. Survival rates have improved in parallel with advances in medical technology and supportive therapies. However, allo-HSCT is a highly intensive and toxic therapy that can lead to an array of late-onset complications. Aside from reducing quality of life, these complications can be life threatening. Of particular interest is the increase in observations of heart disease in long-term survivors of cancer related therapies. Allo-HSCT often involves high dosages of chemotherapy that are known to be cardiotoxic. In addition, long-term survivors of allo-HSCT are vulnerable to heart disease due to high rates of cardiovascular disease and effects associated with GVHD. In spite of this, there is limited data on heart disease in long-term survivors treated with allo-HSCT. The main aims of this PhD thesis were to determine the prevalence of heart disease in long-term survivors of allo-HSCT treated in their youth, and to identify risk factors associated with its occurrence. In addition, investigate the relationship between cardiac function and exercise capacity.

This PhD thesis presents the findings from three studies that comprehensively evaluated the heart in long-term allo-HSCT survivors. Data was collected from a nationwide cohort and included all survivors treated at Oslo University Hospital from 1974 to 2009, were less than 30 years of age at treatment, older than 16 years at examination and had a minimum five years of observation time. In total, 104 patients were included, with an average age of 17 years at transplantation, 17 years of follow-up time and were approximately 35 years old at examination. Survivors undertook clinical examinations, blood tests, echocardiography and CPET. A healthy control population was acquired to compare results from echocardiography.

Survivor characteristics in the cohort revealed multiple risk factors for heart disease. Almost half of the participants received anthracyclines as first-line therapies for malignant disease. The vast majority of survivors received standardized myeloablative conditioning regimes consisting of alkylating agents. GVHD occurred in over half of survivors and was associated with pulmonary disorders. Moreover, the study revealed concerning high rates of traditional cardiovascular risk factors among survivors.

A unique feature of the studies conducted for this PhD thesis was the use of comprehensive and modern ultrasound techniques such as three dimensional imaging and strain imaging from speckle-tracking echocardiography. This increased the validity of the findings, and contributed to higher frequencies of cardiac dysfunction as compared to previous studies that used clinical definitions of heart failure. Furthermore, the higher sensitivity of strain imaging to detect early impairment assisted in identifying patients with subclinical impairments at risk of progressive heart disease. The main finding was significantly reduced left and right ventricular function in survivors compared to healthy controls. Mild to moderate LVSD was found in 44% and most cases were without obvious heart failure symptoms. RVSD was in 14% and strongly associated with co-existing LVSD. Pericardium abnormalities were

observed in 8% and mostly limited to cases of chronic GVHD. Elevated levels of NT-proBNP corresponded to patients with cardiac impairments.

Analyses identified possible risk factors for the high rates of cardiac dysfunction found in this cohort. The cohort's long-follow time likely exacerbated the effects from acquired risk factors and deconditioning, and allowed adequate time for gradual processes of heart failure. The young age at transplantation in study participants may have increased their susceptibility for organ damage or disrupted organ growth. The studies found no differences in frequency of impairments between sexes. The use anthracyclines prior to transplantation was found to be strongly associated with cardiac dysfunction. Reductions in ventricular function and signs of remodeling associated with anthracyclines seemed to increase in a dose-dependent fashion. However, the cardiotoxic effects from anthracyclines also appeared to be present at low cumulative dosages, which suggest there is no safe dosage. Patients with cardiovascular risk factors tended to have LVSD. In particular, hypertension was found to be associated with reduced longitudinal function of the left ventricle by GLS. GVHD is known to have widespread effects, and may have a role in modifying cardiac function. This is intriguing possibility and requires further investigations.

Exercise intolerance and reduced exercise capacity is commonly reported in survivors of cancer related therapies. However, information for exercise capacity survivors of HSCT is scarce, and this research was unique by assessing cardiac functions role when controlled to other central and peripheral factors. CPET found 46% of survivors to have reduced exercise capacity defined by VO_{2peak}. Factors of BMI, reduced physical activity, reduced pulmonary function (by FEV1) and LVSD (by GLS) were found to independently predict reduced VO_{2peak}. Reduced oxygen-pulse supported cardiac dysfunction as a probable factor for abnormal exercise capacity. The greatest levels of cardiac dysfunction corresponded to lowest levels of absolute or predicted values of VO_{2peak}. GLS and predicted-FEV_1 showed similar and fair abilities to correctly identify survivors with reduced exercise capacity.

In summary, this thesis proposes that patients treated with allo-HSCT should receive life-long surveillance with regimes incorporating careful evaluation of heart. The thesis endorses the recommendations given by cardio-oncologist specialists to perform echocardiography shortly after cessation of therapy and routinely afterwards at a minimum frequency of once every five years. Performing echocardiography at regular intervals would aid in identifying temporal changes and facilitate earlier medical interventions. Greater surveillance with echocardiography should be considered in individuals with high risk factors such as exposure to high-doses of anthracyclines and hypertension. The results collectively support the potential benefits in monitoring cardiac function with strain imaging acquired by speckle-tracking echocardiography. In addition, reduced exercise capacity by CPET should be considered as a possible manifestation of systolic dysfunction, irrespective of the presence of respiratory disorders. Health care providers need to act assertive to limit traditional cardiovascular risk factors that are modifiable and important targets for primary prevention. Finally, survivors need to be encouraged to engage in healthy lifestyles that limit the effects of deconditioning.

Norwegian translation

Allogen hematopoietisk stamcelletransplantasjon (HSCT) er en potensiell kurativ terapi for en rekke ondartede og ikke-maligne sykdommer. Overlevelsesratene har forbedret seg parallelt med fremskritt innen medisinsk teknologi og støttende terapier. Allo-HSCT er imidlertid en svært intensiv og toksisk terapi som kan føre til en rekke komplikasjoner. I tillegg til og redusere livskvaliteten, kan disse komplikasjonene være livstruende. Av spesiell interesse er økningen i observasjoner av hjertesykdom hos langsiktige overlevende av kreftrelaterte terapier. Allo-HSCT innebærer ofte høye doser kjemoterapi som er kjent for å være kardiotoksiske. I tillegg er langstids overlevende av allo-HSCT sårbare for hjertesykdom på grunn av høye forekomst kardiovaskulære sykdommer og effekter forbundet med GVHD. Til tross for dette er det begrensede data om hjertesykdom hos langtidsoverlevere behandlet med allo-HSCT. Hovedmålene med denne doktorgradsavhandlingen var å bestemme forekomsten av hjertesykdom hos langtidsoverlevere av allo-HSCT behandling i ungdommen, og å identifisere risikofaktorer knyttet til forekomsten. I tillegg ble pasientene undersøkt for å se på forholdet mellom hjertefunksjon og treningskapasitet.

Denne doktorgradsavhandlingen presenterer funnene fra tre studier som ser på hjertefunksjon hos langstidsoverlevende av allo-HSCT som ble behandlet i ungdommen. Data ble samlet inn fra en landsdekkende kohort og inkluderte alle overlevende behandlet ved Oslo universitetssykehus fra 1974 til 2009, var under 30 år ved behandling, over 16 år ved undersøkelse og hadde minimum fem års observasjonstid. Totalt ble 104 pasienter inkludert, med en gjennomsnittsalder på 17 år ved transplantasjon, 17 års oppfølgingstid og 35 år ved undersøkelse. Overlevende gjennomførte kliniske undersøkelser, blodprøver, ekkokardiografi og CPET. Resultat av ekkokardiografi ble sammenliget med en frisk kontrollpopulasjon.

Klinisk gjennomgang av overlevelseskarakteristika i kohorten viste flere risikofaktorer for hjertesykdom. Nesten halvparten av deltakerne fikk antracykliner som førstelinjebehandling for malign sykdom. De aller fleste overlevende fikk standardiserte myeloablative kondisjoneringsregimer bestående av alkyleringsmidler. GVHD forekom hos over halvparten av de overlevende og var assosiert med lungesykdommer. Videre viste studien høye nivåer av tradisjonelle kardiovaskulære risikofaktorer blant overlevende.

Studiene for denne doktorgradsavhandlingen brukte omfattende og moderne ultralydsteknikker som tredimensjonal avbildning og strain imaging fra speckle-tracking ekkokardiografi. Dette økte validiteten av funnene, og bidro til høyere forekomst av hjertedysfunksjon sammenlignet med tidligere studier som brukte kliniske definisjoner av hjertesvikt. Bruk av strain imaging bidro til å identifisere pasienter med subkliniske funksjonsnedsettelser som riskerer å utvikle hjertesvikt. Hovedfunn var signifikant redusert venstre og høyre ventrikkelfunksjon hos overlevende sammenlignet med friske kontroller. Mild til moderat LVSD ble funnet hos 44% og de fleste tilfeller var uten åpenbare hjertesviktsymptomer. RVSD ble funnet i 14% og ofte i pasienter med LVSD.

Perikardiforstyrrelser ble observert hos 8% og for det meste begrenset til tilfeller av kronisk GVHD. Forhøyede nivåer av NT-proBNP korresponderte med pasienter med hjertefeil.

Denne doktorgradsavhandlingen diskuterer resultater av analyser som identifiserte mulige risikofaktorer for den høye forekomsten av hjertedysfunksjon. To faktorer som muligens har bidratt til høy forekomst av hjertefeil er kohortens unge alder ved behandling, samt lang oppfølgningstid. Den unge alderen kan ha økt følsomheten for organskade eller forstyrret organvekst. Studiene fant ingen forskjeller i hyppighet av funksjonsnedsettelser mellom kjønnene. Bruk av antracykliner før transplantasjon ble funnet å være sterkt assosiert med hjertedysfunksjon. Reduksjoner i ventrikulær funksjon og tegn på remodellering assosiert med antracykliner syntes å øke på en doseavhengig måte. Imidlertid syntes kardiotoksiske effekter fra antracykliner også å være tilstede ved lave kumulative doser. Dette kan tyde på at det ikke finnes en trygg dose. Pasienter med kardiovaskulære risikofaktorer hadde en tendens til å ha LVSD. Spesielt ble hypertensjon funnet å være assosiert med redusert langsgående funksjon av venstre ventrikkel ved GLS. GVHD har utbredte effekter som kan påvirke hjertefunksjonen. Dette krever ytterligere undersøkelser.

Treningsintoleranse og redusert treningskapasitet rapporteres ofte hos overlevende av kreftrelaterte terapier. Dokumentasjon på treningskapasitet for overlevende av HSCT er imidlertid knapp. En av studiene i denne dokorgraden så på hjertefunksjonens rolle relatert til treningskapasiert med andre sentrale og perifere faktorer. CPET fant at 46% av overlevende hadde redusert treningskapasitet definert av VO_{2peak}. Faktorer for BMI, redusert fysisk aktivitet, redusert lungefunksjon (ved FEV1) og LVSD (ved GLS) ble funnet å predikere redusert VO_{2peak}. Redusert oksygenpuls hos overleverne støttet hjertedysfunksjon som en mulig årsak til unormal treningskapasitet. Videre korresponderte de største nivåene av hjertedysfunksjon med de laveste nivåene av absolutte eller predikerte verdier av VO_{2peak}. GLS og predicted-FEV_1 viste lignende evner til å identifisere overlevende med redusert treningskapasitet riktig.

I avslutningen av avhandlingen foreslås det at pasienter behandlet med allo-HSCT skal få livslang overvåking med regimer designet for å innlemme evalueringen av hjertet. Avhandlingen støtter anbefalingene gitt av kardio-onkologspesialist om å utføre ekkokardiografi kort tid etter avsluttet behandling og rutinemessig etterpå med en minimumsfrekvens på en gang hvert femte år. Å utføre ekkokardiografi med jevne mellomrom vil hjelpe til med å identifisere tidsmessige endringer og legge til rette for tidligere medisinske inngrep. Økt overvåking med ekkokardiografi bør baseres på individuell risikovurdering og ta hensyn til sentrale risikofaktorer som eksponering for høye doser antracykliner og hypertensjon. Resultatene støtter fordelene ved overvåking av hjertefunksjon med speckle-tracking ekkokardiografi. I tillegg bør redusert treningskapasitet ved CPET vurderes som en mulig manifestasjon av systolisk dysfunksjon, uavhengig av tilstedeværelse av luftveissykdommer. Tidlig interventsjon av allo-HSCT pasienter anbefales for å begrense effekten av modifiserbare kardiovaskulære risikofaktorer. Til slutt må overlevende oppfordres til å engasjere seg i sunn livsstil som begrenser effekten av dekondisjonering.

5.0 Introduction

5.1 Background in hematopoietic stem-cell therapy

Hematopoietic stem-cell transplantation (HSCT) is a potential curative therapy strategy for the treatment of malignant or non-malignant diseases. The technique was pioneered by Donnall Thomas and results from human trials were first published in 1957 (1). In recent decades, major advances in medical technology have pushed HSCT to the forefront of medical treatment. According to the European Society for Blood and Marrow transplantation, 48512 HSCT were performed in 43581 patients (40.8% allogeneic-HSCT) in Europe during 2019 (2). Malignant diseases are currently the most common indications for allogeneic hematopoietic stem-cell transplantation (allo-HSCT). In recent surveys, acute myeloid leukemia (AML) accounted for 38% of allo-HSCT conducted in Europe (2). In comparison, non-malignant disorders collectively accounted for 14% of allo-HSCT conducted in Europe during the same period (2). However, indications for allo-HSCT are continually diversifying, and it is increasingly being chosen as a treatment option for a variety of malignant cancers and hematological illnesses that include autoimmune and congenital metabolic syndromes.

HSCT involves elimination of diseased cells, replacement with healthy stem-cells and the suppression of the immune system to prevent graft rejection; a process called graft-versus-host disease (GVHD) (3). Today, there exist multiple therapeutic options that can provide varied degrees of myeloablative and non-myeloablative conditioning prior to stem-cell infusion (3, 4). These differ in complexity, intensity and the choice often depends on the underlying disease and status of remission. In non-malignant disease, the total eradication of hematopoiesis is not required and therefore a reduced-intensity conditioning with lower dosages of chemotherapy is sufficient (5). In contrary, for indications of malignant disease, an additional preparative or 'first-line' therapy with radiation or chemotherapies can be necessary. In all instances, the use of chemotherapies comes with the potential risk for adverse effects to organ function.

Effective HSCT requires healthy multipotent stem-cells to replace those that have been destroyed by myeloablative chemotherapy or radiation. The origin of the stem-cells can be from a donor (allogeneic) or derived from the patient's own cells (autologous) that are extracted prior to transplantation and then given back after treatment. Stem-cells are most often harvested from peripheral blood cells, although can also be collected from bone marrow and umbilical cord (2, 5). In allo-HSCT, donor stem-cells are preferably from a family member that genetically shares at least one human leukocyte antigen (HLA) allele to the patient. In an absence of relative donors, an unrelated donor that has similar HLA is desired. Disparity, between donor and receipting cells may evoke an immunological response associated with GVHD. Despite this risk, approximately half of allo-HSCT procedures in Europe during 2019 were performed with unrelated donors (2, 5). Moreover, allo-HSCT has two important advantages to auto-HSCT; the donor stem-cells originate from a healthy individual and therefore eliminate the risk of infusing sick (often cancerous) cells back into

the host. Secondly, these new cells participate in beneficial immune responses (graft-versus-tumor effect) that facilitate in the detection and elimination of diseased cells.

Recovery after stem-cell transplantation can be long and require extended periods of isolation. Initial therapies after transplantation include blood and/or platelets transfusions, antibiotics and immune regulatory drugs. Of particular importance is the use of GVHD prophylaxis with, for example, cyclosporine or treatment of GVHD with potent anti-inflammatory drugs like corticosteroids. Allo-HSCT survivors may require continual medical interventions for disease relapses, secondary cancers, chronic GVHD, infections, inflammations (such as mucositis) and organ damage related to the complications.

Allo-HSCT can be a lifesaving treatment when other therapy options are not available. Unfortunately, this comes at a cost, and a patient may have to endure long-term side effects that reduce quality of life and even shorten life expectancy. These undesired transplant related complications can originate from pre-transplantation therapies, stem-cell origin with risk of GVHD, conditioning regimes and post-transplant therapies related to underlying disease or complications. In most instances, patients receive high dosages of chemotherapy that further increase the risk of collateral organ damage. In spite of this, the long-term health of young recipients of allo-HSCT is not well described. A focus of this thesis was determining the prevalence of late-onset heart disease and identifying risk factors associated with its occurrence.

Norwegian treatment strategies for allo-HSCT

The first bone marrow transplantation in Norway was performed in 1968 by Peter Hjort (5). Although, the early 1980s are described as a pioneering period in establishing stem-cell transplantation in Norway (5). In 1985, Oslo University Hospital (Rikshospitalet) became the national center for allo-HSCT, and remained the single center until 2007 when Haukeland University Hospital began treating adult patients (5, 6). In a period between 1974 to 2014, a total of 267 children (median age of 5.65 years), prominently with malignant diseases (mostly leukemia) were treated with allo-HSCT at Oslo University Hospital (7).

At present, approximately 25 patients under 18 years of age are treated with allo-HSCT at Oslo University Hospital each year. As elsewhere, treatment strategies have been modified over the course of time, and allo-HSCT is increasingly being used in non-malignant diseases. Prior to 1990, stem-cells were harvested from bone marrow, whereas today stem-cells are more commonly harvested from blood (5). Previously, donors were predominantly from family relations, while today the majority are from unrelated donors (5, 7). Radiation therapies have gradually been phased out for some diagnoses and chemotherapy dosages have been reduced in attempt to limit undesirable side effects (6). At Oslo University Hospital, the most common preconditioning regime is chemotherapy with busulfan in combination with cyclophosphamide (7).

Mortality and morbidity in HSCT survivors

Advances in treatment strategies and supportive care have led to improved survival in recipients of allo-HSCT (8). In a large American study, Gooley et al. found a 41% reduction in mortality in patients treated between 1993 to 1997 compared to patients treated between 2003 to 2007 (8). The explanation given for these results was fewer complications due to use of peripheral stem-cells instead of bone marrow, reduction in mismatched donors, less radiation and fewer instances of high-dose chemotherapy (8). The acknowledgement of transplant related complications has led to further refining of regimes resulting in lower toxicity and reduced risk from GVHD (2).

Despite the increased awareness and efforts to reduce complications, life expectancy in allo-HSCT survivors still remains considerably lower than the general population (8-11). This was highlighted by Martin et al., who showed patients that survived at least five years after HSCT had a four to nine-fold increased rate of mortality for the next 30 years, which translated to an estimated 30% lower life expectancy than the general population (10). Worldwide data collected by the Center for International Blood and Marrow Transplant Research (CIBMTR) found patients to have a 85% probability of surviving 10 years after allo-HSCT (11). A Norwegian study from a similar timeframe (1995 to 2012) showed the 10 year survival rate in adult recipients of HSCT treated in Norway was 48% (6). In Norwegian children transplanted from 1974 to 2014, the five year survival rate has been reported to be 61%, with no significant improvement in survival for periods 1974 to 1998 compared to 2003 to 2014 (7). Survival rates in Norway have likely improved in recent years due to newer technologies, changes in treatment strategies and expansion of stem-cell transplantation to other non-malignant diseases.

The main causes of death after allo-HSCT are cancer relapse (in malignant survivors), followed by secondary cancers and GVHD (9-11). Cardiovascular diseases including structural heart disease are increasingly being acknowledged as important contributors to shortened life expectancy after HSCT (9-14). The Bone Marrow Transplant Survivor Study (BMTSS) found cardiovascular related mortality to be two to four times higher in HSCT survivors than in the general population (9, 12, 15). However, specific data on the prevalence and types of structural heart disease in long-term allo-HSCT survivors is limited.

Heart failure and left ventricular systolic dysfunction after HSCT

The types of structural heart disease associated with cancer therapies are diverse and include left ventricular (LV) systolic and diastolic dysfunction, coronary artery disease, right ventricular (RV) dysfunction, valvular heart disease and pericardial abnormalities (16). In long-term survivors (>10 years) of childhood chemotherapy, the St. Jude cohort found the prevalence of all types of cardiac dysfunction to be 56.4%, and specifically for cardiomyopathy (systolic dysfunction by echocardiography) in those treated with anthracyclines or radiation therapy to be 6.2% (14). This study also estimated that 21.6% of these childhood survivors would eventually suffer from cardiomyopathy before 50 years of

age (14). There are fewer comparative studies in long-term survivors of HSCT. This is despite the higher prevalence of cardiovascular risk factors in HSCT survivors compared to survivors of conventional therapies (13).

Heart failure and cardiomyopathy has been reported in 5.6 to 10.8% of patients whom have survived at least 10 years after HSCT (12, 17-19). Armenian et al. examined a cohort of 1244 adult patients and found the incidence of heart failure in auto-HSCT survivors to be 4.8% at five years after HSCT and 9.1% after 15 years (17). A retrospective analysis in 544 individuals by Chow et al. found the 10 year cumulative incidence of cardiomyopathy to be 6% in survivors of allogeneic and auto-HSCT (18). More recently, a cross-sectional study by Murbraech et al. found heart failure in 10.6% and asymptomatic left ventricular systolic dysfunction (LVSD) in 5.2% of long-term (10.2 years) survivors of lymphoma treated with auto-HSCT (19). The variation in reported frequencies of myocardial dysfunction is probably attributed to differences in factors that can modify the outcome of heart disease. These factors include: Age at treatment, follow-up time, severity of presenting disease, treatments and presence of comorbidities and cardiovascular risk factors (figure 1).

Chemotherapy and cardiotoxicity

Cardiotoxicity from chemotherapeutical agents falls into two categories: Category 1 are those that cause irreversible damage such as alkylating agents and anthracyclines, and category 2 are those that cause reversible damage such as monoclonal antibodies like trastuzumab (16). In recipients of allo-HSCT, cardiotoxicity may occur due to 'first-line' treatments with anthracyclines, or alkylating agents used as part of conditioning regimes prior to stem-cell grafting. Common alkylating agents used in conditioning are cyclophosphamide and busulfan. Reports of cardiotoxicity from alkylating agents are rare, but include acute LV dysfunction and pericarditis (20-23). There is limited evidence describing late-onset cardiotoxicity solely attributed to alkylating agents.

Anthracyclines are promoted by the World Health Organization as beneficial and effective therapy for malignant disease in children (24). The most widely used anthracyclines are doxorubin, epirubicin, daunorubicin and idarubin. Doxurubin induces cardiotoxicity by interacting with Topoisomerase-IIβ enzyme to form DNA cleaving complex's resulting in damage and depletion of cardiomyocytes (25). The cardiotoxic potency of anthracyclines is evident from a wealth of clinical studies reporting on the matter (16-19, 26-30). The Childhood Cancer Survivor Study (CCSS) demonstrated the risk of heart failure in long-term cancer survivors increased by five-fold when therapies included anthracyclines (31). In childhood survivors of HSCT, Rotz et al., found anthracyclines as the main explanation for late-onset (after 13 years) reductions in LV systolic function (28).

There is considerable variability in severity, susceptibility and temporal aspects of heart disease after anthracyclines. Detection of cardiotoxicity may occur during, immediately after treatment, within months (short-term) or even years (long-term) after cessation of treatment. This may be due to individual susceptibility and severity of cardiac disease. The immediate

effects may be subtle and cause subclinical impairments, which are difficult to detect and insufficient to concede with symptoms. Evidence for immediate effects from anthracyclines is supported by disturbances in electrical conduction and release of troponin shortly after administration of anthracyclines (32, 33). In addition, magnetic resonance imaging has revealed remodeling processes associated with edema and fibrosis shortly after treatment with high-dose anthracyclines (34).

Reductions in cardiac function after chemotherapy replicate the typical progressive patterns described in heart failure; involving left ventricle dilatation and myocardial thinning (35, 36). This is supported by serial echocardiography that shows continual reductions in cardiac function within the first year after treatment with anthracyclines (37). However, the temporal aspects for cardiac dysfunction are not likely uniform, and influenced by an individual's susceptibility to cardiotoxic therapies and other acquired stress factors. This may explain the increase in observations and severity of cardiac dysfunction in parallel with survival time, and account for the use of terminology such as delayed, late-onset or long-term cardiotoxicity.

Cardiovascular risk factors

Traditional cardiovascular risk factors play an important role in modifying heart disease in long-term survivors of allo-HSCT (17, 18, 38, 39). Cardiovascular risk factors may have a duel effect by acting as the primary instigator for heart disease, and accelerating the progression of myocardial impairment caused by cardiotoxic therapies. Data on frequency of cardiovascular risk factors in HSCT survivors is predominantly derived from the American cohorts: BMTSS and CCSS or St. Jude Cohort (40, 41). These cohorts have generated numerous publications that vary in methodology but are consistent in conclusions. In common, traditional cardiovascular risk factors occur at higher rates among allo-HSCT survivors compared to similar aged normal populations (42, 43), survivors of auto-HSCT (13, 39, 42-45) and survivors of conventional cancer therapies (13, 29).

The frequency of cardiovascular risk factors in allo-HSCT survivors whom have survived at least 10 years varies: Hypertension is reported in 24% to 45% (18, 19, 26, 39, 42-45), hypercholesterolemia in 13% to 52% (13, 18, 42-45), diabetes mellitus in 1% to 14% (18, 39, 43-45), hypothyroidism in 30% (46) and obesity 16% to 21% (39, 43-45). The wide range in reported frequencies can be due to differences in definitions, survivor demographics such as social economics, sex, race, age at transplantation or examination, varying length of follow-up time and therapies.

Graft-versus-host disease

Graft-versus-host disease (GVHD) may arise acutely and persist long after transplantation. Acute GVHD has been reported to affect 40% to 60% (47). Recent prediction models for recipients of allo-HSCT in the USA estimate 42% will develop cGVHD within 3 years, and 66% with aGVHD will develop the chronic form (48). GVHD is characterized by immune

and inflammatory responses that are wide-spread and can affect multiple organs. The most commonly reported targets are the skin, liver and the mouth (48, 49). Survivors of allo-HSCT with active cGVHD have a 1.8 times greater risk of life threatening complications such as diabetes, coronary artery disease and stroke (15). Moreover, cGVHD has been implicated for higher rates of cardiovascular disease and cardiovascular related mortality in allo-HSCT as compared to survivors of auto-HSCT (11, 18).

There are several proposed mechanisms by which GVHD can promote cardiac dysfunction. Chronic inflammation processes initiated by GVHD has been linked to endothelial damage and accelerated atherosclerosis (50). This could explain the increased frequency of coronary artery disease observed in HSCT survivors (44). Inflammatory processes may also explain the association between GVHD and pericardial disease (51-53). Indirectly, cardiac function can be negatively affected by other organ damage induced by GVHD. In particular, the manifestation of bronchiolitis obliterans syndrome (BOS) that results in damage of the small airways and impaired lung capacity (54). Although rare after HSCT, pre-capillary hypertension secondary to BOS may potentially impair the RV function (55). Another important effect from GVHD is the potential promotion of cardiovascular risk factors by secondary responses to organ damage or by extended use of immune suppressive medications such as calcineurin inhibitors (cyclosporine A) (42, 56)

Other risk factors

Other factors that are reported to influence the outcome of heart disease in allo-HSCT survivors are age at treatment (35, 57), years after treatment (time to follow-up) (12, 17, 44, 57, 58), sex (17, 26, 35, 43, 58), iron overload (59), pulmonary disease (54, 55), metabolic disease (60, 61) and lifestyle or deconditioning (45, 60-63). The risk profile in patients treated with allo-HSCT during childhood verse as adults is reported to differ (57, 58, 64). There is evidence to support both claims: Older recipients of allo-HSCT have a higher chance of co-morbidities, while young recipients are at higher susceptibility for toxic therapies due to immature organs (35). Females are reported to have higher susceptibility to cardiotoxicity, possibly related to differences in body fat and metabolism of chemotherapeutical agents (31, 35, 58). Lifestyle factors that include diet, smoking and exercise have an obvious role in promoting heart disease.

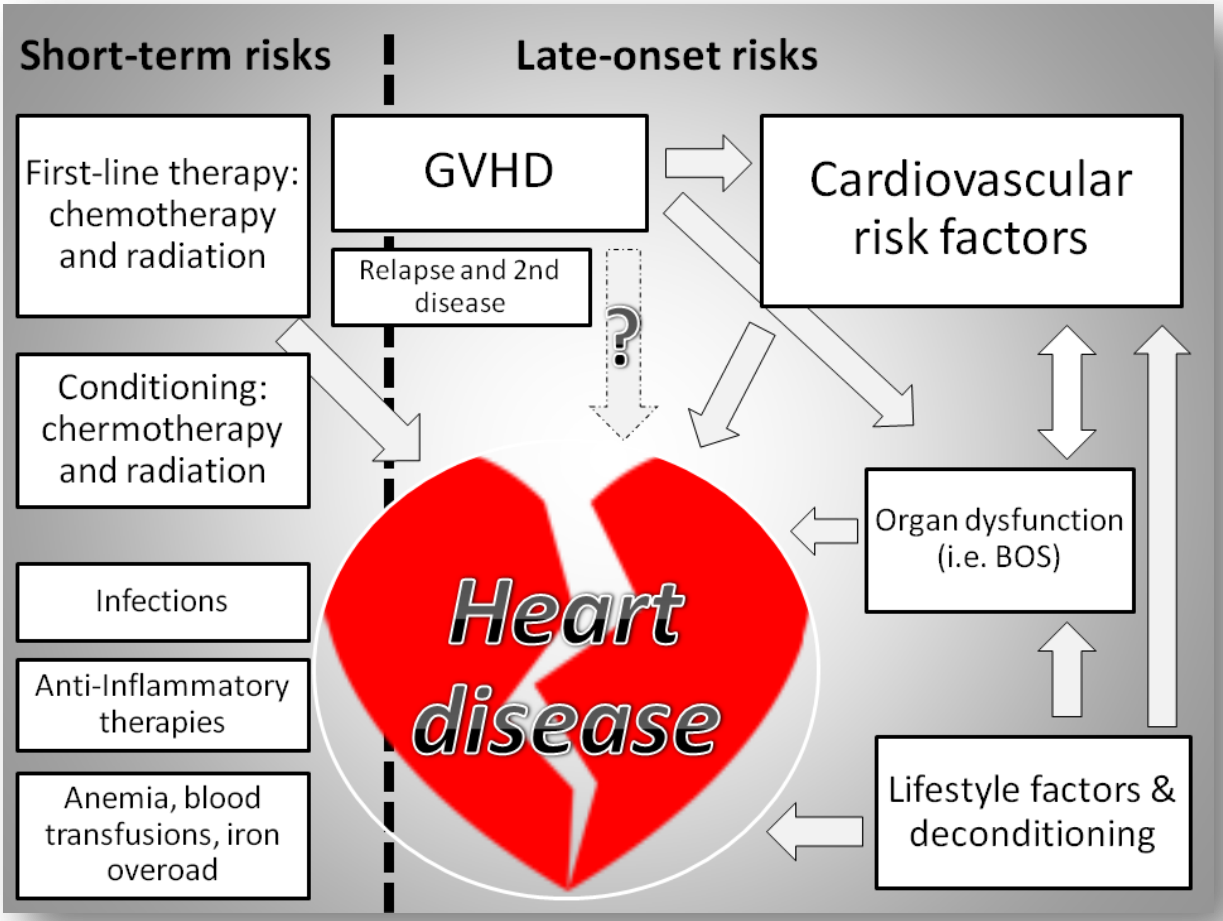

Figure 1: Illustration of potential risk factors and relationships with the outcome of heart disease in long-term survivors of allo-HSCT.

5.2 Echocardiography in the detection of cardiotoxicity

The role of echocardiography in identifying the adverse effects of chemotherapies is well established. Echocardiography is an integral part of the current guidelines published in 2014 by European Association for Cardiovascular Imaging (EACVI) and American Society of Echocardiography (ASE) for identifying and monitoring cardiac function during and after chemotherapy (65). Experts recommend multi-modality ultrasound imaging techniques to increase the sensitivity of detecting cardiotoxicity (65-67). This includes three dimensional (3D) transthoracic echocardiography and speckle tracking echocardiography (STE).

Conventional echocardiography

The fundamental components of echocardiography are two dimensional (2D) grey scale imaging (also called brightness mode or B-mode), motion mode (M-mode) imaging and Doppler wave sampling. These methods provide information on the morphologic characteristics and function. Grey scale images can be used to trace contours and interfaces of

structures such as chamber walls that allow quantification of size. A common application of this is left ventricular ejection fraction (LVEF), which is the volumetric fraction (expressed as percentage) of blood ejected by the left ventricle in systole relative to LV end-diastolic volume. Two dimensional LVEF (2D-LVEF) is used to quantify foreshortening of the myocardium during systole and is a strong prognostic marker of mortality (68). However, 2D-LVEF has limited ability to detect mild myocardial damage, particular after cardiotoxic therapies (65, 69-72).

The Doppler-effect or shift is the change in the frequency of sound waves reflected by moving objects in relation to a stationary object. Measuring the Doppler shift allows quantification of the direction and velocity of blood flow, filling times and changes in chamber pressures. Doppler velocities can be plotted against time to generate flow profiles, and are commonly acquired at the LV outflow tract (LVOT) to calculate cardiac output (CO) and transmitral velocities to evaluate diastolic function. Visualization of blood flow can be achieved by overlaying grey scale imaging with color-coded Doppler velocities.

Another application is myocardial tissue velocities or tissue velocity imaging (TVI) by color-coded imaging or more commonly by pulse wave Doppler. TVI is derived by the filtration and rejection of high range velocities and low amplitude waves created by blood flow, and amplification of low velocities and high amplitude waves created by myocardial motion. In practice, TVI is used to measure myocardial systolic velocity (s') and early-diastolic velocity (e') from sample volumes placed at the annuluses of the atrioventricular valves. The value e' is used to evaluate the recovery or re-coiling abilities of the left ventricle (and possibly the right ventricle) during diastole (73). The ratio of transmitral early-diastolic filling velocity (MV_E) and e' form the parameter of E/e' that is use to estimate filling pressures and correlates well with invasive measures (74, 75).

Speckle tracking echocardiography

The use of speckle tracking echocardiography (STE) has in last two decades evolved from an experimental to a routinely used method in clinical settings. The main principle of STE is the recognition and tracking of reflective patterns ('acoustic speckles') from grey-scale ultrasound generated from structures in the myocardium (76). In doing so, STE can measure the degree of myocardial deformation (shortening or lengthening) during the cardiac cycle. Quantification of myocardial deformation is expressed in percentage of strain (ε) and is calculated by applying the Lagrange formula.

$$\varepsilon\,(_t) = (L(_t) - L_0)/L_0.$$ [Whereby L_0 = initial length, L_t = length at specified time]

The myocardium consists of three layers of different spatial orientated myocytes, which contribute to a unique foreshortening and twisting motion during systole (76). As such, values of strain can be positive or negative and have multiple directional vectors. Strain can be measured in individual myocardial segments, or alternatively multiple segments can be

averaged to attain global values (76). Interpretation is aided by a graphical display of time verse displacement. The overall foreshortening in systole is predominantly in the long-axis direction (base to ventricle apex) and circumferentially (76). The most reliable strain measure for the evaluation of systolic function is global longitudinal strain (GLS) (72, 77, 78). GLS is considered a more sensitive alternative to LVEF for identifying early myocardial impairments and is endorsed for the evaluation of cardiotoxicity (65-67).

5.3 Exercise capacity in allo-HSCT survivors

Exercise intolerance and reduced exercise capacity are readily reported in survivors of cancer therapies (79). The main limiting factors for exercise capacity are cardiac and pulmonary function, hematological capacity and metabolism in skeletal muscle (80, 81). A challenge for clinicians is to eliminate heart disease as a possible explanation. Cardio-pulmonary exercise tests (CPET) enables differential diagnosis for reasons for dyspnea. Moreover, CPET is the gold standard for assessing functional limitation in patients with heart disease (82). In particular, the measure of peak oxygen uptake (VO_{2peak}) is strongly associated with cardiovascular related and all-cause mortality (79, 83, 84).

Evaluation of exercise capacity

The maximal oxygen uptake or consumption (VO_{2peak}) represents the time at which no further incremental changes (plateau) occur despite increasing workload. A major limitation of VO_{2peak} is the rate of oxygen delivery (80, 81). As such, VO_{2peak} is highly dependent on cardiac output (CO) as shown in the Fick's equation:

$$VO_2 = CO \text{ (stroke volume x heart rate)} \times (C_a - C_v)$$

Whereby VO_2 is equal to the product of CO and the difference in arterial oxygen content (C_a) and venous oxygen content (C_v). Changes in stroke volume during exercise are the result of corresponding increases in contractility (by increases in sympathetic nervous system and heart rate) and loading of the left ventricle as described by Frank-Starling mechanisms (80). Cardiac output in healthy persons can increase by four to six-folds during exercise, with the majority of contribution of stroke volume to CO in the initial phases of exercise (81, 85). The parameter oxygen-pulse is the ratio of oxygen uptake (VO_2) to the chronotropic response (heart rate) and reflects the amount of oxygen extraction per heart beat (81, 85). Since, arteriovenous oxygen difference ($C_a - C_v$) in many clinical situations is negligible, a reduction in oxygen-pulse can indicate impairment and reduced ability of the left ventricle to maintain adequate stroke volume during exercise (81).

Reduced exercise capacity in HCST survivors

Reduced exercise capacity by VO_{2peak} in childhood survivors of cancer has been found to give a four-fold increase in hazard rates for death (79). Compared to conventional cancer therapies, there is scarce data describing the role of cardiac function in limiting exercise capacity in survivors of HSCT. In a one year follow-up of 71 adult allo-HSCT survivors, Dirou et al. found 49.3% failed to reach >70% of predicted-VO_{2peak} despite normal 2D-LVEF and few respiratory abnormalities (86). While, in one year follow-up in 43 children treated with allo-HSCT, Vandekerckhove et al. found reductions in VO_{2peak} corresponded to LV dilation, reduced systolic function, elevated filling pressures and was most obvious in those treated with anthracyclines (87). In 20 consecutive long-term HSCT survivors (average 9.8 years), Armenian et al. found significant correlations between abnormal resting GLS (five survivors had GLS >-16%, and all had 2D-LVEF >50%) with VO_{2peak} (38). In long-term lymphoma survivors treated with autologous-HSCT, Murbraech et al. found patients with heart failure, but not asymptomatic LVSD to have reduced VO_{2peak} (19). In the same cohort, but excluding survivors with heart failure, Stenehjem et al. found <80% of predicted-VO_{2peak} to be associated with pulmonary impairments, smoking and reduced physical activity (88). More recently, our research collaborators published findings of pulmonary function in relation to cardio-respiratory fitness in this present cohort (89). In this study, Myrdal et al. found reduced gas diffusion (DLCO), deconditioning and low 2D-LVEF as factors associated with <85% predicted-VO_{2peak} (89).

6.0 Gaps in knowledge

Cardiac function in long-term survivors of HSCT is less well defined as compared to knowledge gathered from conventional cancer therapies. Current descriptions of the late-effects to the heart after HSCT are often from retrospective analyses of registry data and self-reporting questionnaires. In most instances, the cardiac complications from HSCT are described in terms of heart failure that is based on clinical signs and symptoms, with or without imaging diagnostics. When clinical manifestations of heart failure appear, the disease has usually progressed to a stage of irreversible damage. As such, previous studies may fail to identify mild or asymptomatic heart disease and underestimate the risk. Furthermore, the symptoms of heart failure may be masked and confused by other illnesses. Exercise intolerance is readily reported in survivors of chemotherapy. However, more information is required to determine the role of cardiac dysfunction in reducing oxygen uptake and impairing exercise capacity in long-term survivors of allo-HSCT. Relationships between echocardiography and CPET have generated inconsistent results. It is possible that GLS by STE improves detection of systolic dysfunction and assists in establishing reasons for reduced exercise capacity in survivors of allo-HSCT.

7.0 Aims and objectives

It was hypothesized that long-term survivors of allo-HSCT would have a higher likelihood of cardiac dysfunction as a consequence of the intensive therapies and post-transplant complications. This may partially explain the occurrence of exercise intolerance and reduced exercise capacity in these survivors. The main aim of this thesis was to describe cardiac function in long-term survivors of allo-HSCT treated as child, adolescents and young adults (CAYA). Echocardiography and STE were used to achieve these aims. In-turn creating a secondary focuses on the imaging indices for the evaluation of late-onset cardiotoxic effects.

Specific aims

Paper I:
This paper aimed to describe left ventricular structure and function with echocardiography and STE in long-term survivors of allo-HSCT. Furthermore, determine the prevalence and risk factors associated with left ventricular systolic dysfunction (LVSD).

Paper II:
This paper aimed to describe right ventricular structure and function with echocardiography and STE with echocardiography in long-term survivors of allo-HSCT. Furthermore, determine the prevalence and risk factors associated with right ventricular systolic dysfunction (RVSD).

Paper III:
This paper aimed to describe relationships between cardiac function in allo-HSCT survivors and exercise capacity by cardio-pulmonary exercise testing (CPET). Furthermore, investigate if cardiac dysfunction by echocardiography was associated with reductions in oxygen uptake (VO_{2peak}).

8.0 Methods

This current PhD commenced in autumn of 2017 and was part of a nationwide cohort study titled: *'The Norwegian Allo-HSCT survivorship study*' with the main objectives to identify and evaluate the health aspects and long-term complications in young survivors of allo-HSCT. The main project coordinator was Ellen Ruud (MD, PhD) at Oslo University Hospital.

8.1 Design and inclusion criteria

This Norwegian cross-sectional study included all survivors of allo-HSCT performed at Oslo University Hospital who were <30 years of age at HSCT, >16 years (born prior to August 1998) at inclusion and with a minimum of five years of follow-up after allo-HSCT. Oslo University Hospital was the single national center for allo-HSCT, and a complete nationwide cohort was identified by browsing the quality registry. All examinations were completed over a two day hospital stay. Indications for allo-HSCT included malignant and non-malignant diseases. Eleven individuals with mucopolysaccharidosis type 1 (Hurler syndrome) were excluded as these patients may have multi-organ pathology as part of their primary disease. All participants underwent a medical examinations, questionnaires and blood sampling from June 2014 to February 2016. Data from the healthy controls was collected from January 2016 to June 2018.

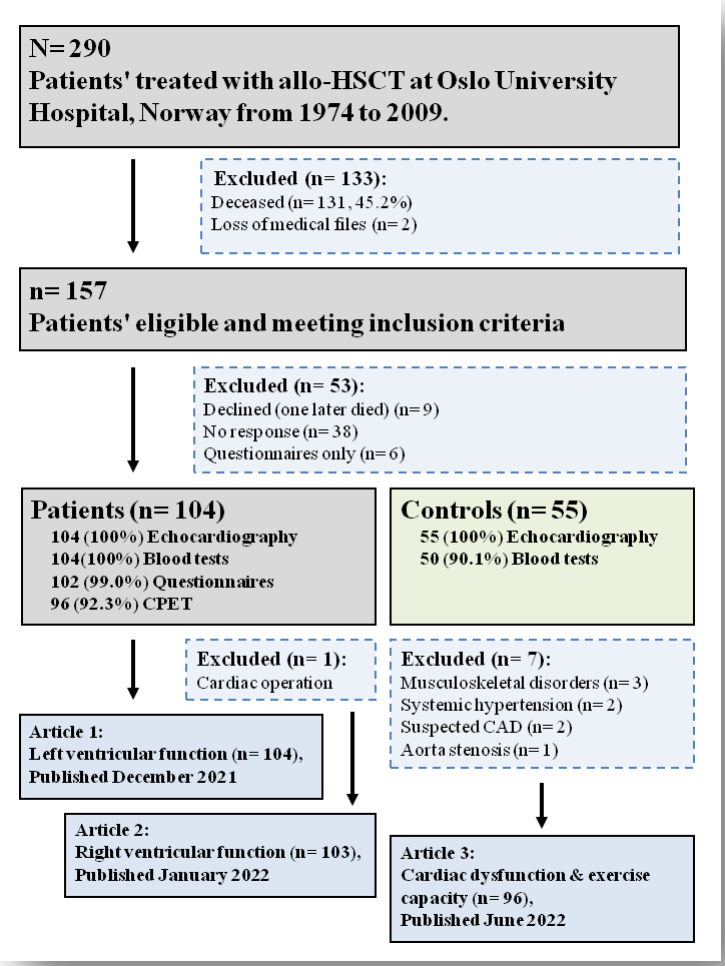

Figure 2: Flow chart.

8.2 Clinical assessment and patient characteristics

Clinical assessment of study participants were made by experienced physicians (P.P.D, L.B and M.M.B) on arrival at Oslo University hospital. Documentation of medical status included: disease type, pre/post transplantation therapies, conditioning regimes, other previous/current medical illness or conditions, symptoms and current medication. Further information on life style factors and health status were obtained from questionnaires. Height, weight and body surface area (BSA) were recorded prior to echocardiography. A standard 12 lead Electrocardiogram (ECG) was taken. Blood samples were collected after overnight fasting and analyzed at Oslo University Hospital laboratory. Biochemistry analysis included hematology tests, as well as kidney, lever, thyroid and cardiac function. N-terminal pro-brain-type natriuretic peptide (NT-proBNP) concentrations were determined by an electrochemiluminescence immunoassay (Roche proBNP II, Roche Diagnostics, Basel Switzerland), and troponin was measured using a high-sensitive immunoassay (Roche hs-TnT). The lowest detectable level for NT-proBNP was 5ng/L and manufacturer's recommendations were used for classifying elevated NT-proBNP according to the age and sex specific cutoffs, and elevated troponin as >14ng/L. Elevated C-reactive protein (CRP) was defined according to laboratory recommendations as >4.0mg/l.

Anthracycline cumulative dosage was converted to isotoxic doses of doxorubicin as recommended in the current Children's Oncology Group (COG) guidelines (90). Dyspnea was classified according to the New York Heart Association (NYHA) (91). Retrospective classifications of acute GVHD were made by Glucksberg scales and chronic GVHD by Schulman scales (92, 93). The National Institutes of Health (NIH) Consensus Criteria's were used to define current status of active GVHD and BOS (49). Diabetes mellitus type-II was defined as hemoglobin HbA1c >6.5% (48mmol/mol), or use of glucose-lowering medication (94). Hypothyroidism was defined by the use of thyroid replacement medication or serum TSH >4mg/l and fT4 <9pmol/L (95). Blood pressures were measured with a Dinamap ProCare 300 Monitor (Criterion, USA), and measured in the supine position, immediately after echocardiography (>30 minutes) and as the average of three measurements. Hypertension was defined as systolic blood pressure >140mmHg or diastolic blood pressure (DBP) >90mmHg, or current use of anti-hypertensive drugs (96). Hypercholesterolemia was defined as LDL >4.1mmol/l (160mg/dl) or use of lipid-lowering medication (97). Anemia was defined as reduced hemoglobin in males <13.5g/dl and females <12g/dl (98). Obesity was defined as body mass index (BMI) $\geq$30kg/m^2 (99).

Quantification of physical activity

Physical activity was quantified from a self-reported questionnaire (HUNT 2/3) undertaken in a Norwegian population and validated against measurements of VO_{2peak}, METS calculations and international physical activity questionnaires (100, 101). This calculated physical activity in a week as the product of weighted scores for the categories of frequency (scores of 0, 0.5, 1, 2.5, 5 ranging from 'never' to 'almost every day'), intensity (scores of 1.0, 2.0, 3.0 ranging from light exercise to 'near-exhaustion') and duration (scores of 0.1, 0.38, 0.75, 1.0 ranging

for <15 minutes to >60 minutes) (101). Higher values (range 0 to 15) reflect greater weekly physical activity. For example: A survivor who exercises two to three times a week, for a total 60 to180 minutes at a moderate intensity has a physical activity score of 3.75. This scoring system generated a numerical scale that was used in comparisons and in multivariable analyses to adjust for the effects of physical activity.

8.3 Echocardiography

Transthoracic echocardiography was performed using General Electric (GE), Vivid E9 scanners, M5S-D / M5Sc-D (1.5 - 4.6 MHz) and 4V-D (1.5 - 4.0 MHz) probes and dedicated software (v113.1.3 Echo-PAC; GE-Vingmed Ultrasound, Horten, Norway). All echocardiograms (patients and controls) were acquired and analyzed by the same experienced and EAVCI accredited investigator (R.J.M). Studies followed current EAVCI and ASE guidelines for image acquisition and evaluation of the LV and RV function (71, 74, 102-104). In addition, the imaging procedures incorporated recommendations for evaluation of cardiotoxicity (65). Echocardiography was comprehensive and used an established image protocol generated for research purposes. Scanner settings were optimized and average measurements made from three consecutive heart cycles. Particular care was made to ensure grey scale imaging with correct orientation, without foreshortening and with appropriate Doppler alignment. Measurements were analyzed off-line and digitally stored.

Evaluation of the left atrium and left ventricle

Standardized grey scale views were obtained in all participants, which consisted of parasternal long-axis, parasternal short-axis (aortic valve and mid-papillary muscle level), apical-4 chamber, apical-2 chamber, apical-3 chamber and subcostal planes. An additional set of apical views with focus on the left ventricle were obtained for calculations of LVEF and GLS. The left atrium (LA) cavity was manually traced with planimetry in the apical-4 and apical-2 chamber grey scale images at end-systole. LA volume was calculated by the biplane disc summation algorithm and divided by BSA to calculate LA volume index (LAVI) (71).

Internal linear dimensions of the left ventricle at end-systole and end-diastole were measured as the average of parasternal long axis and short axis views with M-mode. These measures were verified with 2D caliper measurements. The Devereux cube formula was used to estimate LV cardiac mass and was compared to sex specific cutoffs (71). 2D-LVEF was calculated by manually tracing the endocardial borders and using the modified Simpson's biplane method (71). Recommended sex specific cutoffs for 2D-LVEF were used to define abnormality (males <52%, females <54%) (71). The mean mitral annular plane systolic excursion (MAPSE) was calculated from reconstructed 'anatomical' M-mode from grey scale images taken from the anterior and lateral wall mitral annulus in the apical-4 chamber view.

Pulse wave Doppler was used to measure velocity profiles at the LV outflow track (LVOT), mitral valve (MV), right ventricle outflow track (RVOT) and pulmonary vein (PV). Doppler

profiles taken in the LVOT were used to calculate stroke volume (SV) and cardiac output (CO). TVI Doppler was acquired with spherical sample volumes (radius: 0.4 to 0.5cm^2) placed at the septal and lateral annulus level in the apical-4 chamber view. Average measures of myocardial systolic (s') and early-diastolic velocities (e') were used in analyses. Heart rate was obtained from Doppler tracings.

Diastolic function

In sinus rhythm, pulse wave Doppler measured at the mitral valve (MV) tips was used to calculate: MV early-diastolic filling velocity (MV_E), late-diastolic filling velocity (MV_A), $MV_{E/A}$ ratio, MV deceleration time (MV_{DT}) and Isovolumic relaxation time (IVRT). TVI Doppler was used to obtain e' velocities, and the mean e' was used in the calculation of E/e' ratio. Pulse wave Doppler with low-pass notch filters was used to acquire pulmonary vein (PV) systolic velocity (PV_S), PV diastolic velocity (PV_D), $PV_{S/D}$ ratio, PV atrium contraction velocity (PV_A) and PV_A duration (PV_{Adur}). Algorithms provided in guidelines aided in the categorization of diastolic dysfunction and grading of filling pressures (74). This incorporated: LA size, $MV_{E/A}$ ratio, E/e', $PV_{S/D}$ ratio and tricuspid regurgitation peak velocity. Elevated filling pressure was graded in survivors with LVSD (by reduction in 2D-LVEF and/or GLS).

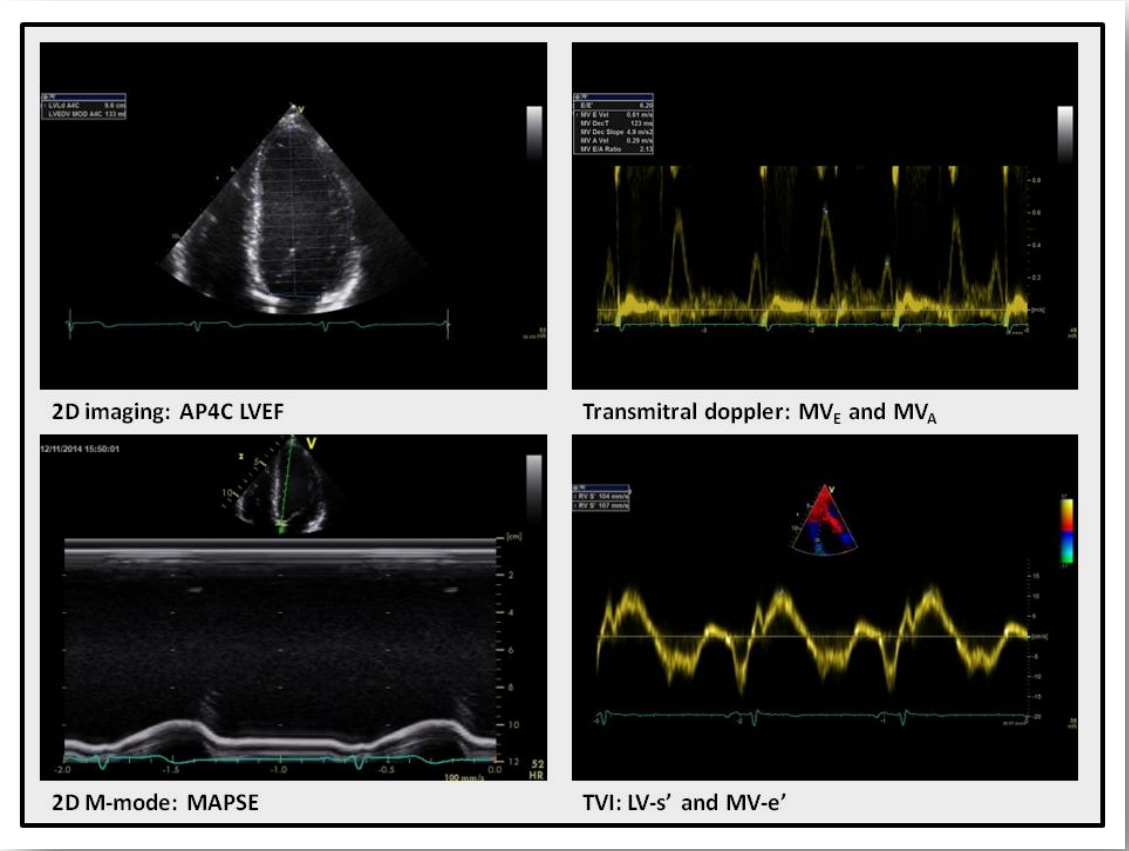

Figure 3: Example of echocardiographical measurements of the left ventricular function.

Evaluation of the right atrium and ventricle

Internal RV dimensions, right atrium (RA) size and measurements of RV function were conducted on apical 4-chamber view focused on the right ventricle (71, 102). Reductions in sector width and depth of grey-scale images resulted in increased frame rate and optimized evaluation of the right ventricle. Planimetry was used to trace RA area in end-systole. RA single-view disc summation was used to calculate RA end-systolic volume (71). Linear dimensions of the right ventricle at end-systole and end-diastole were measured at basal and mid-levels with 2D caliper measurements. RV wall thickness was measured in diastole on a dedicated sub-costal 4-chamber view. Measurements of RA and RV size were indexed to BSA as recommended (71, 102).

Fractional area change (FAC) was acquired by tracing the RV's borders (with consideration for trabeculations) in end-systole and end-diastole. Tricuspid annular plane systolic excursion (TAPSE) was measured with M-mode cursor placed between the annulus to apex and parallel to the free-wall. TVI Doppler with a sample volume (radius: 0.4 to 0.5cm^2) was recorded slightly above the tricuspid annulus to measure RV peak systolic velocity (RV-s'), PV early diastolic velocity (RV-e') and RV index of myocardial performance (RIMP).

Pulse wave Doppler was measured at the RV outflow tract (RVOT). Tricuspid regurgitation peak velocity was measured with continuous Doppler from multiple views. Pulmonary artery peak-systolic pressure (PASP) was estimated from tricuspid regurgitation pressure gradient (TRP) using the Bernoulli equation and adjusting for the RA pressure. RA pressure was estimated to be 5mmHg when the RA was of normal size, and the inferior vena cava was of normal size and had normal respiratory variation (102). In the absence of pulmonary stenosis, elevated PASP was defined as tricuspid regurgitation peak velocity >2.8m/s and/or PASP >35mmHg (102). PASP of 23.3mmHg (groups mean) was allocated to survivors without adequate tricuspid regurgitation or signs of pulmonary hypertension.

Current EAVCI guidelines were used to define the cutoffs for reduced RV function: FAC <35%, TAPSE <17mm, RV-s' <9.5cm/s, RVFWS >-20%, and RIMP >0.54 (105). In the absence of a consensus for definition of RVSD, RVSD was judged to be present when at least two of these parameters were abnormal.

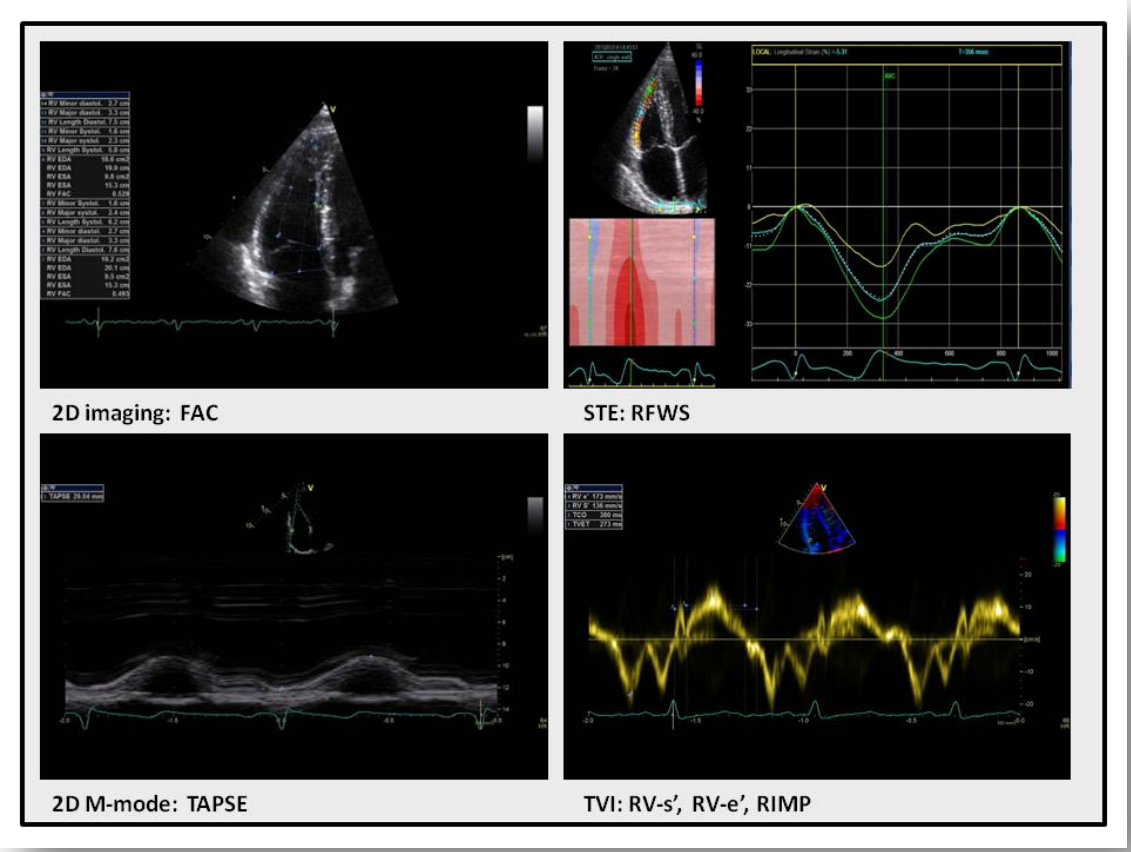

Figure 4: Example of echocardiographical measurements of the right ventricular systolic function.

Evaluation of heart valves

Heart valves were assessed according to recommendations (106, 107). 2D grey scale imaging was used to detect structural abnormalities (i.e. calcification) and color Doppler was applied in multiple views to identify regurgitations. Continuous wave Doppler aided in evaluating of valve stenosis or regurgitation. Valve abnormalities were graded as mild, moderate or severe (106, 107).

Evaluation of the pericardium

The pericardium was evaluated from multiple acoustic views. Pericardial thickness was assessed after careful adjustments of gain settings and non-harmonic ultrasound set at 2.5MHz. Pathology was defined as increased pericardial fluid >0.5cm at end diastole, and/or presence of abnormal thickening or fusion of the visceral and parietal membranes. Further examination was made in cases of suspected pericardial pathology to determine the hemodynamic effects.

Three-dimensional transthoracic echocardiography

Three dimensional volume acquisition of the left ventricle was obtained from the apical-4 chamber view. Optimal images were achieved by adjusting for depth and sector width (60 to 70 degrees), ratio of pyramid (1:1) and cardiac cycles (4 to 6 heart beats). This gave an average volume rate of 39 frames per second (range: 29 to 51fps). End-systole (frame at AV closure) and end-diastole (frame at MV closure) were automatically detected and adjusted when necessary. 3D-LVEF was calculated with semi-automated software for endocardial detection and subsequently manually adjusted after careful examination of long-axes and short-axes views. 3D-sphericity index was calculated as the ratio of LV end-diastolic volume divided by the volume of the sphere with a diameter equal to the longitudinal LV axis in end-diastole.

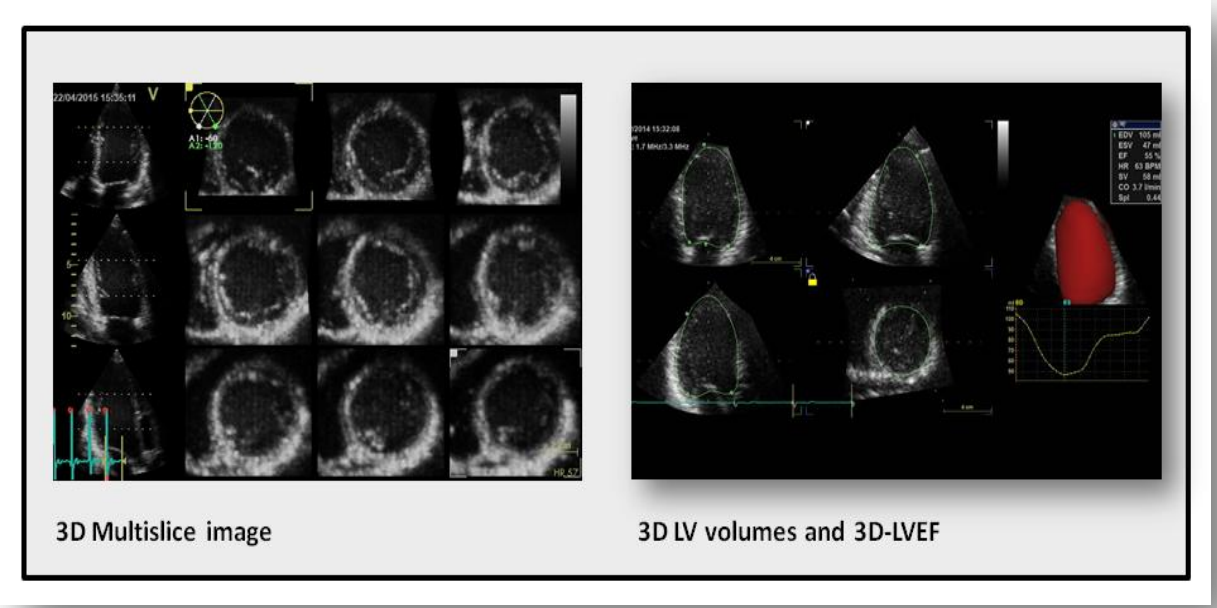

Figure 5: 3D imaging to determine LV volumes and 3D-LVEF.

8.4 Speckle tracking echocardiography

Speckle tracking echocardiography (STE) was conducted on images of good acoustic quality, with avoidance of foreshortening and with consideration for possible ultrasound artifacts. Q-Analysis tracking software was used to calculate GLS. The process entailed manually selecting images, defining endocardial boarders and event timing and automatic tracking software to generate initial results. Region of interest (ROI) was manually adjusted to correctly define the endocardial borders, apex and annular plane and exclusion of the pericardium (103). Strain values were averaged from measures over the ROI width. End of systole was adjusted to coincide with aortic valve closure. Tracking was visually controlled and further manual adjustments made if necessary. Segments judged to have poor tracking or generate unreliable values were excluded. Drift compensation was set at default (middle position). Temporal and spatial smoothing was not manually adjusted. The global longitudinal strain value was calculated as the average of peak systolic values at end-systole recorded from all included segments. Strain was recorded as negative values, in which lower negative values correspond to greater in longitudinal foreshortening. The resulting segmental data was exported and saved in excel.

Evaluation of left ventricular systolic function with STE

STE was conducted in each of the three apical views using similar sector size, depth, heart rate and at an average of 62fps. GLS was calculated as the average of peak systolic strain values in a 17 segment model. The technique allowed no more than three segments to be rejected (no more than one per apical view). There is no current consensus for the cutoff value that separates normal from abnormal GLS. A GLS threshold of ≥-17% was used to define abnormality. This value corresponded to the lower limit of the 95% CI found for GLS in our control group, and is similar to values reported from studies dedicated to determining normal GLS thresholds (108, 109).

Evaluation of right ventricular function with STE

STE was performed on a single focused RV view. Effort was made to align the RV free wall in parallel with the incepting ultrasound. Average frame rate was 69 fps. STE was analyzed on the right ventricle using similar procedures as for the calculation of GLS. Longitudinal strain was calculated by two methods: RV-global longitudinal strain (RV-GLS) as the average value from six segments including free-wall and septum, and RV free-wall strain (RVFWS) as the average of the three free-wall segments only. Results were only included if reliable values were acquired from all segments. Abnormal RV-GLS and RVFWS were categorized as >-20% as recommended, although reference values are uncertain and RVFWS is considered to be higher (105).

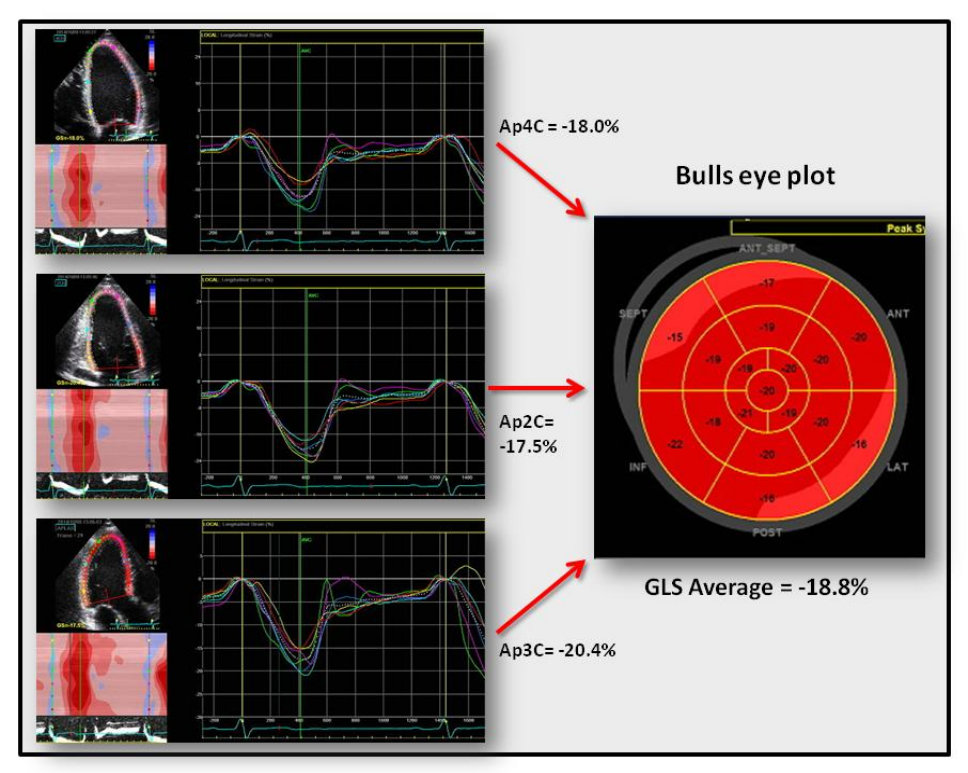

Figure 6: Graphical representation of global longitudinal strain (GLS) acquired with speckle tracking echocardiography (GE medical ultrasound systems). The procedure requires ultrasound images from each of the three standard apical positions: four-chamber (Ap4C), two-chamber (Ap2C) and three-chamber (Ap3C). In each position, the myocardium is divided into six segments. Peak systolic strain measurements are obtained for each segment. The right panel shows time-strain curves for each segment per apical view in relation to timing of end-systole defined as aortic valve closure. GLS is calculated as the average of all peak systolic strain values, and represented by a 'bulls eye' plot (left panel). In this example, GLS is -18.8% which is considered normal (≥-17%).

8.5 Blinding and evaluation of measurement variability

Echocardiography was performed with knowledge that the individual was recruited as a control. The echocardiograms were analyzed on de-identified images, blinded to status and clinical information (including other test results, i.e. CPET), and conducted en-bloc (survivors and controls) after the last inclusion and in random order (aided by a random number generator). Measurement bias was further reduced by measuring LV parameters (i.e. 2D/3D-LVEF and GLS) and RV parameters (i.e. FAC, RV-s', TAPSE, RV-GLS and RVFWS) at separate occasions. Twenty-five echocardiograms from patients and controls were randomly selected to conduct intra-observer variability tests.

8.6 Cardio-pulmonary exercise test

CPET was conducted after echocardiography and was performed and analyzed by experienced personnel. The CPET was conducted on a treadmill (TechnoGym Runrace, Forli, Italy) using the modified Balke protocol (110). Incremental changes in ventilatory parameters were measured at intervals of 30 seconds with Vyntus CPX, (CareFusion, Hoechberg, Germany). Predicted-VO_{2peak} and percent of predicted-VO_{2peak} were calculated by equations that adjust VO_{2peak} by age and sex. These adjustments were derived from a Norwegian multicenter study conducted in 759 healthy subjects that completed a maximal exercise test (111). In 20 to 29 year olds, the normal VO_{2peak} value for males is 48.6 ml/kg/min and females is 40.3 ml/kg/min (111). Reduced exercise capacity was defined as <85% of predicted-VO_{2peak} as recommended in the guidelines (112). Oxygen-pulse was calculated by dividing VO_{2peak} by maximal heart rate, and predicted oxygen-pulse by dividing predicted-VO_{2peak} by maximal heart rate.

8.7 Spirometry

Spirometry was conducted and evaluated according to recommendations (113, 114). Pulmonary function in this cohort has previously been described (89). For this study, we used forced expiratory volume in one-second (FEV_1) to represent lung function. The rational was two-fold; FEV1 is readily attainable and is strongly associated with BOS, which is the most clinically relevant respiratory disorder in this cohort. Percent of predicted-FEV_1 was calculated for each individual using recommended equations that adjust for race, ethnicity, sex and height (115).

8.8 Control group

A control group was recruited from healthy volunteers responding to advertisements. Controls and survivors were of similar ethnicity and race. Efforts were made to obtain a control group with comparative demographic characteristics, especially for proportions of sex and age (similar age clusters). The only exclusion criterion was established cardiovascular disease. Documentation of clinical history was conducted by an experienced physician (A.B.K). Blood tests, ECG and CPET (with cycle protocol) were conducted. All echocardiograms were performed in the same environment, with the same equipment and by the same examiner (R.J.M). On request, volunteers received a report of findings.

8.9 Statistical analyses

Professional statistical advice was given from Oslo Center for Biostatistics and Epidemiology (OCBE). Statistical analyses were made with SPSS version 25-26 (SPSS Inc., Chicago, USA), and a p-value <0.05 was considered significant.

Sample size and power calculations

The sample size was estimated to detect differences between allo-HSCT patients and controls for normally distributed continuous variables. For feasibility, a minimum number of controls were recruited to meet demands. Estimates were made with the expectation of mild effect size differences. In paper I, estimates for 2D-LVEF were based on experience and knowledge gained from previous studies; a 10% mean difference between groups and a 10% in-group standard deviation. In paper II, the expected values for RV parameters (FAC, TAPSE, RV-s', RVFWS and RIMP) in each group were uncertain. Therefore, hypothetical estimates were generated by supplementing the normal mean and standard deviations provided in EAVCI guidelines for the control group, and estimating means in the survivor group to be 10% lower (71). Standard set parameters were α set at 0.05, power >0.80, sampling rate of controls to survivors at approximately 0.5. In total, 55 controls were included to allow for missing data and maintaining a power >0.80.

Descriptive statistics and comparisons

Histograms, measures of skewness and Shapiro-Wilk test were used to review the normality of data. Continuous data were presented as mean ± standard deviation, or as median (25^{th}, 75^{th} percentile). Categorical data were presented as number (percentage). Student's t-test and Mann-Whitney U-test were used for continuous data, and Chi-square or Fisher's exact test for categorical data. In-group comparisons with multiple categories were tested with one-way analysis of variance (ANOVA), one-way analysis of covariance (ANCOVA), Kruskal-Wallis test and three-way contingency tests. Bonferroni post-hoc adjustment was used in these analyses. In paper I and II, the effect of anthracycline dosage was tested by allocating survivors into three groups of similar size: none, low exposure ($<300mg/m^2$) and high exposure ($\geq 300mg/m^2$). Determination of thresholds was based on the median value and was similar to levels used in comparative studies. The selection of controlling covariates was influenced by baseline differences in groups and knowledge of potential confounders for cardiac function. The number of covariates was restricted to limit compromising statistical power.

Propensity scoring

Propensity scoring was used in paper I and II to adjust for differences between the allo-HSCT group and controls when comparing cardiac function. The main aim of propensity scoring

techniques is to reduce selection bias and confounder effects by balancing groups that have different sets of exposures or risks (116). To achieve this, variables known to influence cardiac function (i.e. heart rate) and factors that differed between groups (i.e. DBP) were selected. The advantages propensity scoring gave to this study were the inclusion of all the data and condensing covariates to one variable. This resulted in optimal covariate balancing, higher degrees of freedom, power and precision compared to traditional methods (116). The specific propensity method used was inverse probability of treatment weighting (IPTW). The residual confounding effect was tested, and no trimming or exclusion of extreme scores was performed. Results were presented with beta, standard error and p-value. These tests were conducted with advice from statisticians and the literature (116).

Regressions

Linear regression analyses were used to determine significant linear relationships between explanatory (independent) variables and outcome (dependent) variables for cardiac function and oxygen uptake (i.e. LVEF, GLS, TAPSE and VO_{2peak}). Linear regression was preferred to increase precision, remove generalization and uncertainties that arise with use of binary dependent variables. In paper III, the higher volume of evidence supporting the prognostic value of VO_{2peak} compared to predicted-VO_{2peak} was further justification for its preferential use in regression analyses (82, 85). Continuous variables were standardized (z-scores) that enabled comparison of size and direction of effect (negative or positive).

The final multivariable regression models included in-prior variables considered as clinically important risk factors (i.e. anthracycline dosage), biological factors known to influence the outcome and variables with p-value <0.2 in a univariable regression. In all instances, the patient characteristics of sex and age at examination were included in final prediction models. Missing data was rare and considered random. Assumption testing included histograms, residual plot analyses and assessment of multi-collinearity by Pearson's correlations (R <0.5), Tolerance (>0.6) and Variance Inflation Factor (<1.5). Considerations were made to prevent over-fitting of prediction models and restrict the number of covariates (one variable to approximately ten subject) (117). Results were presented with beta, confidence intervals and p-value. Likewise deletion was used for the data omissions in paper I and II; with the rational that different sets of interaction between covariates may influence the outcome. In paper III, pairwise deletions were considered a more appropriate due to reduction in sample size and imbalances created by exclusions of subjects for CPET.

ROC and AUC

In paper III, Receiver operating characteristics (ROC) were performed and areas under the curve (AUC) calculated. Variables of echocardiography, spirometry and NT-proBNP were tested singularly and in combination. The diagnostic accuracy (AUC) of the variable was the ability to correctly identify patients who have reduced exercise capacity by <85% of

predicted-VO_{2peak}. The optimal GLS threshold was chosen as the value that maximizes sensitivity and specificity.

8.10 Ethical considerations

This study was well designed, had creditable scientific aims and was conducted by a competent research group to ensure high quality and trustworthy results. The study abided to the 3rd general principle in the declaration of Helsinki that ensures the best interests and welfare to the study participants (118). Informed consent was obtained from all participants, and legal obligations associated with conducting medical research in Norway were obeyed (119-121). This project received acceptance by Regional Ethics Committee (REK) south-east in 2014 (reference number 2014/370, extension 25321, till 31.12.2025). A separate application was accepted by REK for acquiring normal controls (reference number 2015/98).

8.11 Funding

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8.12 Author information

All co-authors have fulfilled the ICMJE criteria for authorship. The 'Norwegian allo-HSCT survivorship study' was designed and administrated by E.R. Subsequent design of protocols relevant for evaluation of cardiac function was made by S.A. Data collection was made by R.J.M, O.H.M, P.P.D, L.B, M.M.D, E.B and A.K.B. Echocardiography was conducted by R.J.M, and analyzed by R.J.M and S.A. Professional advice of study methodology and interpretation of results was given by E.R, P.P.D, S.A, L.L.G and J.O.B. Main supervision was given by J.O.B. All authors contributed to reviewing and editing of the published manuscripts.

9.0 Results

In total, 290 patients were treated with allo-HSCT in the timeframe specified for this study. Of these, 131 (45.2%) died prior to study start and two were excluded due to incomplete patient files. One-hundred and fifty-seven were eligible for inclusion and 104 (66.2%) survivors were examined with echocardiography and 96 (61.1%) with CPET. Echocardiograms were reviewed by an experienced cardiologist (S.A) and reports entered into the patient's journal.

9.1 Survivor characteristics

The cohort consisted of 56 (53.8%) females, aged 17.8 ± 9.6 years at allo-HSCT, follow-up time was 17.2 ± 5.6 years and age at examination was 35.0 ± 11.7 years. Information and use of data from non-participants (n= 53) and the deceased was limited due to privacy laws. As such, data on deaths associated with cardiovascular disease is not reported. Non-participants (n= 53) were younger (27.7 years vs. 34.3 years, p =0.001), had shorter follow-up time (13.2 years vs. 16.5 years, p <0.001) and were less likely to be female (30.2% vs. 53.8%, p= 0.005). Stem-cells were obtained from bone marrow in 88 (84.6%) and originated from unrelated donor source in 31 (29.8%) cases. Malignant disease was the indication in 77 (74%), and included 33 (31.7%) with acute myeloid leukemia, 25 (24.0%) with chronic myeloid leukemia and 13 (12.5%) with acute lymphoblastic leukemia. Non-malignant disease was the indication in 27 (26.0%), of which 17 (16.3%) had severe aplastic anemia.

In cases with malignancy, first-line therapies consisting of anthracyclines were used in 47 (45.2%) and mediastinal radiotherapy in 2 (1.9%). Median anthracycline cumulative dosage was 270mg/m^2 and ranged from 45 to 585mg/m^2. A combinations of anthracyclines were used, consisting mostly of daunorubicin in 31 (29.8%), doxorubicin in 25 (24.0%) and mitroxantron in 20 (19.2%). Twenty-four (23.1%) with chronic myeloid leukemia did not receive anthracyclines. Two (1.9%) with severe combined immunodeficiency (SCID) did not receive any form of chemotherapy. The majority received myeloablative conditioning consisting of busulfan (4-5mg/kg/d/po administered over 4 days) in combination with cyclophosphamide (50mg/kg/d/iv over four days or 60mg/kg/d/iv over two days). Anti-thymocyte globulin (ATG) was given in 23 (22.1%) and seven (6.7%) received additional fractionated total body irradiation (TBI, 1.3Gy x2 over five days) during conditioning. Cyclosporine was administrated in 103 (99%) as part of standard GVHD prophylaxis.

Sex differences

Characteristics of age at treatment, age at examination, follow-up time, BMI or heart rate (at echocardiography) did not differ between sexes. Moreover, the frequency of malignancy, treatment with anthracyclines, GVHD, BOS, LVSD, RVSD and exercise capacity was similar

between sexes. Females had higher median (IQR) values for NT-proBNP (69ng/L (41, 143) vs. 24ng/L (13, 55), p <0.001) and more females were classified with elevated NT-proBNP (12.5% vs. 3.8% p= 0.041). Systolic blood pressure was lower in females (119 ± 17mmHg vs. 127 ± 21mmHg, p= 0.041). Hypothyroidism was the only cardiovascular risk factor to differ between sexes, being more common in females (8.7% vs. 1.0%, p= 0.019). Males had larger heart chambers, a few remaining differences in cardiac function after adjusting for SBP and BSA and higher mean values for VO_{2peak} and oxygen-pulse (see appendices).

Dyspnea and physical activity

Functional dyspnea (NYHA class grade II or III) was reported in 28 (27.4%, two were not classified) survivors and was associated with in participants found to have reduced exercise capacity (41.9% vs. 13.2%, p= 0.001). The median (IQR) physical activity score was 3.8 (1.5, 5.0) and significantly correlated with VO_{2peak} (R= 0.323, p <0.001). A trend towards lower levels of physical activity (median values: 2.0 vs. 3.8, p= 0.056) were found in the group with reduced exercise capacity.

Risk factors

Cardiovascular risk factors were common in survivors (figure 7) and relatively absent in controls. DBP at time of examination was mildly higher in survivors verse controls (72 ± 13mmHg vs. 66 ± 8mmHg, p= 0.002). The most prevalent cardiovascular risk factors were hypertension in 42 (40.4%, and 30.8% were currently medicated) and hypercholesterolemia in 16 (15.7%, n= 102, two had elevated triglycerides). Thirty-three (31.7%) were currently using cardiovascular related medications. CRP was elevated in 24 (23.1%) survivors, of which 17 had a diagnosis of GVHD

A detailed description of GVHD in this cohort has recently been published (122). A history of GVHD was identified in 67 (64.4%). Twenty-seven (26.2%) had aGVHD only, 12 (11.5%) had cGVHD only and 28 (26.9%) suffered from both forms (122). At time of examination, 31 (29.8%) survivors had active GVHD according to the current NIH guidelines (122, 123). GVHD was more commonly associated with malignant disease (70.1% vs. 22.1%, p= 0.040) and in those who received first-line therapies with anthracyclines (74.5% vs. 25.5%, p= 0.052). No relationship was confirmed between GVHD and hypertension or hypercholesterolemia. In addition, survivors with GVHD were physically smaller on average, shown by lower BSA (1.80 ± 0.2kg/m^2 vs. 1.89 ± 0.2kg/m^2, p= 0.054) and BMI (23.6 ± 4.3kg/m^2 vs. 26.2 ± 6.0kg/m^2, p= 0.013). Ten (9.6%) survivors were diagnosed with BOS, of which eight also had cGVHD and seven had co-existing LVSD. Seven with cGVHD had pericardial abnormalities (87.5% vs. 12.5%, p= 0.254).

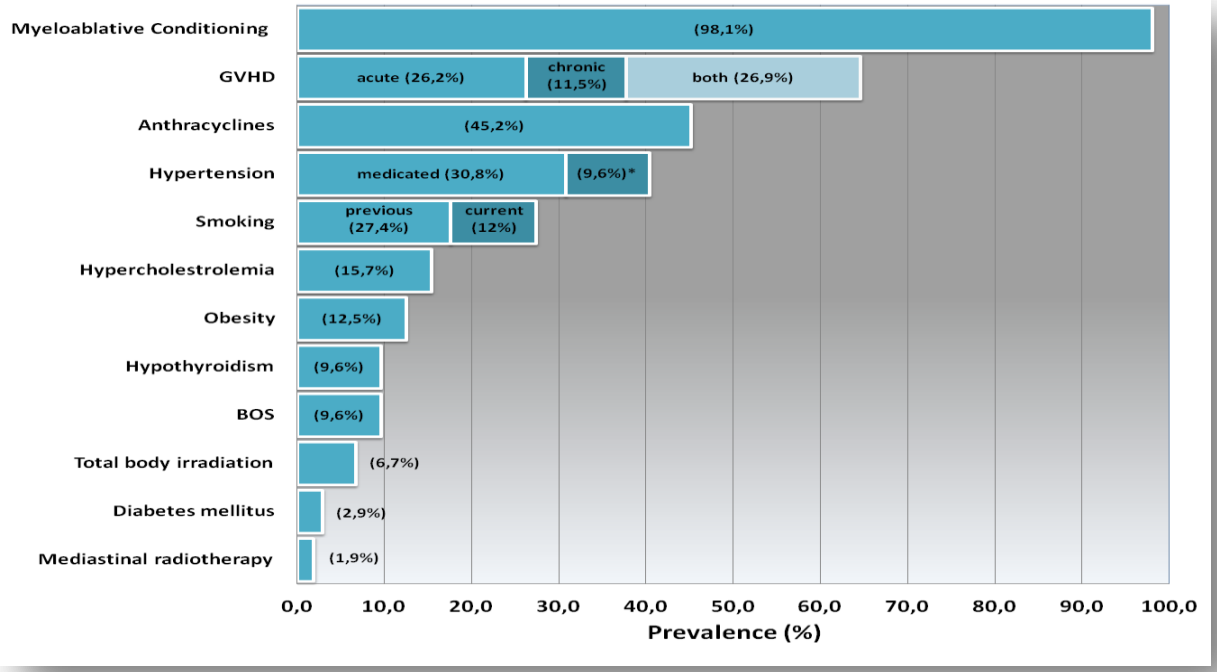

Figure 7: Chart showing prevalence of risk factors. Abbreviations: GVHD: Graft-verse-host disease, BOS: bronchiolitis obliterans syndrome. * Elevated blood pressure and not medicated.

9.2 Echocardiography

Cardiac function

LV function (paper I) and RV function (paper II) were found to be significantly lower in survivors compared to controls after adjustments for potential confounders (table 2 and 3). Significant Pearson's correlations were observed between parameters of LV and RV function (see appendices). LVSD occurred in 46 (44.2%) and was absent in the controls. Patients with and without LVSD were of similar age and follow-up time. The majority of reductions in systolic function were mild and without obvious heart failure symptoms. Thirteen (12.7%) had symptomatic LVSD and 33 (32.4%) had asymptomatic LVSD. Global hypokinesia was the most common phenotype pattern of LVSD. Two individuals had reductions restricted to the septum.

Several parameters related to diastolic function differed between survivors and controls. Diastolic dysfunction in the absence of LVSD was found in seven (6.7%). Elevated filling pressures were classified in 22 (21.2%) survivors with LVSD. Further grading of elevated filling pressures found five (22.7%) with grade I, seven (31.8%) with grade II and ten (45.5%) with grade III. LVSD was not limited to those with cardiovascular risk factors (digital supplement in paper I, published in JACC).

RVSD was categorized in 15 (n= 104) and one control (14.4% vs. 1.8%, p= 0.011). Thirteen (86.7%) with RVSD had co-existing LVSD. In the two cases of RVSD without co-existing

LVSD, one had undergone heart surgery (excluded from later analyses used in paper II) and the other had diastolic dysfunction. Repeated comparative analysis with the removal of survivors with pericardial abnormalities did not alter the significance of the findings associated with RV function (digital supplement in paper II, published in Open Heart).

Table 1: Echocardiogram of the left ventricle

Variable	Allo-HSCT (n= 104)	Control (n= 55)	p-value	Adjusted p-value*
Left atria and ventricular morphology				
IVSd (cm)	0.86 ± 0.17	0.89 ± 0.14	0.274	0.04 (0.03), 0.133
LVIDd (cm/m^2)	2.68 ± 0.32	2.69 ± 0.25	0.984	-0.01 (0.05), 0.887
LVIDs (cm/m^2)	1.87 ± 0.30	1.84 ± 0.2	0.395	-0.40 (0.04), 0.345
LV Mass (g)	130.5 ± 42.7	136.2 ± 35.6	0.406	7.30 (6.1), 0.231
LAVI (ml/m^2)	30.1 ± 9.0	28.6 ± 7.4	0.286	-1.81 (1.3), 0.178
Left ventricular function				
2D-LVEF (%)	55.2 ± 5.8	59.0 ± 2.9	**<0.001**	**3.80 (0.74), <0.001**
3D-LVEF (%)	54.0 ± 5.1 (n= 88)	57.6 ± 2.7 (n= 53)	**<0.001**	**3.42 (0.68), <0.001**
GLS (%)	-17.5 ± 2.2 (n= 100)	-19.8 ± 1.4 (n= 52)	**<0.001**	**-2.13 (0.30), <0.001**
MAPSE (mm)	12.9 ± 2.1	14.9 ± 2.2	**<0.001**	**2.02 (0.34), <0.001**
Diastolic function				
MV_E (m/sec)	67.6 ± 15.3	66.2 ± 15.2	0.579	-0.01 (2.4), 0.997
MV_A (m/sec)	47.9 ± 16.4	41.4 ± 13.6	**0.013**	**-6.60 (2.3), 0.005**
$MV_{E/A}$	1.6 ± 0.8	1.8 ± 0.6	0.198	0.19 (0.12), 0.119
MV_{DT} (m/sec)	162.1 ± 42.7	147.6 ± 29.3	**0.025**	**-15.30 (5.7), 0.008**
MV e' (cm/sec)	10.8 ± 3.2	11.9 ± 3.1	**0.046**	**1.00 (0.50), 0.040**
E/e'	6.6 ± 2.2	5.7 ± 1.4	**0.003**	**-0.73 (0.29), 0.013**
$PV_{S/D}$ (m/sec)	1.0 ± 0.4 (n= 100)	1.0 ± 0.3 (n= 47)	0.936	0.01 (0.06), 0.819

Parameters represent the core results that were used in publications. Data presented as mean ± SD. Comparisons made with Student t-tests. *Propensity scoring and adjusted with covariates of age at examination, BMI, heart rate and diastolic blood pressure. Results presented with beta (Standard error), p-value. Significant p-values (<0.05) are in boldface. **Abbreviations:** E/e': ratio of early-diastolic velocity to mean early-diastolic myocardial velocity, IVSd: Inter-ventricular septum diameter (m-mode) in diastole, FAC: Fractional area change, FEV_1: Forced expiratory volume in one second, GLS: Global longitudinal strain, LV: Left ventricular, LA: Left atrium, LAVI: Left atrium volume indexed, LVEDV: Left ventricular end-diastolic volume, LVEF: Left ventricular ejection fraction, LVIDd: Left ventricular internal end-diastolic dimension, LVIDs: Left ventricular internal end-systolic dimension, MAPSE: Mitral annular plane systolic excursion (average of septum and lateral), MV: Mitral valve, MV_A: Transmitral A-wave velocity, MV_{DT}: Transmitral E-wave deceleration-time, MV e': Left ventricular early-diastolic myocardial velocity (average of septum and lateral), MV_E: Transmitral E-wave velocity, $MV_{E/A}$: Ratio of E-wave to A-wave velocities, $PV_{S/D}$: Ratio of systolic to diastolic pulmonary vein filling velocity, RA-EDV: Right atrium end-diastolic volume, RVIDd: Right ventricular internal dimension in diastole, RVIDs: Right ventricular internal dimension in systole, RVFWS: Right ventricular free-wall strain, RV-GLS: Right ventricular global longitudinal strain, RV-s': Right ventricular peak systolic velocity, RWT: Relative wall thickness, TAPSE: Tricuspid annular plane systolic excursion (average of septum and lateral), TRP: Tricuspid regurgitation pressure gradient and VO_{2peak}: peak oxygen uptake.

Table 2: Echocardiogram of the right ventricle

Variable	Allo-HSCT (n= 103)‡	Control (n= 55)	p-value	Adjusted p-value*
Right atria and ventricle morphology				
RA-EDV (ml/m^2)	22.2 ± 6.8	22.8 ± 4.9	0.560	0.32 (0.96), 0.833
RVIDd basal (cm/m^2)	2.0 ± 0.3	2.0 ± 0.2	0.133	-0.07 (0.04), 0.092
RVIDs basal (cm/m^2)	1.6 ± 0.2	1.5 ± 0.2	0.391	-0.05 (0.03), 0.158
Right ventricular function				
FAC (%)	41.0 ± 5.2	42.2 ± 5.1	0.175	**0.02 (0.01), 0.047**
TAPSE (mm)	20.8 ± 3.7	24.6 ± 3.8	**<0.001**	**3.70 (0.60), <0.001**
RV s' (cm/s)	11.1 ± 2.3 (n= 102)	12.3 ± 2.3 (n= 54)	**0.005**	**1.20 (0.37), 0.001**
RV-GLS (%)	-21.8 ± 3.1 (n= 95)	- 23.1 ± 2.7 (n= 53)	**0.012**	**-1.21 (0.47), 0.012**
RVFWS (%)	-27.1 ± 4.2 (n= 95)	-28.5 ± 3.3 (n= 53)	0.052	**-1.25 (0.61), 0.043**
TRP (mmHg)	18.1 ± 4.0 (n= 76)	16.7 ± 2.6 (n= 29)	**0.034**	-1.11 (0.71), 0.125

Parameters represent the core results that were used in publications. Data presented as mean ± SD. Comparisons made with Student t-tests. ‡ One patient was excluded for comparisons.*Propensity scoring adjusted with covariates of age at examination, heart rate and diastolic blood pressure. Results presented with beta (Standard error), p-value. Significant p-values (<0.05) are in boldface. **Abbreviations:** See Table 1

Factors associated with LVSD and RVSD

A consistent finding was a strong relationship between the use of anthracyclines and reduction in LV (paper I) and RV (paper 2) systolic function. This association was dose-related; with higher anthracyclines cumulative dosages corresponding to the greatest levels of biventricular impairment, signs of LV remodeling and more cases of elevated filling pressures. Significant independent predictors for 2D-LVEF were age at examination, cumulative anthracycline dosage and GVHD. Significant independent predictors for GLS were age at examination, heart rate, cumulative anthracycline dosage and hypertension.

In paper II, RVSD was found to be strongly associated with LVSD. Survivors with RVSD had significantly worse LV function compared to survivors without LVSD (2D-LVEF 49.2 ± 7.2% vs. 56.2 ± 5.0%, GLS -14.6 ± 2.5% vs. -17.9 ± 1.8%, p<0.001). In the multivariable analyses, GLS (more superior than LVEF) was found as a strong independent predictor for reduced RV function.

As a group, survivors with hypertension differed by mildly larger LV diameter, increased cardiac mass, lower GLS (paper I) and diastolic function (see appendices). These remained significantly different after controlling for age and BMI, and to lesser extent after the exclusion of those treated with anthracyclines.

Heart valve function

Frequency and grading of valve regurgitations is presented in table 3. Mitral and tricuspid valve regurgitations were more frequent in allo-HSCT survivors compared to controls. Valve disease of clinical significance was rare: One survivor had mild aortic stenosis (bicuspid) in combination with moderate aortic and mitral regurgitation, one survivor had mild aortic stenosis in combination with mild to moderate regurgitation and one had mild to moderate mitral regurgitation secondary to atrial dilatation.

Table 3: Frequency and grading of valve regurgitation in survivors compared to controls.

	Survivors (n=104)	**Controls (n= 55)**	**p-value**
Aortic valve	8 (7.7%)	2 (3.6%)	0.496
Trivial / Mild	4 / 2 (total= 5.8%)	2 / 0 (total= 3.6%)	0.434
Moderate	2 (1.9%)	0 (0.0%)	0.545
Severe	0 (0.0%)	0 (0.0%)	-
Mitral valve	53 (51.0%)	18 (32.7%)	**0.028**
Trivial / Mild	34 / 17 (total= 49.0%)	9 / 9 (total= 32.7%)	**0.048**
Moderate	2 (1.9%)	0 (0.0%)	0.545
Severe	0 (0)	0 (0)	-
Tricuspid valve	88 (84.6%)	37 (67.3%)	**0.011**
Mild	82 (78.8%)	37 (67.3%)	0.110
Moderate	6 (5.8%)	0 (0.0%)	0.094
Severe	0 (0.0%)	0 (0.0%)	-
Pulmonary valve	86 (83.5%) (n=103)	42 (76.4%)	0.276
Mild	81 (78.6%)	40 (72.7%)	0.403
Moderate	5 (4.9%)	2 (3.6%)	1.000
Severe	0 (0.0%)	0 (0.0%)	-

Values reported as n (%) and p-value calculated with Chi-square or Fishers-exact. Significant p-values (<0.05) are in boldface.

Pericardium abnormalities

Pericardial pathology was found in eight (7.7%) survivors, of which seven also had a diagnosis of cGVHD. Abnormal pericardium was described as localized fibrotic thickening of visceral and parietal layers that gave appearance of fibrotic scarring. In addition, the echocardiograms exhibited no adverse hemodynamic effects. In these individuals, one had medical history of recurring pericarditis, two were previously documented with echocardiography and none had received pericardial effusion drainage.

Other abnormalities

Two survivors had remnant central venous catheters in the right atrium. One individual was operated for a benign tumor in the vicinity of the left atrium and was excluded from analyses involving the right ventricle. Two had suspected coronary artery disease that was followed up with other tests included coronary angiography that revealed one patient with mild

arthrosclerosis. Two with hypertension had dilated ascending aorta. Four had suspected patient foramen ovale. One had borderline pulmonary hypertension (TRP: 36mmHg).

9.3 CPET

In paper III, 96 were tested with CPET. Eight individuals were excluded due contraindications: Three due musculoskeletal disorders, two with systemic hypertension and reduced cardiac function, two with suspected coronary artery disease or abnormalities and one with aortic stenosis. All individuals were exercised to peak effort: Borg scale ≥18 and/or respiratory exchange ratio (RER) ≥1.10. Peak oxygen uptake (VO_{2peak}) was 39.5 ± 6.7 ml/kg/min in males and 33.4 ± 7.5 ml/kg/min in females. Forty-three (45.8%) survivors were found to have reduced exercise capacity (<85% of predicted-VO_{2peak}). These survivors were characterized by younger age, shorter follow-up time, greater BMI, higher frequency of self-reported dyspnea, less engagement in physical activity and higher likelihood of BOS and LVSD. Table 4 summarizes the results from paper III. The greatest levels of cardiac dysfunction corresponded to lowest levels of absolute or predicted values of VO_{2peak} (digital supplement in paper III, published in Journal of Clinical Ultrasound). Survivors with 2D-LVEF <50% had lower percent of predicted-VO_{2peak} (80.0 ± 11.7% vs. 90.2 ± 18.7%, p= 0.050) and trend towards reduced percent of predicted oxygen-pulse (79.3 ± 14.0% vs. 89.6 ± 20.2%, p= 0.071). Forty-four (44.8%) survivors had reduced oxygen-pulse (<85% of predicted oxygen-pulse).

Multivariable linear regression for the prediction of VO_{2peak} (ml/kg/min) was adjusted to physiological factors known to influence the outcome (age, sex and weight) (82). Anthracyclines were negatively correlated with both cardiac and pulmonary function and were not included in the multivariable model. E/e' was considered the most representative parameter for elevated filling pressures. Variables of sex, BMI, physical activity score, reduced pulmonary function (by FEV_1) and reduced LV systolic function (by GLS) were found to be significant independent predictors for VO_{2peak}.

ROC analysis found percent of predicted-FEV_1 (not for unadjusted FEV_1) and GLS to have similar and fair abilities to correctly identify survivors with reduced exercise capacity: Percent of predicted-FEV_1 (AUC: 0.66, 95% CI: 0.55 to 0.77, p= 0.007) and GLS (AUC: 0.64, 95% CI: 0.53 to 0.75, p= 0.014). A GLS cutoff value of -18% gave a sensitivity of 67% and specificity of 62% for correctly identifying survivors with reduced exercise capacity. The prediction abilities increased mildly when tested with the combined probabilities of GLS and percent of predicted-FEV_1 (AUC: 0.70, 95% CI: 0.59 to 0.81, p= 0.001).

Table 4: Comparison of survivors with normal verse reduced exercise capacity

Variable	Normal (>85% predicted-VO_{2peak})	Reduced (<85% predicted-VO_{2peak})	p-value *	Adjusted p-value †
Number	53 (55.2)	43 (44.8)	-	-
Peak heart rate (bpm)	179 ± 15	183 ± 15	0.198	
VO_{2peak} (ml/kg/min)	39.4 ± 6.6	32.3 ± 7.2	**<0.001**	-
range in values	28 to 54	19 to 44		
Predicted Oxygen-pulse (%)	96.4 ± 17.9	77.9 ± 16.8	**<0.001**	-
Anthracycline (yes/no)	21 (39.2)	22 (51.2)	0.258	-
Dyspnea (NYHA II and III)	7 (13.2)	18 (41.9)	**0.001**	-
Predicted FEV_1 (%)	93.13 ± 14.5	82.6 ± 19.8	**0.003**	-
2D-LVEF (%)	56.3 ± 5.5	54.3 ± 6.3	0.095	0.052
3D-LVEF (%)	55.0 ± 4.4 (n= 49)	52.9 ± 5.4 (n= 34)	0.057	**0.046**
GLS (%)	-18.0 ± 1.9 (n= 52)	-17.1 ± 2.0 (n= 43)	**0.022**	**0.043**
E/e'	6.5 ± 2.4	6.4 ± 1.7	0.870	0.867
FAC (%)	40.9 ± 5.0	41.5 ± 5.6	0.576	0.759
RV-s' (cm/sec)	10.9 ± 2.2 (n= 52)	11.3 ± 2.3	0.392	0.633
RVFWS (%)	-27.4 ± 4.5 (n= 51)	-26.4 ± 4.1 (n= 38)	0.319	**0.041**
RV-GLS (%)	-22.1 ± 3.3 (n= 51)	-21.4 ± 3.1 (n= 38)	0.268	**0.021**

Values presented as n (%) or mean ± SD. Significant p-values (<0.05) are in boldface. *Comparison between survivors with normal and reduced exercise capacity made with Student's t-test and Chi-square test. † ANCOVA, adjusting with covariates of age at examination, BMI, heart rate (at echocardiography) and systolic blood pressure (at echocardiography). **Abbreviations:** As table 1

9.4 Biomarkers

NT-proBNP ranged from 5 to 1552ng/L in survivors, and median (IQR) values were significantly higher in survivors as compared to controls (48ng/L (22, 91) vs. 5ng/L (5, 52), p <0.001). Differences were also found between sexes (see above). Elevated levels of NT-proBNP were found in 17 (16.3%) survivors and two controls (4.0%, p= 0.029). In the survivors with elevated NT-proBNP, 12 (70.6% of cases) had cardiac dysfunction consisting of five with biventricular systolic dysfunction (LVSD and RVSD), five with LVSD, one with RVSD and one with diastolic dysfunction. In the five remaining survivors with elevated NT-proBNP, four had enlarged left atrium and one had pericardium abnormalities. Thirty-six with LVSD had normal levels of NT-proBNP. In the two controls with elevated NT-proBNP, one was diagnosed with hyperthyroidism and one had supraventricular tachycardia. NT-proBNP correlated with multiple parameters related to cardiac function, including: Troponin (R= 0.56, p <0.001), LVIDs (R= 0.41, p <0.001), GLS (R= 0.38, p <0.001), Blood pressure (systolic: R= 0.33, p= 0.001, diastolic: R= 0.28, p= 0.004), LVIDd (R= 0.33 p= 0.001), anthracycline dosage (R= 0.32, p= 0.030), 2D-LVEF (R= -0.31, p= 0.001), MAPSE (R= -0.30, p= 0.002),

Oxygen-pulse (R= 0.30, p= 0.003), 3D-LVEF (R= -0.27, p= 0.012), LAVI (R= 0.26, p= 0.021), E/e' (R= 0.23, p= 0.018), IVSd (R= 0.20, p= 0.045). Elevated troponin (>14ng/L) was found in two (1.9%) with LVSD and elevated NT-proBNP.

9.5 Measurement accuracy and intra-observer variability

Good image quality allowed high feasibility. One or more segments were excluded in 41 analyses of GLS: one segment was excluded in 34 analyses, two segments were excluded in five analyses and three segments were excluded in two analyses. Repeatability was assessed in twenty-five randomly selected patients and controls (with sufficient data sets). The same images were analyzed >6 months apart, by the same observer (R.J.M) and software and blinded to the previous result. The average value of three repeated measurements was used to calculate intra-class correlation coefficient (ICC, type A) using two-way mixed and absolute agreements (table 4).

Table 4: Feasibility and intra-observer variability tests.

Variable	Feasibility n (%)*	Absolute differences	ICC coefficient (type A), 95% confidence interval (CI)
2D-LVEF	100.0 (100.0)	0.5 ± 0.5%	0.95, 95% CI: 0.89 to 0.98
3D-LVEF	88 (84.6)	0.8 ± 0.1%	0.93, 95% CI: 0.84 to 0.97
MAPSE	100.0 (100.0)	2 ± 3mm	0.98, 95% CI: 0.95 to 0.99
GLS	100.0 (96.2)	0.4 ± 0.1%	0.96, 95% CI: 0.89 to 0.99
FAC	100.0 (100.0)	1.6 ± 0.3%	0.93, 95% CI: 0.82 to 0.97
TAPSE	100.0 (100.0)	1 ± 4mm	0.98, 95% CI: 0.96 to 0.99
RV-s'	99.0 (100.0)	0.2 ± 1.7cm/s	0.97, 95% CI: 0.97 to 0.99
RV-GLS	92.3 (100.0)	0.2 ± 0.1%	0.90, 95% CI: 0.77 to 0.96
RVFWS	92.3 (100.0)	0.1 ± 0.1%	0.94, 95% CI: 0.87 to 0.96

* n= 104 echocardiograms. **Abbreviations:** See table 1.

9.6 Amendments

Paper 1: for propensity test, beta value for GLS should be negative sign (-2.13)
Paper 1: Beta blockers was used in 3 not 13 individuals.

10.0 Discussion

The primary aim of the study was to describe heart function in a complete nationwide cohort of long-term allo-HSCT survivors treated as children, adolescents or as young adults (CAYA). Comprehensive echocardiography was used to achieve this, and subsequently created a secondary focus on the methodological concepts for evaluation of late-onset cardiotoxicity. This study found cardiac impairment in long-term survivors of allo-HSCT to be more frequent than previously reported. The main risk factors identified in this thesis were pre-transplant anthracyclines and post-transplant cardiovascular risk factors. LVSD was found as an explanatory factor for reduced oxygen uptake. In comparison to conventional cancer therapies, the literature in regard to cardiac function in long-term survivors of allo-HSCT is much scarcer.

10.1 Structural heart disease

Left ventricular systolic dysfunction (LVSD)

Echocardiographical parameters were consistent in finding reduced LV systolic function in comparison to healthy control group. LVSD defined by reduced 2D-LVEF or GLS was found in 44% and in most instances was mild to moderate in severity. This pattern of impairment is suggestive of widespread affection of the myocardium, as expected from cardiotoxic therapies from chemotherapies. However, survivors of HSCT have been shown to be at high risk of atherosclerosis, and given that angiography was not conducted, the possibility of coronary artery disease was not excluded (18, 44). Signs of remodeling including LV dilation and thinner intra-ventricular septum were limited to cases of high anthracycline dosages and advanced systolic dysfunction. This finding is in agreement with results by Armenian et al. who found reduced LV cardiac mass and evidence of increased wall stress in survivors after high-dose anthracyclines (36). In contrast, a sub-analysis of survivors with hypertension showed a tendency to have increased cardiac mass.

Heart failure or cardiomyopathy has been reported to occur in 4.8 to 10.6% of long-term survivors of HSCT (12, 17-19). We found symptomatic LVSD in 12.7% of the entire cohort. A limitation with estimates of heart failure is the risk of overlooking cases of asymptomatic and mild structural or functional abnormalities that are at risk of future deterioration in function. This crucial distinction was recognized in the study by Murbraech et al. who found 10.6% with heart failure and 5.2% with asymptomatic LVSD (asymptomatic with LVEF <50%) in long-term survivors of auto-HSCT (19). In comparison, this study found 32.3% with asymptomatic LVSD (NYHA class I). Although, these studies differed by underlying diseases, stem-cell origin, risk of GVHD, use of radiation, types and dosages of chemotherapies and age of recipients. Moreover, our definition of LVSD used higher thresholds for 2D-LVEF and included GLS that likely contributed to a higher frequency of

mild or subclinical reductions. A possible advantage of our definition of LVSD is higher sensitivity for identifying individuals at risk for progressive cardiac dysfunction. However, it may have conversely increased the occurrence of false positives.

The lack of self-reported symptoms of functional dyspnea (NYHA >class II) is unexpected given the high prevalence of LVSD. This may be due to use of cardio-protective medications, or the mild nature of impairments. An alternative explanation is an inability to distinguish symptoms related to cardiac impairments that are acquired gradually over time, or masked by deconditioning or other complications. Regardless, this observation illustrates the difficulty for clinicians to exclude heart disease in survivors of allo-HSCT by interview and symptoms alone.

Diastolic dysfunction and elevated filling pressures

Diastolic function is dependent on the efficiency of LV relaxation, restoring forces, myocardial and atrial compliance, processes of ventricle filling and atrial contraction (73, 74). The evaluation of diastolic function in cancer survivors is recommended, although the guidelines state that diastolic dysfunction is rarely reported after cardiotoxicity therapies (65). However, impaired relaxation by echocardiography has been reported in long-term childhood survivors of therapies that include anthracyclines (124). Moreover, changes in diastolic function have been observed to precede systolic abnormalities after cardiotoxic therapies (125).

A degree of diastolic dysfunction was expected in this cohort given the long follow-up time, high frequency of arterial hypertension and other cardiovascular risk factors. Patterns of impaired relaxation were observed, although were infrequent and mostly confined to older individuals with hypertension and obesity. In situations with reduced systolic function, there is a higher reliance on compensatory mechanisms to maintain cardiac output (particularly during exercise). The mechanisms of elevated filling pressures are complex and beyond the scope of this thesis. In current guidelines, the categorization of elevated filling pressures is limited to overt systolic dysfunction (2D-LVEF <50%) (74). Identification of elevated filling pressures by these guidelines has a reported accuracy of 87% compared to gold standard invasive methods (75). Applying the guideline algorithms to subjects with LVSD resulted in diagnosing of elevated filling pressures in 21% of this cohort. In most cases, elevated filling pressures were associated with moderately reduced LV systolic function and biventricular dysfunction. The use of cardio-protective medications (such as ACE-inhibitors) possibly blunted both the frequency of elevated filling pressures and afterload effects on RV function and exercise capacity.

In paper II and III, the parameter E/e' was used to control for effects of filling pressure. E/e' is the ratio between early transmitral flow (MV_E) that represents passive filling and increases with elevation of LA pressure, and e' that is related to long-axis relaxation rate, restoring forces and lengthening load (preload) (73). With decreased abilities of the myocardium, the

value e' is reduced resulting in increased E/e' ratio (74). E/e' is proportionate to LA pressure, feasible, highly reproducible and less influenced by LV afterload (74). The similar ages of survivors, few valve disorders, comparable levels of systolic function with few cases of advance systolic dysfunction improved the validity of using E/e' to represent filling pressures in this cohort. Noticeably, E/e' moderately correlated with TRP which is supportive of elevated filling pressures as a factor influencing PASP and RV afterload. E/e' was also found to correlate with GLS (but not with LVEF), which suggests a coupling relationship between systolic and diastolic function. However, our analyses did not reliably establish relationships between E/e' with RV function or oxygen uptake (as discussed later).

Right ventricular systolic dysfunction (RVSD)

There is limited information describing RV function in survivors of cancer related therapies, and to our knowledge, this is the first study assessing the right ventricle in long-term survivors of allo-HSCT. The absence of previous studies is possible related to the complex anatomy and physiology of the right ventricle. Motivation to investigate the welfare of the right ventricle after cancer related therapies is that RV dysfunction is a strong predictor for progression and mortality in heart failure (126, 127). The few echocardiography studies that describe RV function after chemotherapy have predominantly been derived from observational studies, with small samples and describing effects immediately after or within the first two years after completion of treatment (128-131). Furthermore, investigations of the right ventricle with echocardiography have often relied on interpretation from a small selection of available parameters. Tanindi et al. reported on subclinical changes in FAC, TAPSE and s' shortly after anthracycline therapy in 36 patients treated for breast cancer (130). In a recent study by Zhao et al., 3D echocardiography was used to examine recipients of anthracyclines over a one year period, found progressive increases in RV volumes, reductions in both RV ejection fraction and 3D longitudinal strain (128). Christiansen et al. used various echocardiographical parameters, and found reduced RV function in 30% of long-term survivors of childhood lymphoma or acute leukemia treated with anthracyclines and radiotherapies (132).

A finding shared in long-term studies of RV dysfunction is the presence of co-existing LV abnormalities (132, 133). Another repeated observation is a disparity in the level of impairment between ventricles (129, 132-134). Literature on RV function after HSCT is from one source (133). In this study, Murbraech et al. found RVSD in 6.2% of long-term lymphoma survivors treated with auto-HSCT. In comparison, our study found RVSD in approximately 14%, with similar levels of severity and also a strong association with LVSD. The higher prevalence of RVSD (with the same definition) in this cohort is possibly explained by greater levels of co-existing LVSD function, and greater stresses with allogeneic verses autologous stem-cell transplantation.

Important distinguishing features of the right ventricle include the thin myocardial wall predominantly consisting of longitudinal orientated fibers, functioning in the low-resistance

pulmonary circuit and interdependency with anatomical structures shared with the left ventricle (135). Explanations for RV impairment explored in this study were cardiotoxic effects from therapies, secondary effects of LVSD, cardiovascular risk factors, GVHD, increased pulmonary resistance irrespective of LV function and interdependency with anatomical structures shared between ventricles. The cascade of effects starting with cardiotoxicity and LVSD, leading to elevated end-diastolic pressures, elevated filling pressures and eventual increased afterload is a possible pathway for the cases of RVSD in this cohort. The right ventricle is susceptible to sustained elevated afterload due to its lower myocardium mass and reliance on contributions from the shared septum for adequate systolic function (135). The results from our analyses were suggestive, but not conclusive for elevated filling pressures as a contributor to RVSD in this study. The inability to confirm this association may have been influenced by mild degree of effects and sample size, use of cardio-protective medications and detection methods.

The physiological differences between the ventricles are important in explaining the differences in responses to cardiotoxic therapies and cardiovascular risk factors. Pre-transplant anthracycline therapies were strongly associated with observations of LV impairment and were a logical explanation for RV impairment. While, our data also showed a dose-related relationship between anthracyclines and level of RV impairment, it was not possible to discriminate if this observation was directly associated with effects from anthracyclines. The failure to confirm anthracyclines as a negative predictor for RV function is possibly a consequence of dysfunction being confined to those receiving high dosage of anthracyclines or due to modification by LV dysfunction.

Traditional cardiovascular risk factors were not found to be associated with RV function. This may indicate higher tolerance in the right ventricle to effects from the tested cardiovascular risk factors and partially explain the disparity in ventricular dysfunction. The risk of elevated pulmonary vascular resistance was amplified by the occurrence of GVHD (136). However, pre-capillary pulmonary hypertension was not evident by echocardiography, and GVHD with or without BOS (n= 10) were not identified as predictor of RV function. Pericardial abnormalities (n= 8) can impose RV function, and therefore was treated as a potential risk factor for RVSD (135). However, examination of these cases and repetitive testing excluding pericardial disease found that this was not the main explanatory factor for RVSD.

Pericardial disease

Pericardial disease is well a described complication of chemotherapy (65). The most common aliment of the pericardium is acute pericardial effusion. Previous rates of pericardial effusion in HSCT survivors are reported to be 1% to 21% (51-53, 137-140). Abnormal thickening of the pericardium was described in approximately 8% of this cohort, none had received drainage procedures and no significant hemodynamic effects were observed. The possible risks for pericardial effusion in this cohort were underlying disease, cyclophosphamide, infections, iron-overload, thrombotic microangiopathy and GVHD (20-23, 51-53, 137-141).

Our data suggests survivors with cGVHD to be at higher risk for pericardial abnormalities. This is in agreement with other reports that show cGVHD to be strongly related to recurrent and persistent pericardial effusion (137, 138).

Valvular heart disease

Valve disease is common in survivors of radiotherapy (142). Valve lesions after chemotherapy are more commonly secondarily to chamber remodeling, or caused by other complications such as infections (65). Endocarditis associated with HSCT is rare, despite heighten risk due to blood transfusions, pancytopenia, GVHD and use of venous catheters (143). Valve disease has been reported to occur at a higher frequency in auto-HSCT survivors treated with anthracyclines (144). Clinically relevant valve disease was very rare in this cohort. The higher frequency of mitral and tricuspid valve regurgitations in survivors compared to controls is possibly secondary to chamber remodeling.

10.2 Exercise capacity and cardiopulmonary dysfunction

Oxygen uptake in participants in this cohort was found to be lower than values from a comparative healthy population (111). Reduced exercise capacity by CPET was identified in 46% of this cohort. This cohort included a complex array of risk factors for reduced oxygen uptake. In order to objectively assess the role of cardiac function, tests were adjusted for central and peripheral factors that could influence oxygen uptake. Explanatory factors included physiological characteristics (age and sex) and lifestyle factors such as obesity and reduced physical activity or fitness. Sedentary lifestyles in this cohort are possibly a repercussion of deconditioning over an extended period. These factors are commonly associated with reduced exercise capacity in previous comparative studies (88, 89).

Comprehensive echocardiography identified specific phenotypes that were associated with reduction of oxygen uptake. LV Systolic dysfunction and pulmonary dysfunctions were found to have similar levels of association with reduced oxygen uptake. As mentioned earlier, systolic dysfunction is likely related to impaired myocardial contractility induced by anthracyclines and the secondary effects from cardiovascular risk factors such as increased afterload due to hypertension. Pulmonary dysfunction may have arisen from complications associated with chemotherapy, and/or the effects of BOS secondary to chronic GVHD (89). In systolic heart failure, reduced VO_{2peak} is principally the consequence of inadequate blood flow to skeletal muscles secondary to insufficient cardiac output (84, 85). In this study, clinically relevant reductions in oxygen uptake were mostly reserved to cases with obvious cardiac dysfunction (2D-LVEF <50%). Findings of reduced oxygen-pulse corresponded well with reductions seen in systolic function by echocardiography and thus supported cardiac dysfunction as a likely contributor for reduced exercise capacity.

CPET is considered the gold standard for assessing reasons of reduced functional capacity (82-84). Peak oxygen uptake has been shown to give valuable prognostic information on heart disease and all-cause mortality (82-84). According to risk stratification classifications for patients with heart failure, VO_{2peak} <10ml/kg/min indicates high risk and VO_{2peak} >20ml/kg/min corresponds to lower immediate risk and better short-term prognosis (145). While, percent-predicted VO_{2peak} <50% has previously been shown to be indicative of poor prognosis in patients with heart failure (146). These predictions are likely to differ in survivors of allo-HSCT whom have additional high levels of risk factors and comorbidities. One study, in childhood cancer survivors found reduced exercise capacity (<85% of predicted-VO_{2peak}) resulted a four-fold increase in hazard rate for death (79). To our knowledge, there are no studies describing the prognostic impact of mild to moderate reduced oxygen uptake in long-term survivors of HSCT. Although, the ability to identify cardiac dysfunction as a cause for exercise intolerance or reduced oxygen uptake (even if mild) has medical value and potential prognostic benefits.

10.3 Risk factors for heart disease after allo-HSCT

Plausible explanations for the high frequency of cardiac dysfunction in this study population include young age at HSCT, long follow-up/exposure time, pre-transplantation therapies, conditioning therapies and post-transplant risk factors. First-line therapies with anthracyclines were the strongest risk factor for late-onset cardiac dysfunction in this cohort. However, anthracyclines were not able to account for 17% of LVSD cases. Other risk factors such as cardiovascular risk factors and potentially GVHD driven inflammation processes may also have relevance for late-onset heart disease. The relationship between the outcomes of cardiac dysfunction (LVSD, RVSD) and the main factors found associated with their occurrence is illustrated in figure 6.

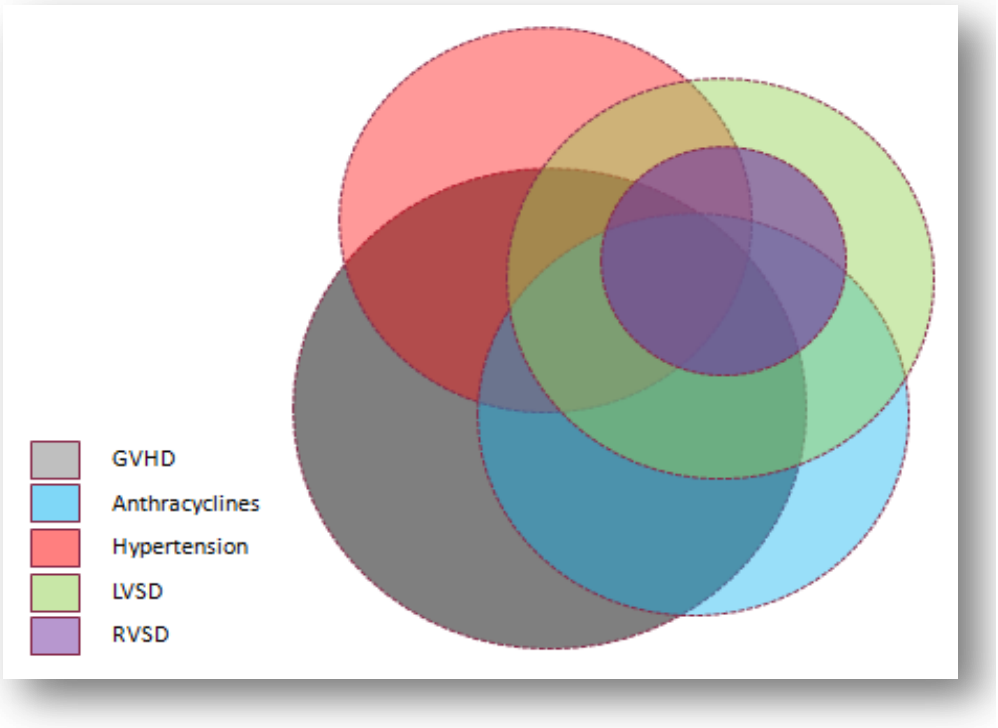

Figure 6: One-hundred and four survivors of allo-HSCT were included in cohort. Risk factors for heart disease included: Myeloablative conditioning in 102 (98.1%), anthracyclines prior to allo-HSCT in 47 (45.2%), Graft-versus-host disease (GVHD) in 67 (64.4%) and hypertension in 42 (40.4%). At time of examination, left ventricular systolic dysfunction (LVSD) was found in 46 (44.2%) and right ventricular systolic dysfunction (RVSD) in 15 (14.4%). Thirteen (12.5%) had bi-ventricular dysfunction.

The levels of cardiac dysfunction in this study may have been influenced by temporal aspects of age at HSCT and the long follow-up time. This is relevant since cardiac dysfunction after chemotherapy has been shown to worsen and increase in frequency in parallel with observation time (12, 17, 44, 57). Long follow-up time in this cohort facilitates processes of cardiotoxicity, prolongs effects of deconditioning and increases vulnerability for acquiring risk factors. The majority of prior studies have examined either childhood survivors or older adults. This study mostly comprised of a timeframe from children to adulthood that is usually characterized by rapid growth and development. It is plausible that immature organs are more susceptible to chemotherapies and may disrupt organ growth (35). If true, this would support the concept of premature or accelerated aging that has been used to explain the high levels of comorbidities in childhood survivors of cancer therapies (64).

The risk for cardiotoxicity and frequency of late-onset heart disease has been reported to differ between sexes (17, 26, 31, 35, 43, 58). In this cohort, cardiovascular disease (with exception of hypothyroidism) and structural heart disease was equally distributed between sexes.

The role of chemotherapy

Almost half (45.2%) of the entire cohort received anthracyclines prior to allo-HSCT for treatment of malignant disease. In addition, myeloablative conditioning with alkylating agents were used in conditioning prior to stem-cell transplantation. The inclusion timeframe of this cohort coincided with a shift from radiation to chemotherapeutical agents as the primary method to provide myeloablation. As such, several of the older participants received treatment with mediastinal radiation or TBI, although too few to accurately determine their impact. In this cohort, conditioning regimes were used in all except two participants and were fairly standardized despite the heterogeneous reasons for allo-HSCT in our cohort. This limited the confounding, but prevented the ability to test the effects of conditioning. However, the lack of recent data suggests alkylating agents to have a low risk of late-onset cardiotoxicity.

In agreement with other studies, anthracyclines were found o be a vital explanatory factor for late-onset cardiac dysfunction in allo-HSCT survivors. Moreover, it is assumed that patients with non-malignant indications for allo-HSCT are exposed to lower cardiotoxic risk. The reductions in LV and RV systolic function with signs of remodeling were shown to be dependent on anthracycline dosage. This finding is consistent with many other studies with similar anthracycline dosage thresholds (250 to 300mg/m^2) (17, 19, 26, 27, 30, 31, 35-37, 58, 147). In a cohort of 830 children in the Netherlands, Van Dalen et al. estimated that one in every ten child treated with doses >300mg/m^2 will eventually develop heart failure (58). Mulroney et al. demonstrated that the long-term risk of heart failure in childhood survivors almost doubles from anthracyclines dosage of <250mg/m^2 to dosages of >250mg/m^2 (31). In another study, Armenian, et al. reported an odds ratio of 9.5 for heart failure in HSCT survivors who had received dosages >250 mg/m^2 (17). It should be noted, that the recommended isotopic doxorubicin conversion rates were altered in 2018 and the cutoff that defines low to high risk is an approximation (90). While categorization based on dosage is clearly useful to stratify risk, the potential for anthracyclines to induce cardiotoxicity at lower dosages suggests that there is no safe dosage (27, 30-32, 148).

Given the knowledge of the mechanisms for cardiotoxicity, it would be reasonable to expect anthracyclines to induce global myocardial affection. In spite of this, the results from this study revealed a disproportionate degree of ventricle affection. One interpretation of this finding is the right ventricle is more tolerant to anthracyclines. This would be in agreement with an experimental study, which found irreversible cellular damage and myocyte remodeling in the left ventricle while only mild affection to the myocardium belonging to the right ventricle after exposure to daunorubicin (134). From this study, it seems the negative effects from anthracyclines on the right ventricle are conditional on the presence of LV impairment. As discussed previously, physiological differences between the ventricles may be instrumental in explaining susceptibility to cardiotoxic therapies.

The role of cardiovascular risk factors

This cohort found a concerning high frequency of cardiovascular risk factors. These were at levels much higher than equivalent aged controls, and at similar levels to comparative publications (13, 18, 19, 26, 29, 39, 42-46). The high rates of cardiovascular risk factors are one explanation for greater frequency of structural heart disease in HSCT survivors as compared to survivors of conventional cancer therapies (13, 29). Cardiovascular risk profiles are dynamic and rates likely to increase with aging. In this cohort, hypertension and hypercholesterolemia were associated with older age and longer survival time. Also, survivors with multiple cardiovascular risk factors had a tendency to have more severe grades of LVSD and biventricular dysfunction. However, the exact contribution of these cardiovascular risk factors is difficult to attend, especially given that many were receiving cardio-protective therapies and the exact temporal aspects of their origin are unknown.

Hypertension was the most common cardiovascular risk factor being found in 40.4% and 30.8% were currently using antihypertensive treatment. A similarly high prevalence of hypertension (24 to 45%) has been reported in long-term HSCT survivors (>10 years) treated at various ages (18, 19, 26, 42, 43, 45). Compared to similar aged healthy persons, these levels are considered to be considerably high. Surveys conducted in the general population by the Norwegian HUNT 3 study found the prevalence of elevated systolic blood pressure (>140mmHg) in persons under 39 years of age to be approximately 15% (149). Hypertension is known to cause myocardial fibrosis, reduced ventricular compliance and in-turn reductions of systolic and diastolic function. The relevance of hypertension in adult survivors of childhood cancer is illustrated by a 19-fold (RR 19.4) increase in incidence rate of heart failure (150). In this study, hypertension was associated with reduced GLS. Moreover, patients with hypertension had increased cardiac mass and signs of elevated filling pressures.

The finding of hypercholesterolemia occurring in 15% was the second most prevalent cardiovascular risk factor in this study. While not found to be significantly associated with reduced cardiac function, its presence was often associated with other risk factors and findings of more serious cardiac impairments. This cohort had lower rates of obesity and diabetes mellitus compared to the American studies (39, 43, 45). Differences in lifestyle factors and social economics may in part explain the differences. Sedentary lifestyles after HSCT are recognized as a significant factor for elevated rates of coronary artery disease (63).

The role of GVHD

GVHD was reported in 64% of survivors in this study and was associated with multiple organ damage (122). It remains unclear the extent or role GVHD has with cardiac impairment in this cohort. It is possible that high rates of cardiovascular risk factors in allo-HSCT recipients are partially driven by the inflammation caused by GVHD (18, 29, 45, 56). Hypercholesterolemia and hypertension are more frequently associated with GVHD (18, 29, 45, 56). This relationship was not observed in this cohort. In support of previous findings, a non-significant

but numerically striking proportion of pericardial pathology was observed in sufferers of cGVHD (51-53).

Given that GVHD has widespread effects, a statistical association between GVHD and cardiac function could have been foreseen. However, it was not anticipated to find the dichotomous value of GVHD as a positive (instead of a negative) predictor for 2D-LVEF. The reasons for this finding are uncertain, and possibly a consequence of the regression model and survivor's characteristics in those with GVHD. Possible interactions were considered and removal of GVHD did not affect the significance of the other parameters. Survivors with GVHD were smaller in stature and had lower levels of fitness compared to those without GVHD. It is possible GVHD promotes deconditioning and growth distributions. Regardless, the role of GVHD in modifying cardiac function is intriguing and requires further investigations.

10.4 Use of Natriuretic peptides

The European Society of Cardiology promotes the use of troponin for detection of early myocardial cardiotoxic damage, and NT-proBNP for late-onset cardiotoxic damage (151). Several studies have shown elevated troponin levels shortly after chemotherapy to be related with subsequent cardiac dysfunction (33, 69, 152, 153). In this cohort, an elevated level of troponin was found in only two individuals and was expected given the long follow-up time. NT-proBNP is associated with myocardial stretch and wall stress caused by remodeling during heart failure and filling pressures, and as such was the most relevant biomarker in this study (151). Elevated levels of NT-proBNP have been shown to predict late-onset LV remodeling in children treated with anthracyclines (154). Similarly, the St. Jude Lifetime cohort found elevated NT-proBNP to be common among long-term survivors (>10 years) exposed to cardiotoxic therapies and was associated with increased risk of future cardiomyopathy (153). Recently, a meta-analysis by Michel et al. found elevated NT-proBNP after chemotherapy to give a mild significant increased risk (OR 1.7) of predicting LV dysfunction (152). NT-proBNP has also been shown to increase the sensitivity to identify cardiotoxicity when used in combination with GLS (155).

This study was not designed to calculate the predictive abilities of NT-proBNP. In this cohort, elevated levels of NT-proBNP corresponded to a small portion of survivors with obvious LV dysfunction and signs of remodeling. However, the accuracy of NT-proBNP to identify individuals with mild cardiac impairments was limited. It is possible that the usefulness of NT-proBNP was blunted by cardio-protective therapies, natural variability or due to uncertainty with defining abnormality in asymptomatic patients. The role of NT-proBNP in identifying and predicting cardiotoxicity risk remains unsure, and is dependent on timing in relation to therapy (151, 155).

10.5 Echocardiography considerations

Echocardiography is a noninvasive, cost effective and readily available technique. However, there are several limitations with echocardiography that need to be addressed when interpreting results. The most important technical limitation for all modalities of ultrasound is the acoustic image quality that varies between subjects, equipment and expertise of the investigator. Discipline is required to avoid analyzing untrustworthy images with acoustic artifacts and apical foreshortening caused by ultrasound angulations.

This study benefited from good image quality, comprehensive protocols, use of same equipment and experienced investigators. These factors explain the high feasibility and reproducibility in this study. It is important to recognize that cardiac function with echocardiography is influenced by factors that alter loading and heart rate (103). Moreover, echocardiography is not a true measure of 'contractility' that is dependent on mechanisms of force generated in myocardial cells. Furthermore, the forces generated during myocyte contraction occur early, while most measures of echocardiography are measured later in systole.

How best to evaluate LV systolic function with echocardiography?

Early detection of heart disease is crucial for effective medical intervention and prevention of progressive and unrecoverable heart disease. Evaluation of systolic function has traditionally relied on the use of 2D-LVEF, which is an easy concept to grasp and highly feasible. However, there are several disadvantages with LVEF that limit its accuracy and reliability in detecting systolic dysfunction. An obvious source for inaccuracy is the geometric assumptions of LV shape used in the calculation of 2D-LVEF. Three-dimensional echocardiography overcomes this limitation and judged more accurate for calculating ejection fraction (71, 72, 104). In this study, values of 3D-LVEF were on average lower than 2D-LVEF, had better correlation with GLS and greater association with VO_{2peak} than 2D-LVEF. An established problem with LVEF is the high inter- and intra-observer variability (67, 72). This in-part is due to operator interpretation and definition of the endocardial borders. In contrast, GLS has better reproducibility and lower re-test variability compared to 2D or 3D-LVEF (67, 72).

It is generally recognized that longitudinal myocardial fibers are more susceptible to damage and cardiotoxic therapies. Moreover, there is ample evidence supporting the superior sensitivity of GLS over LVEF to detect systolic dysfunction after chemotherapies (62, 65, 67, 69, 70, 72, 77, 78). Hence, GLS is often referred to as an early marker of cardiotoxicity. In contrast, LVEF measures the summation of deformation, with the largest contribution from circumferential shortening, and has lower proficiency to detect mild impairments of the longitudinal fibers. This deficiency is possibly augmented by compensatory mechanisms by normally functioning myocardial fibers. As such, reductions in LVEF coincide with advanced heart failure (65, 70). Given these factors, it is not surprising that GLS has better prognostic

capabilities than LVEF (67, 77, 78). The summation of these factors has consequently led to the endorsement of GLS by experts in cardio-oncology (65, 156).

This study was not designed to address mortality and has no evidence to support the prognostic value of GLS. However, given the findings from this study, it is foreseeable that GLS has potential to stratify risk in long-term survivors of HSCT. The use of GLS increased the number of subclinical impairments being identified, which contributed to an overall higher prevalence of systolic dysfunction than comparable studies. Findings with GLS were supported by correlations with NT-proBNP that confirmed the presence of elevated wall stress. In addition, GLS was superior to 2D/3D-LVEF in predicting VO2peak that is strongly associated with progressive heart failure and mortality (79, 82-84). This relationship was found despite GLS being measured at rest.

The use of MAPSE should be considered in situations whereby image quality is not ideal for GLS, and when the only requirement is to estimate long-axis foreshortening. MAPSE has less technical constraints than GLS, but measures global deformation in one plane. In fact, adjusting MAPSE by LV mid-chamber length will often generate similar values to GLS. As seen in this study, MAPSE was significantly lower in survivors compared to controls, showed dose-related relationship with anthracyclines and expectantly strongly correlated with GLS. However, MAPSE was more variable in the association with RV impairments and VO_{2peak}.

Limitations with GLS

There are several unresolved problems with STE that affect the credibility of measurements such as GLS. STE has high demands for frame rate, good image quality and temporal stability of tracking patterns (103). It also relies on the trustworthiness of software to accurately distinguish and track myocardial fiber motion (that human eyes cannot follow). Accuracy is dependent on an optimal relationship between sampling rate (or frame rate), lateral resolution and heart rate (103). This may partially explain why speckle tracking seems best in structures parallel (without curvature) with the ultrasound beam. Foreshortening will overestimate GLS and adjusting ROI or timing of end-systole can markedly alter the results. In this study, peak systolic strain was used, that in the majority of cases coincided with timing of end-systole due to the global impairments, few instances of regional wall abnormalities and absence of dyssynchrony. To reduce the risk of errors, tracking was scrutinized and manually adjusted. While this relies on the proficiency from training, it is currently judged to result in better accuracy than fully automated GLS (157).

A remaining challenge with STE is the lack of consensus in defining absolute cutoffs and defining causes of variability (108, 109). Central to the problem are inter-vendor differences in tracking software, post-processing capabilities and adjustments in strain algorithms for factors that introduce natural variation (103, 108, 109). Physiological factors that potentially influence GLS are age, sex, body size, heart rate and blood pressure (108, 109). These problems are undoubtedly further exacerbating the risk of false-positive categorization when

selecting cutoffs for abnormality. There is a degree of uncertainty ('grey area') for GLS values from -17 to -18%, as there is for 2D-LVEF from 50 to 53%. For this reason, this study relied on linear regression that gave better accuracy for identifying predictors of LV function. For descriptive purposes, the GLS cutoff of ≥-17% was chosen based on experience, previous studies and our normal data that was acquired using the same equipment and operator. In comparison, the JUSTICE study found normal GLS (in GE scanners e7 and e9) to be -21.3 ± 2.1%, and translates to similar cutoffs to define abnormality (109). In paper 1, we referred to the thresholds presented from a meta-study by Yingchoncharoen et al. who combined results of various vendors and found normal GLS to be -19.7% with 95% confidence interval of -20.4% to -18.9% (108). Given the unresolved uncertainties, a more conservative value range of -16% to -18% has been suggested to indicate cardiotoxicity (158). The cardio-oncology council and EAVCI /ASE are currently working to standardize GLS techniques and clarify what constitutes cardiotoxicity with GLS (159, 160). In borderline cases, we endorse follow-up examinations with the same scanner manufacture and with emphasis on temporal change in absolute values.

Right ventricular function by echocardiography

Echocardiography of the right ventricle is technically more challenging than of the left ventricle due to its position in the thorax, complex anatomy and its sensitivity to changes in load. The mean values in our normal control group were in the lower limits of the reference values given in the guidelines (71). The increased sensitivity to physiological factors may be responsible for natural variation in measurements of RV function. The reduction in FAC was less significant than the drop in parameters reflecting longitudinal shortening. This is possibly explained by FAC representing a composite measure of myocardial foreshortening (analogous to 2D-LVEF), and is influenced by the interactions between ventricles through the septum. The advantage with TAPSE, RV-s' and RVFWS is their ability to quantify the function of the longitudinal myocardial fibers that are the most abundant fiber type in the right ventricle. TAPSE and RV-s' are easy to attain, but measure myocardial shortening in one dimension and are angle dependent.

Only recently has dedicated software for strain imaging of the RV function been introduced. Measuring RV strain has been shown to be beneficial in several types of myocardial disease, including arrhythmogenic RV cardiomyopathy (161), pulmonary hypertension (162) and ischemic disease (163). In similarity with GLS, RV strain has been shown to be superior in detecting subtle changes in function and has been defined as a good prognostic marker (126, 127, 164, 165). RV strain using the same techniques and vender software as this study has been reported to give good reproducibility (166). However, at present, RV longitudinal strain has not been incorporated in any recommendations, including for detection of cardiotoxicity. The technical limitations of GLS are amplified when performing strain on the right ventricle. Firstly, the algorithms devised for the GLS may not be applicable to the right ventricle that differs in anatomy and sensitivity to loading. The thin walls and geometry of the right ventricle (especially apical segments of septum) can create dubious tracking. Normal

thresholds for the RV strain are not well defined. The RV strain values obtained from our controls were very similar to those described by Morris et al. in a large multi-center cohort evaluating RV function in healthy and individuals with heart failure (164). The higher absolute strain values in the right compared to the left ventricle can be explained by lower afterload and a greater proportion of longitudinal fibers. The lower values for RV-GLS compared to RVFWS are due to the inclusion of segments from the inter-ventricular septum. This may have contributed to the higher frequency of reduced RV-GLS than reduced RVFWS. Therefore, RVFWS is deemed a more sensitive measure of RV function than RV-GLS, and was the reasoning for not including RV-GLS in paper 2.

Echocardiography and prediction of exercise capacity

Previous studies have shown relationships between functional capacity with echocardiographical measurements of systolic function (19, 38, 79, 167, 168), diastolic function (169) and RV function (170). However, there is inconsistency in the reported abilities of echocardiography to predict exercise capacity. One reason is the preferential use of LVEF (with its limitations) to establish relationships with exercise capacity (VO_{2peak}). Murbraech et al. reported lower VO_{2peak} in auto-HSCT survivors classified with heart failure and 2D-LVEF <50% (19). Smart et al. found correlation between elevated filling pressures and VO_{2peak} and not with LVEF in an elderly sample with moderate to severe reductions in LVEF (171). Hassleberg et al. found GLS to be superior in predicting VO_{2peak} in heart failure patients with moderately depressed 2D-LVEF and in patients with heart failure and 2D-LVEF >50% (168). This study also found GLS to be superior to 2D-and 3D-LVEF for predicting $VO_{2peak,}$ but in a population with milder systolic dysfunction and with co-exiting pulmonary disease. We are not aware of previous reported associations between GLS and reduced exercise capacity in long-term survivors of allo-HSCT. Furthermore, it is likely that the level of association between parameters of systolic function and exercise capacity were underestimated due to the exclusion of several patients with heart disease for CPET.

Our results align well to results collected from the St. Jude Lifetime cohort that examined exercise capacity in 1041 long-term adult survivors of childhood cancer (79). In this study Ness et al. found GLS and not 2D-LVEF or diastolic dysfunction to predict reduced exercise capacity (<85% of predicted-VO_{2peak}) as well as identifying other explanatory factors such as reduced pulmonary function by FEV_1 (79). Moreover, they found reduced GLS (> 1.5 SD of normal mean measures from JUSTICE study, or >-18.2%) with GE scanners gave an odds ratio of 1.7 for reduced exercise capacity (79, 109). Our ROC analysis suggested -18% as the most appropriate cut-off for correctly identifying reduced <85% of predicted-VO_{2peak}.

In sub-group analyses, RV function (by RV strain) was found to be lower in subjects categorized with reduced exercise capacity. However, these survivors had co-existing LVSD and the effects of reduced RV function were conditional and not significant enough to independently predict VO_{2peak}. Parameters that represent diastolic function (measured at rest) were significantly correlated with VO_{2peak}. It is rational that elevated filling pressures

measured by E/e' can have a role in limiting exercise capacity. However, evidence supporting this with E/e' has been inconsistent. A meta-analysis by Sharifov et al. concluded that E/e' was not reliable for assessing changes in filling pressure during exercise (172). In the multivariable regressions that were conducted in this study, no significant associations with diastolic function could be confirmed. It is possible that loading changes are responsible, or simply caused by interactions with other covariates such as BMI and age.

CPET has an ideal role in screening long-term survivors of allo-HSCT who present with unclear origins for exercise intolerance of functional dyspnea. In allo-HSCT survivors, the possibility of both pulmonary and cardiac impairments can complicate deciphering causes for reduced exercise capacity. While, CPET can indicate the cardiac limitations, it cannot distinguish specific mechanisms for reduced oxygen uptake. In these instances, echocardiography is required to clarify and provide vital information for medical interventions. Compared to echocardiography, CPET has greater participation requirements, safety concerns and moderately hindered by obesity and degree of physical fitness. Echocardiography (with GLS) should be considered when findings of CPET are inconclusive, irrespective of pulmonary pathology and symptoms. As demonstrated in this study, GLS when measured at rest had superior ability to predict VO_{2peak} compared with NT-proBNP and other tested echocardiographical parameters.

11.0 Study design limitations

The main strength of this study is the nationwide inclusion and comprehensive clinical examination. The main limitation to this study is the cross-sectional design that cannot describe causality and is unable to determine temporal aspects of myocardial damage or conclude if deterioration of LV function is a part of a progressive continuum. It acknowledged that this cohort is limited by survival bias by representing long-term survivors who escaped the most serious complications. The participation rate for those eligible for inclusion was 66.2%, which is considered satisfactory given the severity of disease and long observation time. The causes of death, in particularly the number of deaths attributed to solely cardiovascular disease prior to study start was not accessible. The cardiovascular disease status of non-study participants has not been recorded. Registry data showed potential response bias, with non-participants being younger with shorter follow-up, possibly resulting in over-estimation of cardiac dysfunction in this sample.

It is recognized that the results are limited to certain generalizations. The cohort contained subjects with heterogeneous indications for transplantation. This was necessary to obtain a valid sample size. Importantly, the conditioning regimes were fairly standardized and the confounding effects from first-line therapies were considered. The results may not apply to older recipients of allo-HSCT. The external validity of the findings may not directly be transferable to other countries with differing social economic status and race.

Considerable efforts were made to limit measurement bias and to increase the validity of the results. Detection of mild cardiac effects is limited by sample size and evaluation methods. This limitation was reduced by careful statistical analyses and by use of sensitive ultrasound techniques. Advantageously, the study obtained a comparative normal control sample for echocardiography. Normal controls were recruited without cardiovascular disease. This selection bias was addressed by adjusting for baseline differences in comparisons and conducting subgroup analyses with exclusion of cardiovascular disease. Sample size estimates for controls were acceptable. The exception was for FAC whereby a smaller difference than expected was found between groups and a possible type II error could not be excluded.

Echocardiography was performed at rest and this needs to be taken into consideration when evaluating correlations with VO_{2peak}. Five participants with cardiovascular disease were exempted from CPET. This introduced selection bias and likely led to an underestimation of the predictive power of echocardiography and NT-proBNP on exercise capacity. A small percentage of survivors were using beta blockers under CPET, although peak exercise was attended and therefore considered to have little effect on test outcome. CPET was conducted in the controls, although by different personnel, equipment and using a cycle protocol and thus was not used for comparisons.

12.0 Summary of findings

- Long-term survivors of allo-HSCT are at increased risk of heart disease due to pre-transplant therapies with anthracyclines, conditioning therapies and post-transplant risk factors that include traditional cardiovascular risk factors and GVHD.

- LVSD found in 44% was the most common form of functional heart disease found in long-term allo-HSCT survivors. One third of cases of LVSD were symptomatic. RVSD found in 14% and was associated with LVSD.

- Anthracyclines were strongly associated with biventricular systolic dysfunction. The effects were dose-dependent. Hypertension occurred in 40% and was associated with reduction in longitudinal LV function

- Exercise intolerance and reduced exercise capacity by VO_{2peak} measured by CPET in survivors of allo-HSCT was found to be associated with reductions in GLS measured by STE.

13.0 Clinical implications

Allo-HSCT is a potential lifesaving treatment option for many young sufferers of severe disease. However, this complex and intensive therapy comes with a heavy burden of therapy related complications. The detrimental long-term impact of chemotherapeutical therapies to the heart is now widely accepted. This is a concerning problem that has led to a surge of research into the field of cardio-oncology in recent years. Norway has followed the example of other countries by introducing cardio-oncology specialization, and becoming a member of Nordic Cardio-oncology Society which was established in 2019. With increased use and expansion of allo-HSCT to other diseases, it is important to establish unified recommendations to aid health professionals to provide optimal care.

This study raises several clinically relevant questions such as implications for treatment, and changes in follow-up regimes to ensure adequate screening and monitoring for heart disease. The high frequency of cardiac dysfunction found in this study is in-part due to more sensitive detection methods that were used. It is uncertain what the clinical relevance of subclinical reductions in LV systolic function has had and will have for a long-term survivor of allo-HSCT. This presents a challenge for clinicians, who need to decide on an appropriate action, i.e. whether to intervene at an earlier stage of dysfunction with cardio-protective medicines to prevent irreversible damage, or monitor by repeated testing to determine temporal status and eliminate the possibility of measurement error. The PRADA study guided by echocardiography has reported marginal benefits with prophylactic cardio-protective medications such as ACE and beta-blockers (173). Longer follow-up studies are required to determine if this beneficial trend persists.

Changes in routines, regimes and follow-up protocols take time to implement. However, there is good concordance between countries in patient selection and surveillance for childhood cancer survivors (174). The European Society of Cardiology (ESC), European Society of Medical Oncology (ESMO) and International Children's Oncology Cancer Harmonization group give guidance to screening and monitoring methods (16, 155, 174). Currently in Norway, there is no formal guidance on frequency or types of cardiac evaluation that should be implemented during follow-up. Regardless, survivors require life-long surveillance that includes routine and careful scrutiny of symptoms and blood tests associated with cardiovascular disease. However, clinicians need to be alert to the difficulties associated with defining origins to symptoms and the limitations with NT-proBNP in detecting cardiac dysfunction in long-term survivors.

Echocardiography provides a safe, efficient and accurate method to detect cardiac dysfunction. Echocardiography has an obvious role in screening of heart function in long-term survivors of cancer related therapies. The recommendation by ASE and EACVI to incorporate GLS in imaging protocols has been a positive initiative (65). The use of GLS to determine cardio-protective intervention has shown potential short-term therapeutic benefits (175). However, the presence of different phenotypes of cardiac dysfunction in long-term

survivors suggests that imaging protocols need to be comprehensive. The frequency of RVSD was low in this cohort, but acquiring these measurements may identify survivors that require more frequent surveillance and assertive cardio-protective interventions. CPET is useful for screening for causes that limit exercise capacity. However, when reasons for abnormality are inconclusive, echocardiography (with GLS) should be considered, irrespective of pulmonary pathology and symptoms.

The timing of follow-up in childhood survivors is recommended to occur no later than two years after chemotherapy and should be repeated a minimum of once every five years (174, 176). Performing echocardiography at these regular intervals would aid in identifying temporal changes and facilitate earlier medical interventions. The European Society of Cardiology published new guidelines this year for surveillance of adult HSCT survivors (177). It recommended echocardiography before stem-cell transplantation and afterwards at three and 12 months, and continually at yearly intervals in selected patients (177). Examining all recipients of allo-HSCT with echocardiography at one year or less intervals may not be feasible, and unlikely beneficial given that our results showed mostly mild reductions in LV function after a very long follow-up. If availability is restricted, the decision should be based on individual risk evaluation, and guided by an evidence based classification system. Regimes for children and adolescent patients should differ to adult patients. It is important to recognize that risk varies according to age at transplantation and increases with time. Thus, patients must not be forgotten after declared cured and life-long surveillance is imperative.

This study has identified several risks factors that constitute valid reasons for echocardiography. From our results, we suggest patients exposed to anthracyclines prior to HSCT would benefit most from echocardiography. Furthermore, the risk of cardiotoxicity seems proportional to cumulative anthracycline dosage. Cardiovascular risk factors occur at concerning high rates in long-term survivors, and possibly exacerbate the risk of cardiac dysfunction in long-term survivors. Of particular importance is hypertension that should be carefully monitored, managed and considered as an indication for screening with echocardiography. GVHD is a serious condition, which may increase the risk of cardiovascular disease. Exercise intolerance or reduced capacity by CPET should be considered as a possible manifestation of systolic dysfunction, irrespective of the presence of respiratory disorders.

Allo-HSCT is evolving with improved technologies and treatment strategies, resulting in improved survival and reduced complications. The goal of a chosen regime is to achieve a therapeutic balance between adequate toxicity to eliminate the diseased cells and to weaken immune system to allow engraftment of transplanted cells, but without causing damage to neighboring organs. To ensure optimal care, health care specialists need to be educated in the risk of late-onset heart disease associated with toxic therapies, importance of stem-cell selection and need to act assertive to limit the impact of modifiable risk factors. In addition, patient counseling on healthy lifestyles and recovery programs involving exercise to limit effects of deconditioning should be part of follow-up regimes.

14.0 Future perspectives

Research into cardio-oncology in the last decade has lead to awareness of cardiovascular risks associated with conventional and stem-cell transplantation therapies. However, new therapeutic methods continually appear and indications for treatment are diversifying. As such, there is an on-going need to be vigilant and to ensure the cardiovascular risk is adequately studied. Future research should be prospective and longitudinal in design. This would aid in determining the timing and rate of cardiac impairment, discover the prognostic value of GLS and establish the long-term benefits of earlier cardio-protective interventions and assertive prevention of risk factors. As identified in this thesis, further research is essential to improve the standardization of deformation imaging and three-dimensional echocardiography. Additionally, there is limited knowledge on the occurrence and relationship of vascular disease and inflammatory effects (associated with GVHD) with heart disease in long-term allo-HSCT survivors.

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16.0 Appendices

Appendices 1: Echocardiography and CPET comparisons between sexes.

	Male (n= 48)	Female (n= 56)	p-value	p-value*
Left ventricular function				
2D-LVEF (%)	55.3 ± 5.7	55.0 ± 6.0	0.809	0.874
3D-LVEF (%)	53.8 ± 4.8 (n= 42)	54.2 ± 5.3 (n= 46)	0.739	0.494
GLS (%)	-17.1 ± 2.3 (n= 47)	-17.8 ± 2.1 (n= 53)	0.092	0.144
MAPSE (%)	13.4 ± 2.0	12.5 ± 2.1	**0.023**	0.126
LV-s' (cm/sec)	8.4 ± 1.7	7.7 ± 1.6	**0.028**	**0.021**
Cardiac index (l/min/m^2)	2.7 ± 0.4	2.5 ± 0.4	**0.016**‡	-
MV_{EA} (cm/sec)	1.6 ± 0.7	1.6 ± 0.9	0.865	0.196
MV_{DT} (cm/sec)	160 ± 42	164 ± 44	0.709	0.036
E/e'	6.5 ± 2.1	6.7 ± 2.2	0.777	0.230
Right ventricular function				
FAC (%)	40.8 ± 5.4	41.1 ± 5.1	0.745	0.555
RVFWS (%)	26.4 ± 4.4 (n= 43)	27.5 ± 4.4 (n= 53)	0.210	0.211
TAPSE (%)	21.5 ± 4.0	20.0 ± 3.4	**0.042**	0.237
RV-s' (cm/sec)	12.0 ± 2.4 (n= 47)	10.4 ± 1.8	**<0.001**	**<0.001**
TRP (mmHg)	18.9 ± 3.8 (n= 34)	17.5 ± 4.1 (n= 43)	0.118	0.286
CPET				
Peak heart rate (bpm)	185 ± 14	178 ± 16	**0.023**‡	-
VO_{2peak} (ml/kg/min)	39.5 ± 6.7	33.4 ± 7.5	**<0.001**‡	-
Predicted-VO_{2peak} (%)	85.5 ± 13.2	91.5 ± 21.2	0.096‡	-
Oxygen-pulse (ml/beat)	16.3 ± 4.0	12.3 ± 2.4	**<0.001**‡	-
Predicted Oxygen-pulse (%)	84.1 ± 19.8	91.5 ± 19.1	0.065‡	-

The parameters listed represent the core parameters for left and right ventricular function. Data presented as mean ± SD or n (%). Comparisons made with student t-tests and significant p-values (<0.05) are in boldface. *Echocardiography variables adjusted by ANCOVA and with variables of SBP and BSA. ‡Unadjusted p-value. **Abbreviations:** See table 1.

Appendices 2: Pearson's bivariate correlations between echocardiographical parameters.

		Left ventricle					
		Fractional shortening	MAPSE	2D-LVEF	3D-LVEF	LV-GLS	E/e'
Right ventricle	FAC	0.308‡	0.290‡	0.401*	0.333‡	-0.355*	-0.017
	TAPSE	0.198†	0.479*	0.311‡	0.340‡	-0.432*	0.076
	RV-s'	0.292‡	0.356*	0.276†	0.342‡	-0.273†	0.139
	RV-GLS	-0.433*	-0.338‡	-0.463*	-0.470*	-0.593*	0.026
	RVFWS	-0.301‡	-0.179	-0.234†	-0.194	-0.441*	-0.111
	RIMP	-0.186	-0.153	-0.189	-0.093	-0.368*	0.135
	TRP	-0.139	-0.042	-0.066	-0.018	0.141	0.356‡
	E/e'	-0.030	-0.277‡	-0.087	-0.124	0.241†	-

*p <0.001, ‡ p <0.005 and † p <0.05. 3D-LVEF (n= 88), LV- GLS (n= 100), RV-s' (n= 103), RV-GLS (n= 96) and RVFWS (n= 96), RIMP (n= 100), TRP (n= 77). Parameters of strain are negative values. **Abbreviations:** See Table 1

Appendices 3: Echocardiography in survivors with and without hypertension

Parameter	**No Hypertension (n= 62)**	**Hypertension (n= 42)**	**p-value ***	**Adjusted p-value †**
Age (years)	30.0 ± 10.5	42.2 ± 9.3	**0.002**	-
BMI (kg/m^2)	23.4 ± 5.1	26.1 ± 4.7	**0.013**	-
SBP / DBP (mmHg)	115 ± 12 / 67 ± 10	132 ± 23 / 80 ± 13	**<0.001**	-
Left atria and ventricular morphology and function				
LA area (cm^2)	16.7 ± 3.3	18.5 ± 3.8	**0.010**	0.811
LAVI (ml/m^2)	29.0 ± 8.2	31.7 ± 10.0	0.140	-
LVIDd (cm)	4.8 ± 0.5	5.0 ± 0.5	**0.037**	**0.041**
LVIDs (cm)	3.3 ± 0.4	3.5 ± 0.6	0.235	0.068
LVEDV (ml)	110.9 ± 26.9	122.6 ± 33.8	0.051	**0.039**
LVEDV index (ml/m^2)	61.3 ± 13.3	66.9 ± 12.9	**0.037**	-
IVSd (cm)	0.80 ± 0.12	0.95 ± 0.19	**<0.001**	**0.008**
LV mass (g)	114.9 ± 29.7	153.7 ± 48.4	**<0.001**	**<0.001**
LV mass indexed (g/m^2)	63.6 ± 11.4	80.1 ± 21.2	**<0.001**	-
RWT (%)	0.28 ± 0.05	0.32 ± 0.06	**0.001**	0.359
3D-spherical index (%)	0.34 ± 0.07 (n= 51)	0.32 ± 0.07 (n= 37)	0.190	0.694
Left ventricular systolic and diastolic function				
2D-LVEF (%)	54.9 ± 4.8	55.6 ± 7.7	0.511	0.340
3D-LVEF (%)	53.8 ± 4.0 (n= 51)	54.7 ± 6.2 (n= 37)	0.305	0.626
GLS (%)	-17.9 ± 1.6 (n= 60)	-16.9 ± 2.7 (n= 53)	**0.034**	**0.002**

MAPSE (mm)	13.1 ± 1.8	12.7 ± 2.5	0.283	0.137
LV-s' (cm/sec)	8.3 ± 1.6	7.6 ± 1.7	**0.044**	0.295
MV_{EA}	1.7 ± 0.6	1.4 ± 1.0	**0.007**	0.336
MV_{DT} (m/sec)	153 ± 40	176 ± 44	**0.048**	0.814
MV e' (cm/sec)	12.1 ± 2.8	8.9 ± 2.8	**<0.001**	**0.008**
E/e'	6.0 ± 1.5	7.5 ± 2.6	**0.002**	**0.038**
Right ventricular function				
FAC (%)	41.4 ± 5.1	40.4 ± 5.4	0.337	0.138
RVFWS (%)	27.3 ± 3.9 (n= 43)	26.4 ± 5.2 (n= 53)	0.363	0.603
TAPSE (mm)	20.8 ± 3.5	20.6 ± 4.1	0.829	0.323
RV-s' (cm/sec)	11.1 ± 2.2	11.2 ± 2.4 (n= 41)	0.695	0.630
TRP (mmHg)	17.3 ± 3.0 (n= 51)	20.1 ± 4.7 (n= 26)	**0.007**	**0.001**
CPET				
VO_{2peak} (ml/kg/min)	36.4 ± 8.0 (n= 59)	36.0 ± 7.4 (n= 37)	0.824	0.109
% of predicted-VO_{2peak}	86.6 ± 18.9 (n= 59)	92.1 ± 17.8 (n= 37)	0.148	0.747
Oxygen-Pulse (ml/beat)	13.2 ± 3.2 (n= 59)	15.6 ± 4.1 (n= 37)	**0.004**	**0.035**
% of predicted Oxygen-pulse	83.4 ± 18.9 (n= 59)	95.2 ± 19.0 (n= 37)	**0.005**	0.776

The parameters listed in the table are non-exclusive and represent the core parameters for left and right systolic function. Data presented as mean ± SD or n (%). * Comparisons made with students t-test. † Parameters adjusted with ANCOVA using covariates of age at examination and BMI. Significant p-values (<0.05) are in boldface. **Abbreviations:** See table 1.

17.0 Published Papers

I Left ventricular systolic function in long-term survivors of allogeneic hematopoietic stem cell transplantation.

II Impaired right ventricular function in long-term survivors of allogeneic hematopoietic stem-cell transplantation.

III Reduced exercise capacity is associated with left ventricular systolic dysfunction in long-term survivors of allogeneic hematopoietic stem-cell transplantation.

I

JACC: CARDIOONCOLOGY VOL. 2, NO. 3, 2020

ORIGINAL RESEARCH

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

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ABSTRACT

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

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

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

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

CONCLUSIONS LVSD is common in long-term survivors of allo-HSCT treated in their youth. Pre-HSCT therapies with anthracyclines, age, heart rate, hypertension, and graft versus host disease are associated with measures of LV function. (J Am Coll Cardiol CardioOnc 2020;2:460–71) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a complex and potentially curative therapy for malignant and nonmalignant diseases. It is increasingly being used, particularly in younger individuals (1). Improvements in protocols and supportive therapy have resulted in improved initial survival rates (2). However, long-term survivors of allo-HSCT face high rates of life-debilitating complications (3–6). In patients that survive beyond 5 years, mortality rates are 4 to 9 times higher than the general population, corresponding with a 30% shorter life expectancy regardless of age at transplantation (7). The incidence of heart failure (HF) has been reported to be between 5.6% to 10.8% in those who have survived at least 10 years after HSCT (4,5,8,9), and the risk of cardiovascular (CV) related mortality is 2 to 4 times higher in HSCT survivors than in the general population (4). The relative role of different risk factors in the development of left ventricular systolic dysfunction (LVSD) in long-term allo-HSCT survivors is not well defined, especially in those treated during childhood, adolescence, and young adulthood. Furthermore, few studies have comprehensively evaluated heart function in long-term survivors of allo-HSCT. This observational study aimed to evaluate cardiac function in a population recruited nationwide who underwent allo-HSCT at a young age. Modern echocardiography techniques, including 3-dimensional (3D) imaging and speckle tracking echocardiography, were used to assess left ventricular (LV) systolic function.

METHODS

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

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

ECHOCARDIOGRAPHY. Transthoracic echocardiography studies were performed using Vivid E9 scanners and dedicated software (Echo-PAC version 113.1.3, GE-Vingmed Ultrasound, Horten, Norway). All echocardiograms were acquired and analyzed by the same experienced investigator (R.J.M) en-bloc, and in

ABBREVIATIONS AND ACRONYMS

allo-HSCT = allogeneic hematopoietic stem cell transplantation

CV = cardiovascular

GLS = global longitudinal strain

GVHD = graft versus host disease

LVEF = left ventricular ejection fraction

LVSD = left ventricular systolic dysfunction

NYHA = New York Heart Association

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

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TABLE 1 Patient Characteristics

	Allo-HSCT (n = 104)	Control Subjects (n = 55)	p Value
Female	56 (53.8)	29 (52.7)	0.893
Height, m	1.72 ± 0.09	1.74 ± 0.09	0.053
Weight, kg	72.5 ± 17.5	73.3 ± 12.3	0.730
Body surface area, m^2	1.83 ± 0.23	1.88 ± 0.18	0.179
Body mass index, kg/m^2	24.5 ± 5.1	24.1 ± 3.4	0.530
Age at allo-HSCT, yrs	17.8 ± 9.6	–	–
Age at examination, yrs	35.0 ± 11.7	36.4 ± 10.6	0.460
Years to follow-up	17.2 ± 5.6	–	–
Systolic blood pressure, mm Hg	122 ± 19	117 ± 11	0.074
Diastolic blood pressure, mm Hg	72 ± 13	66 ± 8	0.002
Heart rate, beats/min	69 ± 11	68 ± 12	0.629
Malignant disease	77 (74.0)		
Acute lymphoblastic leukemia	12 (11.5)		
Acute myeloid leukemia	32 (30.8)		
Chronic myeloid leukemia	26 (25.0)		
Other malignant	7 (6.7)		
Nonmalignant disease	27 (26.0)		
Severe aplastic anemia	17 (16.3)		
Metabolic/immunodeficiencies	10 (9.6)		
Pre-transplantation therapy			
Mediastinal radiotherapy	2 (1.9)		
Anthracyclines	47 (45.2)		
Cumulative anthracycline dosage, mg/m^2	270 (140, 435)		
Myeloablative conditioning			
Chemotherapy, busulfan/cyclophosphamide	95 (91.3)		
Chemotherapy + total body irradiation	7 (6.7)		
None	2 (1.9)		
GVHD	67 (64.4)		
Acute GVHD	27 (26.2)		
Chronic GVHD	12 (11.5)		
Both acute and chronic GVHD	28 (26.9)		

Values are n (%), mean ± SD, or median (25th, 75th percentiles). Percentages may not equal 100% given rounding

allo-HSCT = allogeneic hematopoietic stem cell transplantation; GVHD = graft-versus-host-disease.

random order after the last patient inclusion. After acquisition and prior to analyses, all echocardiograms were deidentified, and the investigator was blinded to all of the patient's medical records. Two-dimensional (2D) left ventricular ejection fraction (LVEF), 3D-LVEF, and global longitudinal strain (GLS) were measured on separate occasions to reduce bias. Studies followed current guidelines for evaluation of LV function and cardiotoxicity (14,15). Measurements were averaged from 3 consecutive heart cycles.

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

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

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

CONTROL GROUP. A control group was recruited from healthy volunteers responding to advertisements. The sample size was estimated to detect differences between allo-HSCT patients and control subjects for the normally distributed variables 2D-LVEF and GLS. With 104 patients, an expected 10% SD in-group and α of 0.05, it was determined that 52 control subjects were required at a power of 0.83 to demonstrate a 5% difference in 2D-LVEF between groups. We included 55 control subjects to allow for a small buffer and still obtain a power >0.80. Control individuals were selected to obtain comparative group characteristics for race, ethnicity, sex, age, height, and body mass index (BMI). The only exclusion criterion was established CV disease.

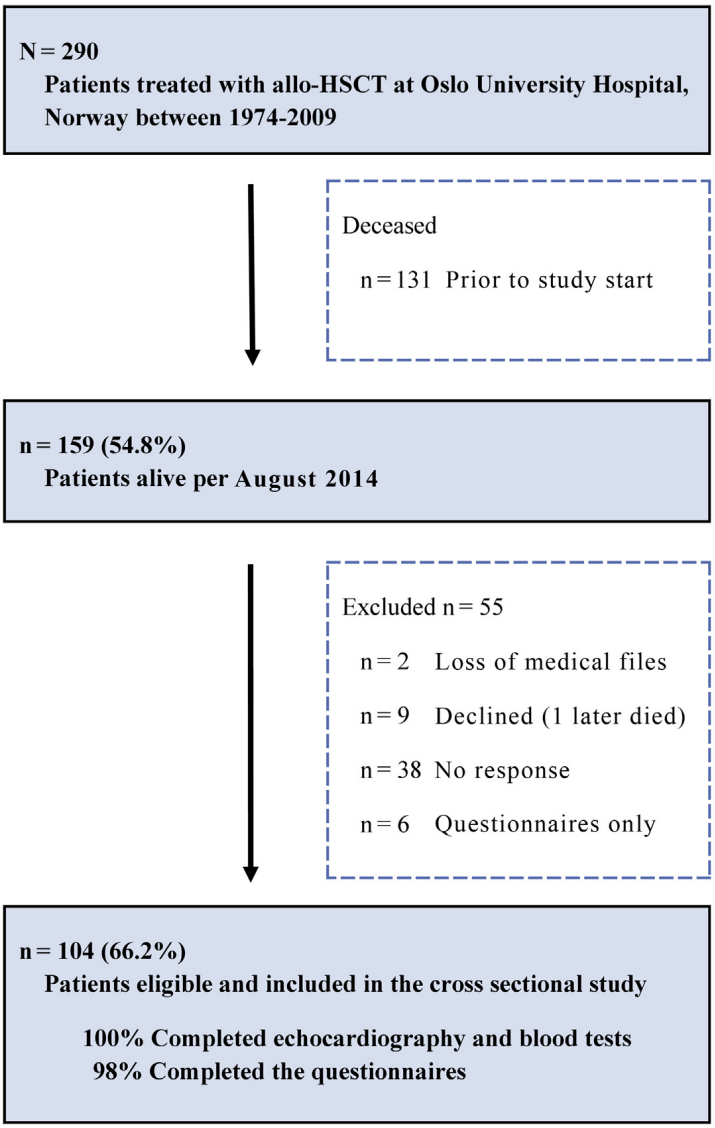
STATISTICAL ANALYSIS

Continuous data are presented as mean ± SD if normally distributed, or as median (25th, 75th percentile) if asymmetrically distributed. Categorical data are presented as numbers and percentages. Allo-HSCT survivors and control subjects were compared with the Student's *t*-test for continuous data, and chi-square or Fisher exact test for categorical data. To adjust for any differences between the allo-HSCT group and control subjects when analyzing cardiac function, inverse-probability weighting (propensity scoring) was performed. In general linear regression analyses, the observations were weighted in each group by the inverse of the probability of being in that group with a given set of covariates: age, BMI, heart rate, and diastolic blood pressure (DBP). Multivariable linear regression analyses were conducted to determine significant predictors for the primary outcome variables of 2D-LVEF and GLS in survivors. Covariates included: age, height, BMI, heart rate, cumulative anthracycline dosage, sex, mediastinal radiation, total body irradiation, malignancy, hypertension, diabetes mellitus, hypercholesterolemia, hypothyroidism, smoking, and GVHD (acute and/or chronic). All continuous variables were standardized, and p values, beta, and corresponding 95% confidence intervals were reported. The final regression models considered multicollinearity, and contained all variables with $p < 0.20$ in a univariable regression and/or variables considered as clinically important risk factors for LVSD. Likewise, deletion was used for the data omissions: 2 for smoking in the univariable test, and 2 for hypercholesterolemia (high triglycerides). To evaluate the effects of anthracycline dosage, patients were allocated into 3 groups according to cumulative anthracycline dosage: none, moderate (<300 mg/m^2), and high (≥300 mg/m^2). Chi-square, Kruskal-Wallis test, and 1-way analysis of covariance with Bonferroni post hoc analyses were used to identify group differences. Analysis of covariance was used to adjust for age, BMI, heart rate, and DBP. Statistical analyses used SPSS version 25 (SPSS Inc., Chicago, Illinois), and a p value <0.05 was considered significant.

RESULTS

PATIENT DEMOGRAPHICS AND ALLO-HSCT TREATMENT CHARACTERISTICS. Patients characteristics are shown in Table 1. In total, 290 patients were treated during the time period specified for inclusion. Of these, 131 died prior to study start (total deaths 45.2%) and 2 were excluded due to incomplete patient files. Non-participants (n = 53) were younger (age 27.7 years vs. 34.3 years; p = 0.001), had shorter follow-up time (13.2 years vs. 16.5 years; p < 0.001), and were more likely men (37 men vs. 16 women; p = 0.005). A total of 104 participants (66.2% of eligible survivors) accepted the study invitation, provided informed consent, and completed clinical examinations (Figure 1). Malignant disease occurred in 74% (55.8% women), and was the indication for anthracycline chemotherapy in 45.2% (59.6% women) and mediastinal radiotherapy in 2 (1.9%). Median isotoxic cumulative anthracycline dosage was 270 mg/m^2 (range 45 to 585 mg/m^2). Stem cells were obtained from the

FIGURE 1 CONSORT Diagram: Illustration of Patient Recruitment



In total, 104 (66.2%) of eligible survivors were evaluated with comprehensive echocardiography.

TABLE 2 Clinical Assessment

	Allo-HSCT (n = 104)	Control Subjects (n = 55)	p Value
New York Heart Association functional class*			
I	74 (72.5)	55 (100)	<0.001
II	16 (15.7)	0 (0.0)	0.002
III	12 (11.8)	0 (0.0)	0.009
Comorbidities			
Hypertension	42 (40.4)	1 (1.8)	<0.001
Diabetes mellitus	3 (2.9)	0 (0.0)	0.552
Hypothyroidism	10 (9.6)	0 (0.0)	0.016
Hypercholesterolemia	16 (15.4)	0 (0.0)	0.002
Smoking (current/previous)*	10 (9.8)/18 (17.6)	2 (3.6)/11 (20.0)	0.165/0.717
Cardiovascular medications	33 (31.7)	0 (0.0)	<0.001
Statins	4 (3.8)	0 (0.0)	–
Calcium-channel blockers	13 (12.5)	0 (0.0)	–
Beta-blockers	13 (12.5)	0 (0.0)	–
Angiotensin-converting enzyme inhibitors	7 (6.7)	0 (0.0)	–
Angiotensin receptor blockers	13 (12.5)	0 (0.0)	–
Laboratory parameters			
Troponin T >14 ng/l	3 (2.9)	0 (0.0)†	0.551
Elevated NT-proBNP‡	17 (16.3)	2 (4.0)†	0.029
HDL cholesterol, mmol/l§	1.5 ± 0.4	1.7 ± 0.5†	0.051
LDL cholesterol, mmol/l§	3.1 ± 0.8	3.0 ± 0.9†	0.433

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

allo-HSCT = allogeneic hematopoietic stem cell transplantation; HDL = high-density lipids; LDL = low-density lipids; NT-proBNP = N-terminal pro-B-type natriuretic peptide.

bone marrow in 84.6%, and 29.8% had unrelated donors. In total, 95 (91.3%) received a myeloablative conditioning regimen consisting of busulfan in combination with cyclophosphamide. The total dosage of busulfan was 4 to 5 mg/kg/day administered orally over 4 days, combined with 50 mg/kg/day of cyclophosphamide administered intravenously over 4 days or 60 mg/kg/day over 2 days. In total, 23 (22.1%) received antilymphocyte globulin and 7 (6.7%) fractionated total body irradiation during conditioning. Cyclosporine was administrated in 99% as part of standard GVHD prophylaxis. Resolved GVHD was identified in 29 (43.3%) of those with prior acute and/or chronic GVHD.

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

ECHOCARDIOGRAPHY AND LVSD. 2D-LVEF was feasible in all patients, 3D-LVEF in 85%, and GLS in 96%. The majority of echocardiography parameters for systolic function were significantly reduced in allo-HSCT survivors compared with control subjects: 2D-LVEF (55.2 ± 5.8% vs. 59.0 ± 2.9%; $p < 0.001$), 3D-LVEF (54.0 ± 5.1% vs. 57.6 ± 2.7%; $p < 0.001$), GLS (−17.5 ± 2.2% vs. −19.8 ± 1.4%; $p < 0.001$), and MAPSE (12.9 ± 2.1 mm vs. 14.9 ± 2.2 mm; $p < 0.001$) was all reduced in survivors compared with control subjects (Table 3, Central Illustration). These group differences remained significant after controlling for baseline differences in age, BMI, heart rate, and DBP (Table 3). After exclusion of participants with established CV disease (45 survivors and 1 control), LVEF and GLS remained significantly impaired in survivors compared with control subjects, despite survivors being considerably younger (Supplemental Table 1). In total, 46 (44.2%) allo-HCST survivors had LVSD in contrast to none in the control group. No sex differences were found for the prevalence of reduced LVEF, GLS, or LVSD. Allo-HSCT survivors had significantly ($p < 0.001$) smaller LV end-diastolic volumes (indexed 2D LV end-diastolic volume) and smaller cardiac mass compared to controls. LVSD was described as mild to moderate global hypokinesis in most instances. Septal hypokinesis was described in three survivors (2.9%), including one symptomatic individual found to have significant coronary disease by angiography.

TABLE 3 Echocardiography Assessment

	Allo-HSCT (n = 104)	Control Subjects (n = 55)	p Value	Inverse-Probability Weighting Beta (Standard Error), p Value*
IVSd, mm	0.86 ± 0.17	0.89 ± 0.14	0.274	
IVSd >12 mm	4 (3.8)	2 (3.6)	0.957	
Indexed LV mass index, g/m^2†	70.5 ± 18.0	73.5 ± 12.5	0.230	
Relative wall thickness	0.30 ± 0.06	0.29 ± 0.05	0.263	
Indexed LVIDd, cm/m^2	2.68 ± 0.32	2.69 ± 0.25	0.984	
Indexed LVIDs, cm/m^2	1.87 ± 0.30	1.84 ± 0.20	0.395	
Indexed 2D-LVEDV, ml/m^2	63.6 ± 13.3	71.9 ± 13.9	0.000	
Indexed 2D-LVESV, ml/m^2	28.8 ± 8.3	29.6 ± 6.5	0.562	
Indexed 3D-LVEDV, ml/m^2	71.5 ± 13.4	75.2 ± 12.8	0.083	
Indexed 3D-LVESV, ml/m^2	33.0 ± 8.6	31.7 ± 7.7	0.374	
3D sphericity index	0.33 ± 0.7	0.33 ± 0.6	0.652	
Cardiac index, l/min/m^2	2.62 ± 0.44	2.76 ± 0.47	0.082	–
Fractional shortening, %	30.6 ± 5.7	31.6 ± 4.0	0.210	1.08 (0.78), 0.169
2D-LVEF, %	55.2 ± 5.8	59.0 ± 2.9	<0.001	3.80 (0.74), <0.001
2D-LVEF ♂<52%, ♀<54%	33 (31.7)	0 (0.0)	<0.001	–
2D-LVEF <50%	17 (16.3)	0 (0.0)	<0.001	–
3D-LVEF, %	54.0 ± 5.1	57.6 ± 2.7	<0.001	3.42 (0.68), <0.001
3D-LVEF ♂<52%, ♀<54%	29 (27.9)	0 (0.0)	<0.001	–
MAPSE, mm	12.9 ± 2.1	14.9 ± 2.2	<0.001	2.02 (0.34), <0.001
s′ velocity, cm/s	8.0 ± 1.7	8.9 ± 1.7	0.002	7.72 (2.66), 0.004
GLS, %	−17.5 ± 2.2	−19.8 ± 1.4	<0.001	2.13 (0.30), <0.001
GLS ≥−17%	34 (32.7)	0 (0.0)	<0.001	–
Pericardial pathology	8 (7.7)	0 (0.0)	0.051	–

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

allo-HSCT = allogeneic hematopoietic stem cell transplantation; GLS = global longitudinal strain; IVSd = interventricular septal end-diastolic dimension; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; LVIDd = left ventricular internal end-diastolic dimension; LVIDs = left ventricular internal end-systolic dimension; MAPSE = mitral annular plane systolic excursion, average of septum and lateral; NT-proBNP = N-terminal pro-B-type natriuretic peptide; s′ velocity = tissue Doppler peak systolic velocity, average of septum and lateral.

Univariable and multivariable linear regression analyses are shown in **Tables 4 and 5**. Statistically significant independent predictors for 2D-LVEF were age, cumulative anthracycline dosage, and GVHD. Significant independent predictors for GLS were age, heart rate, cumulative anthracycline dosage and hypertension. Anthracycline therapy was found to be a strong predictor of impaired LVEF and GLS. Further analysis confirmed a dose dependent relationship between anthracycline dose and reduction in 2D-LVEF and GLS, after adjusting for age, BMI, heart rate, and DBP (**Table 6**). Those who received higher anthracycline dosages tended to have greater evidence of cardiac dysfunction and adverse remodeling.

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

DISCUSSION

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

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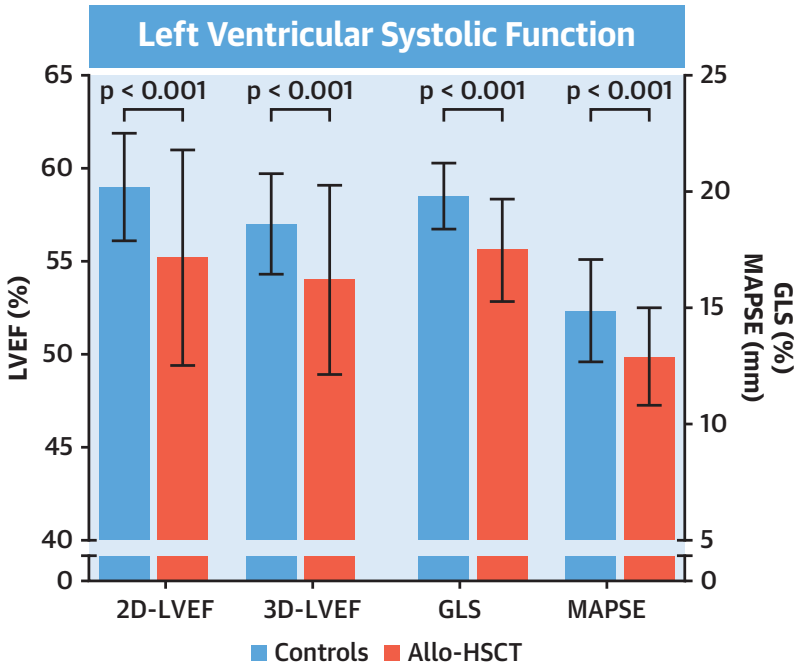
CENTRAL ILLUSTRATION **Left Ventricular Systolic Function in Long-Term Survivors Treated as Children, Adolescents, and Young Adults With Allo-HSCT**



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

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) survivors were found to have significantly reduced left ventricular systolic function with echocardiography compared with healthy control subjects. Most cases were described as mild to moderate global hypokinesis with few cases of valve disease. Significant independent predictors of left ventricular systolic function were age, anthracyclines, graft versus host disease, heart rate, and hypertension. 2D = 2-dimensional; 3D = 3-dimensional; LVEF = left ventricular ejection fraction; GLS = global longitudinal strain; MAPSE = mitral annular plane systolic excursion.

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

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

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

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

	Univariable			Multivariable		
	β	95% CI	p Value	β	95% CI	p Value
Sex	0.05	−0.34 to 0.44	0.809	−0.06	−0.42 to 0.30	0.739
Age, yrs*	0.27	0.09 to 0.46	0.005	0.29	0.07 to 0.52	0.011
Height, m	−0.08	−0.28 to 0.17	0.423			
Body mass index, kg/m^2	0.18	−0.01 to 0.38	0.062	0.06	−0.14 to 0.26	0.548
Heart rate, beats/min	−0.01	−0.20 to 0.19	0.952			
Mediastinal radiation	−0.64	−2.07 to 0.77	0.370			
Cumulative anthracycline dosage, mg/m^2	−0.41	−0.59 to −0.23	<0.001	−0.46	−0.63 to −0.28	<0.001
Total body irradiation	0.26	−0.52 to 1.03	0.517	−0.20	−0.91 to 0.51	0.580
Malignancy	−0.22	−0.66 to 0.23	0.339			
Hypertension	0.13	−0.27 to 0.53	0.511	−0.18	−0.60 to −0.24	0.393
Diabetes mellitus	−0.68	−1.84 to 0.48	0.248			
Hypercholesterolemia	0.06	−0.48 to 0.61	0.822			
Hypothyroidism	0.12	−0.55 to 0.78	0.727			
Smoking, current/previous	0.11	−0.34 to 0.55	0.632			
Graft versus host disease, acute and/or chronic GVHD	0.29	−0.12 to 0.69	0.164	0.64	0.26 to 1.03	0.001

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

allo-HSCT = allogeneic hematopoietic stem cell transplantation; GVHD = graft versus host disease.

survivors treated in their youth (23). The cardiotoxic effect of anthracycline was also confirmed in this study; anthracycline treatment was a significant independent predictor for the reduction of 2D-LVEF and GLS. Moreover, the reduction in systolic function and adverse LV remodeling were dose-dependent. These observations are in agreement with other studies that have used similar thresholds of cumulative anthracycline dosage (>250 to 300 mg/m^2) (8,9,19,21,23).

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

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

	Univariable			Multivariable		
	β	95% CI	p Value	β	95% CI	p Value
Sex	0.38	−0.06 to 0.73	0.092	0.22	−0.16 to 0.59	0.249
Age, yrs*	−0.06	−0.26 to 0.14	0.548	−0.24	−0.45 to −0.02	0.031
Height, m	0.07	−0.14 to 0.27	0.523			
Body mass index, kg/m^2	0.01	−0.21 to 0.22	0.964			
Heart rate, beats/min	0.33	0.13 to 0.52	0.001	0.34	0.15 to 0.53	0.001
Mediastinal radiation	0.68	−0.74 to 2.10	0.343			
Cumulative anthracycline dosage, mg/m^2	0.19	−0.01 to 0.39	0.059	0.25	0.07 to 0.44	0.009
Total body irradiation	0.40	−0.44 to 1.24	0.345	0.20	−0.64 to 1.04	0.638
Malignancy	0.38	−0.07 to 0.82	0.095			
Hypertension	0.48	0.08 to 0.87	0.019	0.50	0.04 to 0.96	0.035
Diabetes mellitus	0.49	0.93 to 1.91	0.493			
Hypercholesterolemia	0.66	0.11 to 1.22	0.020	0.44	−0.14 to 1.02	0.134
Hypothyroidism	−0.37	−1.03 to 0.29	0.271			
Smoking, current/previous	0.20	−0.25 to 0.65	0.372			
GVHD, acute and/or chronic GVHD	−0.02	−0.44 to 0.39	0.919	−0.29	−0.69 to 0.11	0.158

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

Abbreviations as in Table 4.

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TABLE 6 Dose-Related Responses to Anthracycline Used in Pre-Treatment Therapies in allo-HSCT Survivors

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

Values are median (25th, 75th percentiles), n (%), or mean ± SD. *Significant difference between anthracycline ≥300 mg/m² and <300 mg/m² in Bonferroni post hoc analysis (<0.05). †Significant difference with both treatment groups (anthracycline ≥300 mg/m² and <300 mg/m²) with no anthracycline group in Bonferroni post hoc analysis (<0.05). ‡No significant difference between treatment groups in Bonferroni post hoc analysis. §Echocardiographical parameters are adjusted for covariates of age, heart rate, body mass index, and diastolic blood pressure. ‖Significant difference between anthracyclines ≥300 mg/m² and no anthracycline in Bonferroni post hoc analysis (<0.05). ¶ASE cube formula (14).

Abbreviations as in Table 3.

Any potential differences in reported CV risk factor prevalence are likely associated with cardiotoxic exposures, recipient age, and observation time. In our study, hypertension and hypercholesterolemia were more frequently observed in survivors with older age and longer survival time, suggesting that risk profiles may alter with aging after HSCT. One concerning finding from our cross-sectional analyses was that hypertension was prevalent in 40.4%, but the use of antihypertensive therapy in only 30.8%. A similarly high prevalence of hypertension (24% to 45%) has been reported in long-term survivors (>10 years) treated with HSCT at various ages (5,9,20,21,24,25). The significance of hypertension in contributing to prevailing cardiac disease in HSCT survivors has previously been shown (5,21). However, in our study, given that the onset and duration of hypertension is unknown, it is unclear if the cumulative consequences of hypertension were fully manifested. Indeed, the echocardiograms showed little evidence of concentric remodeling beyond a small but significant difference in indexed LV end-diastolic volume. However, hypertension is known to cause myocardial fibrosis that leads to reduced longitudinal shortening of the heart (27). This may explain why hypertension was found to be an independent predictor of GLS.

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

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

has been shown to be associated with a higher risk of CV death (5). It is thought that the chronic inflammation processes instigated by GVHD results in endothelial damage leading to accelerated atherosclerosis (29). We did not find GVHD (acute or chronic) to be consistently associated with LV systolic function, and found a very modest association with increased LVEF ($\beta = 0.64$; 95% CI: 0.26 to 1.03) and no association with GLS. GVHD may have an indirect role in altering cardiac function through the development of CV risk factors. Moreover, a higher proportion of pericardial pathology was observed in patients with GVHD. Although not statistically significant, it is numerically striking, may have clinical relevance possibly affecting systolic function, and supports the few previous reports on this matter (30). This is an area that deserves further study.

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

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

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

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

CONCLUSIONS

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

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PERSPECTIVES

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

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

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

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

Supplement Table 1: Comparison of allo-HSCT survivors and controls without cardiovascular disease

Variable	Allo-HSCT N= 59	Controls N = 54	p value	Adjusted p value *
Age (years)	30.1 ± 10.7	36.5 ± 10.6	0.002	
Gender (female)	36 (55.4)	29 (53.7)	0.432	
BMI (kg/m^2)	23.4 ± 5.1	24.1 ± 3.4	0.432	
Anthracyclines	26 (44.1)	0 (0)	0.000	
Systolic BP (mmHg)	116 ± 12	117 ± 11	0.746	
Diastolic BP (mmHg)	67 ± 11	66 ± 8	0.535	
HR (bpm)	68 ± 11	68 ± 12	0.994	
IVSd (cm)	0.80 ± 0.1	0.89 ± 0.1	0.001	0.09 (0.03), 0.001
LV cardiac mass (g) †	115.7 ± 30.2	135.8 ± 35.8	0.002	19.60 (6.60), 0.004
LVIDd (cm)	4.8 ± 0.5	5.0 ± 0.4	0.313	0.17 (0.08), 0.032
LVIDs (cm)	3.4 ± 0.4	3.4 ± 0.4	0.31	0.04 (0.07), 0.573
2D-LVEDV (ml)	111.7 ± 27.2	134.7 ± 31.4	<0.001	21.7 (5.75), 0.000
2D-LVESV (ml)	51.0 ± 13.2	55.1 ± 14.2	0.092	3.76 (2.67), 0.162
Fraction shortening (%)	30.5 ± 4.8	31.5 ± 4.0	0.200	1.46 (0.83), 0.081
2D-LVEF (%)	54.6 ± 4.6	59.1 ± 2.9	<0.001	4.50 (0.71), 0.000
3D-LVEF (%)	53.3 ± 3.9	57.6 ± 2.7	<0.001	4.25 (0.66), 0.000
s' velocity (cm/s)	8.3 ± 1.6	8.9 ± 1.7	0.046	6.02 (3.13), 0.057
MAPSE (mm)	13.2 ± 1.9	14.9 ± 2.2	<0.001	1.71 (0.40), 0.000
GLS (%)	-17.9 ± 1.6	-19.8 ± 1.4	<0.001	1.81 (0.30), 0.000

Number displayed as means ± SD or number (%). Allo-HSCT survivors (45) and control (1) were excluded due to presence of hypertension, hypercholesterolemia, diabetes mellitus and / or receiving cardiovascular medicines.
* Inverse-probability weighting (covariates: age, heart rate, body mass index and diastolic blood pressure). Results presented as beta (standard error), p value.
† ASE cube formula (14)
Abbreviations: BMI: Body Mass Index, BP: Blood Pressure, GLS: Global Longitudinal Strain, HR: Heart Rate, IVSd: Inter-Ventricular Septum end-diastolic dimension, LV-cardiac mass: Left Ventricular cardiac mass, LVIDd: Left Ventricular Internal end-diastolic Dimension, LVIDs: Left Ventricular Internal end-systolic Dimension, LVEDV: Left Ventricular End-Diastolic Volume, LVEF: Left Ventricular Ejection Fraction, LVESV: Left Ventricular End-Systolic Volume, MAPSE: Mitral Annular Plane Systolic Excursion (average of septum and lateral), s' velocity: Tissue Doppler peak systolic velocity (average of septum and lateral).

II

openheart

Impaired right ventricular function in long-term survivors of allogeneic haematopoietic stem-cell transplantation

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ABSTRACT

Aims Survivors of allogeneic haematopoietic stem-cell transplantation (allo-HSCT) are at higher risk of cardiovascular disease. We aimed to describe right ventricular (RV) systolic function and risk factors for RV dysfunction in long-term survivors of allo-HSCT performed in their youth.

Methods and results This cohort included 103 survivors (53% female), aged (mean±SD) 17.6±9.5 years at allo-HSCT, with a follow-up time of 17.2±5.5 years. Anthracyclines were used as first-line therapy for 44.7% of the survivors. The RV was evaluated with echocardiography, and found survivors to have reduced RV function in comparison to a group of healthy control subjects: Tricuspid annular plane systolic excursion, (TAPSE, 20.8±3.7 mm vs 24.6±3.8 mm, p<0.001), RV peak systolic velocity (RV-s', 11.2±2.3 cm/s vs 12.3±2.3 cm/s, p=0.001), fractional area change (FAC, 41.0±5.2% vs 42.2±5.1%, p=0.047) and RV free-wall strain (RVFWS, −27.1±4.2% vs −28.5±3.3%, p=0.043). RV systolic dysfunction (RVSD) was diagnosed in 14 (13.6%), and was strongly associated with progressive left ventricular systolic dysfunction (LVSD). High dosages of anthracyclines were associated with greater reductions in RV and LV function. Multivariable linear regressions confirmed global longitudinal strain to be a significant independent predictor for reduced RV function.

Conclusion Impaired RV function was found in long-term survivors of allo-HSCT who were treated in their youth. This was associated with progressive left ventricle dysfunction, and pretransplant therapies with anthracyclines. The occurrence of RVSD was less frequent and was milder than coexisting LVSD in this cohort.

Key questions

What is already known about this subject?

► Survivors of allogeneic haematopoietic stem-cell transplantation (allo-HSCT) are at elevated risk of heart disease due to high-dose chemotherapies, graft-versus-host disease and high rates of cardiovascular risk factors. Despite this, there is scarce information on right ventricular (RV) function.

What does this study add?

► This study demonstrates that RV function is reduced in survivors of allo-HSCT.
► The occurrence of RV systolic dysfunction is strongly associated with co-existing left ventricular systolic dysfunction.

How might this impact on clinical practices?

► Examination of the right ventricle with echocardiography should be performed in survivors of high-dose anthracyclines and coexisting left ventricular dysfunction.
► Allo-HSCT survivors identified with reduced RV function may benefit from more frequent surveillance and assertive cardioprotective interventions.

INTRODUCTION

Allogeneic haematopoietic stem-cell transplantation (allo-HSCT) is increasingly used as a curative therapy for severe malignant and non-malignant disorders in young patients. Advances in treatment strategies have improved survival in the immediate years after transplantation.[1] A growing concern in long-term survivors is a 2–4 times higher cardiovascular mortality compared with the general population.[2 3] Acquired heart disease can be attributed to pretransplant chemotherapy, transplant-related conditioning, graft-versus-host disease (GVHD) and elevated levels of cardiovascular risk factors.[2–5]

Chemotherapy-induced cardiotoxicity in the form of left ventricle systolic dysfunction (LVSD) is well acknowledged. In contrast, information regarding the therapeutic consequences of HSCT to right ventricular (RV) function is scarce,[6] and to our knowledge, is absent in those exposed during childhood, adolescence and young adulthood (CAYA). The level of functional impairment after chemotherapy is reported to differ between the ventricles.[6–8] Moreover, RV function has been shown to be a strong predictor for progression and mortality in heart failure.[9 10]

This observational study aimed to evaluate RV function, determine the prevalence

of RV systolic dysfunction (RVSD), and identify factors predicting its occurrence in a cohort that underwent allo-HSCT at a young age.

METHODS

Study design

Oslo University Hospital is Norway's national centre for allo-HSCT. A complete nationwide cohort was identified by browsing the hospital registry. The eligibility criteria were allo-HSCT at Oslo University Hospital, <30 years at transplantation, >16 years at inclusion and a minimum observation time of 5 years. Survivors with Hurler syndrome were excluded due to multi-organ pathology as a part of their primary disease.

Clinical assessment

Anthracycline cumulative dosage was calculated by recommended conversion rates to isotoxic doses of doxorubicin.[11] Dyspnoea was classified according to the New York Heart Association (NYHA).[12] Blood pressures were measured after ≥30 min in the supine position, immediately after echocardiography, as the average of three measurements. Blood samples were collected after overnight fasting and analysed at the hospital's laboratory. N-terminal pro-brain-type natriuretic peptide (NT-pro-BNP) concentrations were determined by an electrochemiluminescence immunoassay (Roche Diagnostics, Switzerland). The lowest detectable NT-pro-BNP value was 5 ng/L, and elevated levels were defined by age-specific and gender-specific cut-offs as recommended by the manufacturer. Diabetes mellitus type II was classified as haemoglobin glucose >6.5% (48 mmol/mol) or use of glucose-lowering medication. Hypothyroidism was defined by the use of thyroid replacement medication or serum thyroid-stimulating hormone >4 mg/L and free thryroxine-4 <9 pmol/L. Hypertension was defined as current use of anti-hypertensive drugs, systolic blood pressure >140 mm Hg or diastolic blood pressure (DBP) >90 mm Hg. Hypercholesterolaemia was defined as low-density lipoprotein >4.1 mmol/L (160 mg/dL) or use of lipid-lowering medication. Acute GVHD and chronic GVHD (cGVHD) were graded by the Glucksberg and Shulman criteria.[13 14] Bronchiolitis obliterans syndrome (BOS) was defined as recommended.[15]

Echocardiography

Transthoracic echocardiography was performed using Vivid-E9 scanners and software (V.113.1.3-EchoPAC, GE-Vingmed Ultrasound, Norway). A comprehensive protocol was designed to evaluate cardiac function, and data from this cohort were used to describe LV and RV function. All echocardiograms were acquired and analysed by the same experienced investigator (RJM). Analyses were conducted en bloc after completion of the last inclusion, on deidentified images, in random order and blinded to patient's medical records. RV and LV indices were measured at separate occasions to reduce bias. Twenty-five echocardiograms from patients and controls were randomly selected for intraobserver variability tests.

The study followed European Association of Cardiovascular Imaging (EAVCI) recommendations for image acquisition and analyses.[16 17] Scanner settings were optimised and measurements made from three consecutive heart cycles. Internal RV dimensions, right atrium (RA) area/volume, and measures of RV function were performed on modified apical four-chamber view focused on the RV. Particular care was made to ensure appropriate orientation and without foreshortening. Further adjustments to sector width and depth of grey-scale images increased resolution. Average frame rate was 69/s. For fractional area shortening (FAC), the RV's borders were traced in end-diastole and end-systole, with consideration of trabeculations. RV posterior-wall thickness was measured in diastole on a dedicated subcostal four-chamber view. Tricuspid annular plane systolic excursion (TAPSE) was measured with anatomical M-mode placed between the annulus and apex, and parallel to the free-wall. Tissue velocity imaging (TVI) with Doppler was recorded from the annulus to calculate RV peak systolic velocity (RV-s') and RV index of myocardial performance (RIMP). Speckle tracking echocardiography (STE) was used to obtain RV free-wall strain (RVFWS) as the average peak systolic value from three free-wall segments. Region of interest was manually adjusted for apex, annular plane and myocardial borders. Tracking was visually controlled. Results were only included if reliable values were acquired from all segments.

Current EAVCI guidelines were used to define the cut-offs for reduced RV function: FAC <35%, TAPSE <17 mm, RV-s'<9.5 cm/s, RVFWS >-20% and RIMP >0.54.[16] In the absence of a consensus for definition of RVSD, we considered RVSD to be present when at least two of these parameters were abnormal.

Tricuspid regurgitation pressure (TRP) was measured with continuous wave Doppler from multiple views. Pulmonary artery systolic pressure (PASP) was calculated from TRP using the Bernoulli equation plus RA pressure estimated by size and respiratory variation of the inferior vena cava.[17] Elevated PASP was defined as TRP >2.8 m/s and/or PASP >35 mm Hg.[17] In survivors without adequate TR or signs of pulmonary hypertension, PASP of 23.3 mm Hg (group mean) was allocated.

LVSD was defined as reduced 2D-LVEF (male <52%, female <54%) and/or reduced global longitudinal strain (GLS) ≥-17%. Diastolic function was evaluated according to guideline algorithms.[18] Tricuspid and pulmonary valve pathology were graded according to recommendations.[19] Pericardial pathology was defined as increased pericardial fluid >0.5 cm, and/or presence of abnormal thickening or fusion of the visceral and parietal membranes.

Control group

Calculations of sample size were made for TAPSE, RV-s', RVFWS and FAC, using EACVI reference values for means and SD in controls.[16] To identify a 10% difference

in group means with α=0.05 (two sided) and power >0.8, a minimum of 48 controls were required. To accommodate for eventual missing data, 55 healthy controls of similar race and ethnicity were recruited from advertisements. Efforts were made to obtain control group with a comparative patient characteristics. The only exclusion was established cardiovascular disease.

Statistical analyses

Statistical analyses were performed with SPSS V.26 (SPSS), and p<0.05 was considered significant. Shapiro-Wilk test was used to assess normality. Continuous data are presented as mean±SD, or as median (25th, 75th percentile) in cases of asymmetric distribution. Categorical data are presented as number (percentage). Allo-HSCT survivors (n=103) and controls (n=55) were compared with Student's t-test and Mann-Whitney U-test for continuous data, and χ^2 or Fisher's exact test for categorical data. Inverse probability of treatment weighting method (propensity scoring) was used to adjust for imbalances between the groups in covariates that potentially influence RV function: Age at examination, heart rate (HR) and DBP.

Predictors for RV function (TAPSE, RV-s', RVFWS) were determined by multivariable linear regression analyses. Continuous variables were standardised. The final prediction model included priori selected variables considered to be important determinants for RV function and covariates with p<0.20 in univariable regression. Assumption testing included histograms, residual plots and multicollinearity assessed by Pearsons correlations, tolerance and variance inflation factor (VIF). Over-fitting was avoided. Likewise deletion was used to handle the few randomly missing data.

Dose-dependent effects from anthracyclines on RV function were tested by allocating survivors into three groups according to dosage: none, low (<300 mg/m^2) and high (300 mg/m^2). Allocation of dosage cut-offs was based on median values, and was comparative to previous studies. Analysis of covariance (ANCOVA) with age at examination, HR, body mass index and DBP as covariates, and χ^2, both with Bonferroni post hoc analysis, were used in these in-group comparisons.

RESULTS

In total, 290 patients were treated with allo-HSCT in the time frame specified for this study. Of these, 131 (45.2%) died prior to study start and two were excluded due to incomplete patient files. One-hundred and fifty-seven were eligible for inclusion, and 104 (66.2%) survivors were initially included and examined with echocardiography (figure 1). One patient had previous heart surgery to remove a benign tumour from the left atrium and was excluded from statistical analyses. The cohort consisted of 103 survivors, 55 (53.4%) females, aged 17.6±9.5 years at allo-HSCT, and with a follow-up time to examination with echocardiography of 17.2±5.5 years. Non-participants (n=54) were younger (27.7 years), had shorter follow-up time (13.2 years), and were more commonly male (68.5%).

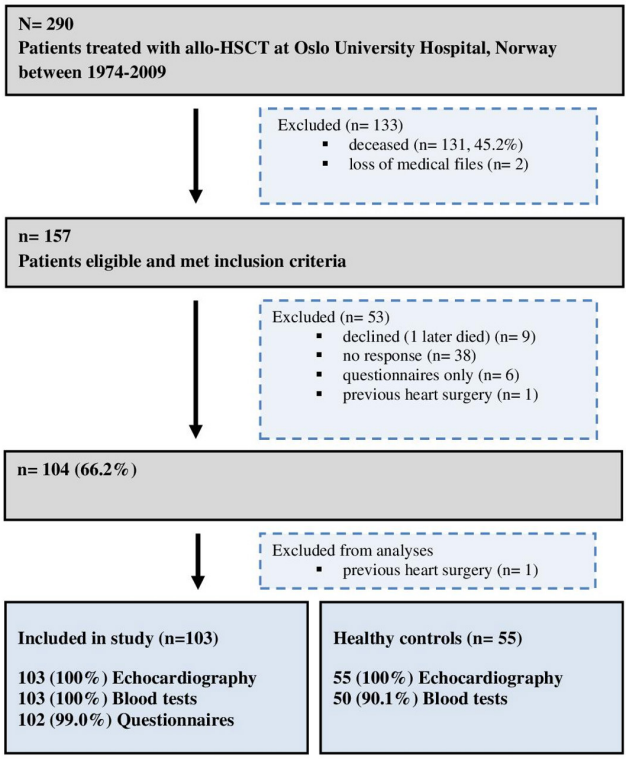

Figure 1 Flow chart of study participants. allo-HSCT, allogeneic haematopoietic stem-cell transplantation.

Survivor characteristics and treatments

Survivor characteristics are shown in table 1. Malignancy was the primary disease in 76 (73.8%, 55.3% were female), and was the indication for anthracyclines in 46 (44.7%, 58.7% were female) and mediastinal radiotherapy in two (1.9%). Median cumulative anthracycline dosage was 270 mg/m^2 (45–585 mg/m^2). The majority received myeloablative conditioning consisting of busulfan (4–5 mg/kg/day/po administered over 4 days) in combination with cyclophosphamide (50 mg/kg/day/intravenously over 4 days or 60 mg/kg/day/intravenously over 2 days). Seven (6.8%) received fractionated total body irradiation (TBI, 1,3 Gy × 2 over 5 days). History of GVHD was identified in 67 (65.0%) survivors. Ten (9.7%) were diagnosed with BOS, of which 80% also had chronic GVHD. Currently used medication included ACE-inhibitors and/or angiotensin receptor blockers in 20, calcium blockers in 13, beta-blockers in two and statins in four. In comparison to controls, survivors had a higher prevalence of cardiovascular risk factors (table 1), and a higher DBP (72 mm Hg vs 66 mm Hg, p<0.001). Risk factors in survivors were distributed equally between sexes, with the exception of hypertension (17 female vs 24 male, p=0.048) and hypothyroidism (8 female vs 1 male, p=0.025).

Table 1 Survivor characteristics

Variable	Allo-HSCT	Controls	P value
Number	103	55	
Gender (female)	55 (53.4)	29 (52.7)	0.936
Body mass index (kg/m^2)	24.5±5.1	24.1±3.4	0.478
Age at allo-HSCT (years)	17.6±9.5		
Time to follow-up (years)	17.2±5.5		
Age at examination (years)	34.8±11.6	36.4±10.6	0.401
Systolic blood pressure (mm Hg)	123±19	117±11*	**0.036**
Diastolic blood pressure (mm Hg)	72±13	66±8*	**<0.001**
Heart rate (bpm)	69±11	68±12	0.59
Malignant/non-malignant disease	76 (73.8)/27 (26.2)		
Mediastinal radiotherapy	2 (1.9)		
Anthracyclines	46 (44.7)		
Cum. dosage (mg/m^2)	270 (130, 435)		
Myeloablative conditioning:			
Chemotherapy (Bu/Cy)	94 (91.3)		
Chemotherapy+TBI	7 (6.8)		
None	2 (1.9)		
Graft-versus-host disease (GVHD)	67 (65.0)		
Acute GVHD	27 (26.2)		
Chronic GVHD	12 (11.7)		
Acute and chronic GVHD	28 (27.2)		
New York Heart Association			
Class-I	74 (73.3)†	55 (100.0)	**<0.001**
Class-II	16 (15.8)†	0 (0)	**0.002**
Class-III	11 (10.7)†	0 (0)	**0.008**
Class-IV	0 (0)†	0 (0)	
Risk factors:			
Hypertension	41 (39.8)	1 (1.8)	**<0.001**
Diabetes mellitus	3 (2.9)	0 (0)	**0.552**
Hypothyroidism	9 (8.7)	0 (0)	**0.028**
Hypercholesterolaemia	15 (14.9)†	0 (0)	**0.003**
Bronchiolitis obliterans syndrome	10 (9.7)	0 (0)	**0.015**
Smoking (current/previous)	10 (9.9)/17 (16.5)†	2 (3.6)/11 (20.0)	**0.216/0.622**
Laboratory parameters:			
NT-pro-BNP (ng/L)§	47 (22, 84)	5 (5,52)¶	**<0.001**
Elevated levels**	16 (15.5)	2 (4.0)¶	**0.038**

Data presented as mean±SD, median (25th, 75th) or n (%). Calculated with Student's t-test, Mann-Whitney and χ^2/Fisher's exact test. Statistically significant values in boldface p<0.05.
*n=54.
†n=101.
‡
§Lowest recordable value=5 ng/L.
¶n=50.
**18–44 years: male >86 ng/L, female >130 ng/L, 45–54 years: male >121 ng/L, female >249 ng/L.
allo-HSCT, allogeneic haematopoietic stem-cell transplantation; NT-pro-BNP, N-terminal pro-brain-type natriuretic peptide; TBI, total body irradiation.

RV morphology and function

The RV and RA were structurally similar between survivors and controls, with the exception of indexed RV mid-wall diameter that was mildly, but significantly larger in survivors (table 2). Male survivors had larger heart chambers, and significantly greater values for RV-s' (male 12.0±2.4 cm/s vs female 10.4±1.8 cm/s, p<0.001) and larger values for TAPSE (male 21.5±3.3 mm vs

Table 2 Comparisons of right ventricular function in survivors and controls

Variable	Allo-HSCT (n=103)	Control (n=55)	P value	Adjusted P value*
RA morphology				
RA area (cm/m^2)	8.0±1.5	8.3±1.4	0.254	0.445
RA-EDV-index (mL/m^2)	22.2±6.8	22.8±4.9	0.560	0.833
Dilated RA-EDV†	14 (13.6)	5 (9.1)	0.407	
RV morphology				
RVIDd basal (cm/m^2)	2.0±0.3	2.0±0.2	0.133	0.092
RVIDd mid-wall (cm/m^2)	1.7±0.3	1.6±0.2	**0.005**	**0.001**
RVIDs basal (cm/m^2)	1.6±0.2	1.5±0.2	0.391	0.158
RVIDs mid-wall (cm/m^2)	1.3±0.2	1.1±0.2	**0.001**	**<0.001**
RV length (cm/m^2)	4.1±0.5	3.9±0.4	0.119	0.145
RV wall-thickness (mm)	39±4 (n=90)	38±4 (n=48)	0.111	0.168
RV-EDA (cm/m^2)	11.0±2.3	10.8±1.0	0.538	0.428
Dilated RV-EDA‡	32 (31.1)	9 (6.4)	**0.045**	-
RV-ESA (cm/m^2)	6.5±1.4	6.2±1.1	0.227	0.068
RV function				
Fractional area change (%)	41.0±5.2	42.2±5.1	0.175	**0.047**
FAC <35%	11 (10.7)	2 (3.6)	0.222	-
TAPSE (mm)	20.8±3.7	24.6±3.8	**<0.001**	**<0.001**
TAPSE <17mm	13 (13.0)	1 (1.8)	**0.020**	-
RV s' (cm/s)	11.1±2.3	12.3±2.3	**0.005**	**0.001**
RV-s' <9.5cm/s	26 (25.5)	8 (14.8)	0.124	-
RV-e' (cm/s)	10.0±2.7	11.7±2.5	**<0.001**	**<0.001**
RV-e' <7.8cm/s	18 (17.6)	1 (1.9)	**0.004**	-
RIMP	0.45±0.09	0.45±0.07	0.851	0.649
RIMP >0.54	17 (17.2)	3 (5.6)	**0.042**	-
RVFWS (%)	−27.1±4.2	−28.5±3.3	0.052	**0.043**
RVFWS ≥20%	3 (3.2)	0 (0)	0.553	-
TRP (mm Hg)	18.1±4.0	16.7±2.6	**0.034**	0.125
PASP >35 mm Hg	3 (2.9)	0 (0)	0.552	-
LV function				
2D-LVEF (%)	55.2±5.9	59.0±2.9	**<0.001**	**<0.001**
GLS (%)	−17.5±2.2	−19.7±1.4	**<0.001**	**<0.001**
E/e'	6.6±2.2	5.7±1.4	**0.003**	**0.011**

*Adjusted p values by propensity scoring. Statistically significant values in boldface (p<0.05).
†Dilated RA in males if >32 mL/m^2 and in females if >27 mL/m^2.
‡Dilated RV-EDA in males if >12.6 cm^2/m^2 and in females if >11.5 cm^2/m^2.
Allo-HSCT, allogeneic haematopoietic stem-cell transplantation; 2D, two dimensions; E/e', ratio of early-diastolic velocity to mean early-diastolic myocardial velocity; FAC, fractional area change; GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; PASP, pulmonary arterial systolic pressure; RA, right atrium; RA-EDV, RA-end-diastolic volume; RIMP, Right Ventricular Index of Myocardial Performance; RV, right ventricular; RV-e', RV early-diastolic velocity; RV-EDA, RV-end-diastolic area; RV-ESA, RV-End-Systolic Area; RVFWS, RV-free-wall strain; RVIDd, RV-internal dimension in diastole; RVIDs, RV-internal dimension in systole; RV-s', RV-peak systolic velocity; TAPSE, tricuspid annular plane systolic excursion; TRP, tricuspid regurgitation pressure.

female 20.1±3.3 mm, p=0.056). Measured parameters on systolic RV function were clearly lower in survivors of allo-HSCT as compared with controls, with the exception of RIMP: TAPSE (20.8±3.7 mm vs 24.6±3.8 mm, p<0.001), RV-s' (11.1±2.3 cm/s vs 12.3±2.3 cm/s, p=0.001), FAC (41.0%±5.2% vs 42.2±5.1%, p=0.047) and RVFWS (−27.1±4.2% vs −28.5±3.3%, p=0.043) (table 2 and figure 2). Significant linear correlations were found between parameters of RV and LV function (see online supplemental table 1). Higher anthracycline dosages corresponded with greater biventricular functional impairments, and more cases of elevated filling pressures, indicating a dose-dependent relationship (table 3). RVSD was found in two survivors whom had received high-dose (>300 mg/m^2) anthracyclines and TBI conditioning. Neither of the two survivors whom had received mediastinal radiotherapy had registered RVSD.

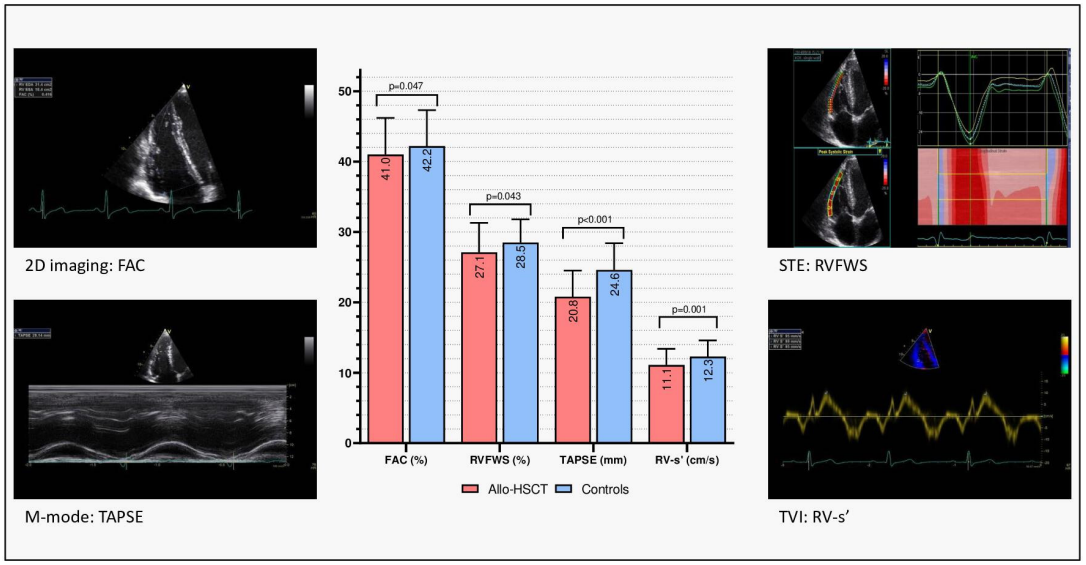

Figure 2 Comparison of right ventricular systolic function between allo-HSCT survivors and healthy controls. Presented with means±SD and adjusted p values. 2D, two dimensions; allo-HSCT, allogeneic haematopoietic stem-cell transplantation; FAC, fractional area change; M-mode, motion-mode ultrasound; RVFWS, Right ventricular Free-Wall Strain (absolute value), RV-s', right ventricular peak systolic velocity; STE, speckle tracking echocardiography; TAPSE, tricuspid annular plane systolic excursion; TVI, tissue velocity imaging.

RVSD was identified in 14 survivors (64.3% were female) and one control (13.6% vs 1.8%, p=0.016), and was less common than LVSD 46 (44.7%). Thirteen (92.9%) survivors with RVSD had coexisting LVSD. The survivors with RVSD had significantly worse LV function than survivors without LVSD (2D-LVEF 48.6±7.1% vs 56.2±5.0%, p<0.001 and GLS −14.3±2.4% vs −17.9±1.8%, p<0.001). The median value for NT-pro-BNP was significantly higher in survivors with RVSD, compared with survivors without RVSD (71 ng/L (46,281) vs 43 ng/L (22,82), p=0.043). Despite these findings, the frequency of dyspnoea (NYHA class ≥II) did not differ in survivors with RVSD compared with survivors without RVSD (21.4% vs 27.6%, p=0.754). Elevated LV filling pressure was identified in 22 (21.4%) survivors with LVSD. E/e' remained significantly higher in survivors than in controls (6.6±2.2 vs 5.7±1.4, p=0.011) after controlling for age at examination, HR and DBP. In survivors, E/e' correlated with TRP (R=0.357, p<0.001). Diastolic dysfunction in the absence of LVSD was present in seven (6.8%) survivors. Individuals with BOS did not

Table 3 Dose-related effects of anthracyclines on right ventricular function

Variable	None	Low-dose <300 mg/m^2	High-dose >300 mg/m^2	Adjusted p value*
Number	57 (55.3)	24 (23.3)	22 (21.4)	
FAC (%)	41.6±4.2	42.0±4.3	38.3±7.6	**0.033**[d]
TAPSE (mm)	21.3±3.6	20.4±3.5	20.0±4.0	0.303[a]
RV-s' (cm/s)	11.7±2.1	10.8±2.4 (n=23)	10.2±2.3	**0.045**[a]
RIMP	0.45±0.09 (n=55)	0.46±0.09 (n=23)	0.44±0.11	0.955[a]
RVFWS (%)	−27.7±3.6 (n=50)	−27.7±4.6 (n=23)	−25.2±4.7	**0.034**[b,d]
TRP (mm Hg)	17.8±3.6 (n=44)	18.7±3.8 (n=15)	18.3±5.3 (n=17)	0.656[a]
2D-LVEF (%)	57.0±4.7	54.5±7.1	51.1±5.0	**<0.001**[b,d]
GLS (%)	−17.9±2.1 (n=55)	−17.0±2.2 (n=23)	−16.8±2.2 (n=21)	**0.024**[d]
Elevated filling pressures	10 (17.5)	2 (8.3)	10 (45.5)	**0.002**[b,d]

Data presented as mean±SD, or n (%). Calculations with χ^2.
*Analysis of covariance (ANCOVA) adjusted for age at examination, heart rate, body mass index, diastolic blood pressure. Statistically significant values in boldface (p<0.05). Post hoc Bonferroni: [a]No significant difference between treatment groups. [b]Significant difference (<0.05) between 'high dose >300 mg/m^2' and 'low dose <300 mg/m^2'. [c]Significant difference (<0.05) between 'high dose >300 mg/m^2' and 'low dose <300 mg/m^2' with 'no anthracycline'. [d]Significant difference (<0.05) between 'high dose >300 mg/m^2' and 'no anthracycline'.
2D, two dimensions; FAC, fractional area change; GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; RA, right atrium; RIMP, Right ventricular index of myocardial performance; RV, right ventricular; RVFWS, RV-free-wall strain; RVIDd, RV-Internal Dimension in diastole; RV-s', RV-peak systolic velocity; TAPSE, tricuspid annular plane systolic excursion; TRP, tricuspid regurgitation pressure.

statistically differ in RV function, and none had RVSD (p=0.350).

Predictors of RV function

Linear regressions to identify predictors of TAPSE, RV-s' and RVFWS are presented in table 4. Assumptions for regressions were satisfied; Pearsons correlations were <0.6, tolerance was >0.6 and VIF <1.5 indicating no multicollinarity. In the multivariable analyses, LV systolic function by GLS (statistically more significant than 2D-LVEF) was identified as a strong, independent predictor for reduced RV function.

Valve disease, pulmonary hypertension and pericardial abnormalities

Trivial or mild TR was found in 82 survivors and 37 controls (79.6% vs 67.3%, p=0.087). Moderate TR was found in only five survivors (4.9% vs 0%, p=0.164), and no severe regurgitations were observed. Trivial or mild pulmonary regurgitation (PR) was found in 80 survivors and in 40 controls (78.4% vs 72.7%, p=0.422). Moderate PR was found in five survivors and in two controls (4.9% vs 3.6%, p=1.000).Severe PR was not observed. Mildly elevated PASP (≤40 mm Hg) was found in three survivors (2.9%, two with RVSD). Abnormal pericardium seen as localised fibrotic thickening without haemodynamic consequences was observed in eight (7.8%) survivors. Statistical comparisons after exclusion of these individuals did not alter the significance of the results (see online supplemental table 2). Seven cases were associated with cGVHD, and none were found in survivors with RVSD. One had a medical history of recurrent pericarditis, two were previously diagnosed by echocardiography, but none had received invasive treatment like pericardiocentesis or pericardectomy.

Measurement variability

Good image quality allowed high feasibility: FAC and TAPSE in 100%, RV-s' in 99.0%, RIMP in 97.1%, RVFWS in 92.2% and TRP in 73.8%. For intraobserver variability, the same images were analysed >6 months apart by the same observer and software, blinded to the previous result. The average value of three repeated measurements was used to calculate intraclass correlation coefficient (ICC). The ICC-type A (two-way mixed and absolute agreement) and mean difference±SD were: FAC (0.93, 1.6±0.3%), TAPSE (0.98, 0.1±0.4 cm), RV-s' (0.98, 0.1±1.7 cm/s) and RVFWS (0.94, 0.1±0.1%).

DISCUSSION

In this study, we showed that long-term survivors of allo-HSCT treated as CAYA can acquire biventricular dysfunction. To our knowledge, this is the first study assessing RV function in allo-HSCT survivors. RVSD was diagnosed in 14%, of which 93% had coexisting LVSD. LV systolic function by GLS was the strongest independent predictor of RV function.

Literature on the effects of chemotherapy on the RV is scarce, especially after HSCT. Tanindi *et al* reported on subclinical changes in RV function by echocardiography in adults, shortly after the completion of therapies including anthracyclines.[20] Christiansen *et al* found reduced RV function by echocardiography in 30% of long-term survivors of childhood lymphoma or acute leukaemia treated with anthracyclines and radiotherapies.[7] In comparison, Ylänen *et al* used cardiac magnetic resonance and detected RV impairment in 27% of long-term childhood survivors of therapies with anthracyclines.[21] The same author, later reported on reduced longitudinal function of the RV by STE in a related population.[22] The most comparable study to ours is by Murbraech *et al*, who found RVSD in 17 (6.2%) by echocardiography in 274 long-term adult survivors of lymphoma treated with anthracyclines and/or radiotherapy prior to autologous-HSCT.[6] In similarity to our findings, they found a strong association between RVSD and LVSD, and identified anthracyclines as a common risk factor.[6] In contrast, our cohort had more reduced LV function, differed in underlying diseases, treatment regimes, stem-cell origin with risk of GVHD, and were treated in their youth. These factors may explain the higher levels of RV systolic impairment and a higher prevalence of RVSD found in our cohort.

Important features of the RV include the thin myocardial wall predominantly consisting of longitudinal orientated fibres, and functioning in the low-resistance pulmonary circuit. The differences in anatomy and physiology between ventricles may lead to varying responses to stress factors such as cardiotoxic therapies. Our study found RV impairment to be milder than the effects on LV function. Disparity in ventricular dysfunction has previously been reported from other cancer survivorship studies.[6 7] Moreover, an experimental study showed daunorubicin to cause greater degrees of cellular damage in the myocardium of the LV as compared with the RV.[8]

The strong correlation between LV and RV function may be explained by common exposure to risk factors (age, cardiotoxic agents, metabolic diseases and GVHD). An alternative explanation is increased RV afterload secondary to LV failure, and/or interdependency with anatomical structures (myocardial fibres and pericardium) shared between the ventricles.[23] Arterial hypertension was found in 40% of our cohort, and can be presumed to reduce LV function by increased afterload, but have minimal direct effects on RV function. Indeed, we have previously identified arterial hypertension as a significant predictor of decreased LV function in this cohort.[5] On the other hand, pulmonary diseases may lead to increased pulmonary vascular resistance and increase RV afterload irrespective of LV function. However, neither pulmonary disease nor arterial hypertension were identified as significant predictors of RV function in this study.

The anatomical position of the RV increases its vulnerability to radiotherapy. In this cohort, very few patients were exposed to radiotherapy, and thus we could not identify radiotherapy as a predictor of cardiac dysfunction. Alkylating agents can potentially instigate cardiac

Table 4 Multivariable linear regression for prediction of right ventricular function

	TAPSE (cm)						RV-s' (cm/s)						RVFWS (%)					
	Univariable			Multivariable (n=99, r^2=0.36)			Univariable			Multivariable (n=98, r^2=0.36)			Univariable			Multivariable (n=93, r^2=0.30)		
	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value
Gender (reference=female)	0.37	−0.01 to 0.76	0.056	0.50	0.14 to 0.85	**0.007**	0.73	0.36 to 1.11	**<0.001**	0.73	0.38 to 1.08	**<0.001**	0.32	−0.06 to 0.71	0.101	0.31	−0.04 to 0.66	0.084
Age at examination (years)	0.14	−0.06 to 0.34	0.161	0.08	−0.12 to 0.27	0.447	−0.00	−0.20 to 0.20	0.984	−0.17	−0.37 to 0.02	0.079	0.06	−0.14 to 0.25	0.567	0.19	−0.00 to 0.38	0.053
Body mass index (kg/m^2)	0.19	−0.01 to 0.38	0.056	0.14	−0.8 to 0.35	0.208	0.25	0.06 to 0.44	**0.011**	0.21	0.00 to 0.43	**0.048**	−0.10	−0.32 to 0.12	0.383	−0.28	−0.50 to −0.05	**0.017**
Heart rate (bpm)	−0.25	−0.44 to −0.06	**0.011**	−0.09	−0.28 to 0.10	0.335	0.03	−0.17 to 0.23	0.782	0.14	−0.05 to 0.32	0.143	0.15	−0.04 to 0.34	0.121	0.01	−0.17 to 0.20	0.885
Anthracyclines dosage (mg/m^2)	−0.11	−0.30 to 0.09	0.270	0.07	−0.12 to 0.26	0.451	−0.23	−0.42 to −0.03	**0.022**	−0.02	−0.20 to 0.16	0.857	−0.14	−0.05 to 0.33	0.149	0.05	−0.14 to 0.23	0.620
Hypertension	−0.07	−0.41 to 0.39	0.974	–	–	–	0.10	−0.30 to 0.51	0.610	–	–	–	0.12	−0.28 to 0.52	0.556	–	–	–
Hypercholesterolaemia	−0.32	−0.88 to 0.23	0.250	–	–	–	−0.19	−0.74 to 0.37	0.503	–	–	–	0.20	−0.38 to 0.78	0.501	–	–	–
GVHD (including BOS)	−0.36	−0.76 to 0.05	0.081	−0.34	−0.72 to 0.05	0.085	−0.25	−0.66 to 0.16	0.222	−0.28	−0.65 to 0.10	0.147	−0.03	−0.44 to 0.38	0.900	−0.15	−0.54 to 0.23	0.430
GLS (%)	−0.44	−0.62 to −0.26	**<0.001**	−0.48	−0.67 to −0.29	**<0.001**	−0.27	−0.45 to −0.08	**0.006**	−0.43	−0.62 to −0.24	**<0.001**	0.45	0.27 to 0.62	**<0.001**	0.41	0.23 to 0.60	**<0.001**
2D-LVEF (%)	0.32	0.13 to 0.50	**0.001**	–	–	–	0.28	0.09 to 0.47	**0.004**	–	–	–	−0.24	−0.42 to −0.05	**0.011**	–	–	–
E/e'	0.08	−0.12 to 0.27	0.431	–	–	–	0.14	−0.06 to 0.34	0.158	0.19	0.00 to 0.38	**0.048**	−0.12	−0.33 to 0.08	0.232	–	–	–
PASP (mm Hg)	0.15	−0.05 to 0.34	0.133	0.18	−0.05 to 0.29	0.177	0.13	−0.07 to 0.32	0.191	0.04	−0.13 to 0.21	0.659	−0.04	−0.23 to 0.15	0.675	–	–	–

All continuous variables are standardised. GLS and RVFW are entered as negative values. Statistically significant values in boldface (p<0.05).
BOS, bronchiolitis obliterans syndrome; 2D, two dimensions; E/e', ratio of early-diastolic velocity to mean early-diastolic myocardial velocity; GLS, global longitudinal strain; GVHD, graft-versus-host disease; LVEF, left ventricular ejection fraction; PASP, pulmonary arterial systolic pressure; RV-e', RV early-diastolic velocity; RVFWS, RV-free-wall strain; RV-s', RV-peak systolic velocity; TAPSE, tricuspid annular plane systolic excursion.

dysfunction. In this cohort, the conditioning regimes were standardised and the effects of alkylating agents are unknown. Anthracyclines are well known to cause myocyte degradation and were administered in first-line therapies to approximately half of the survivors in this cohort. Therapies with anthracyclines are reported to increase the risk of late-onset heart failure by a fivefold in survivors treated in their youth.[24] We observed a dose-related relationship between anthracyclines and level of RV impairment as illustrated in table 3. However, in regression analyses, a significant effect from anthracyclines was absent, possibly due to the main effects being confined to the 22 survivors receiving the highest doses, and possibly due to modification by LV function in the multivariable analyses. It seems that anthracyclines (particular at higher dosages) are a catalyst for pathways leading to progressive bi-ventricular dysfunction. The disparity in ventricle dysfunction implies RV affection in survivors of allo-HSCT is not solely dependent on anthracyclines.

The main contributor to systolic function in the RV is foreshortening of the longitudinal fibres in the free-wall.[23] However, RV performance is also reliant on traction (pulling) forces generated by LV myocardium and function of the pericardium.[23] This cohort had a high percentage (approximately 45%) of mild to moderate LVSD (by LVEF or GLS). Echocardiographical parameters of right and left ventricular function showed consistent linear relationships, and GLS remained a strong predictor of reduced RV function when controlled for risk factors. Elevated LV filling pressures was found in approximately 21% of the patients, and in almost 48% of the individuals with LVSD, despite frequent use of antihypertensive and/or cardioprotective medication. E/e' was significantly higher in survivors than in controls, and there was significant correlation between E/e' and TRP; both reflecting increased RV afterload, and likely involved in the observed RV impairment.[23]

When compared to conventional therapies, survivors of HSCT have greater risk of heart disease, partially due to higher frequencies of cardiovascular risk factors and GVHD.[25 26] Traditional cardiovascular risk factors were common in this cohort and comparable to previous HSCT studies.[2–4 6] However, in this study, none of the cardiovascular risk factors were associated with RV function. This may indicate higher tolerance in the RV to effects from cardiovascular risk factors and partially explain the disparity in ventricle dysfunction. GVHD may affect cardiac function by processes of inflammation, indirectly by respiratory complications (BOS) or modification of risk factors. Neither GVHD nor BOS were identified as predictors of RV function. GVHD was associated with pericardial abnormalities that potentially can alter RV function by effects on preload and ventricular interdependency. However, repeated statistical comparisons after exclusion of cases with pericardial disease did not alter the significance of the results.

Echocardiography of the RV is technically challenging due to its position in the thorax, complex anatomy and its sensitivity to changes in load. While our definition of RVSD was highly specific it remains limited by potential inaccuracies with use of cut-off values. We observed gender differences that were not accounted by with the recommended cut-offs. Also, it is plausible that techniques for the detection of RVSD are not as accurate as those for the detection of LVSD. TAPSE and RV-s' have excellent repeatability, although are angle dependent and measure myocardial shortening in only one dimension. We found the reduction in FAC to be less significant than the drop in parameters reflecting longitudinal shortening. This is possibly due to FAC representing a composite measure of myocardial contractions in all dimensions, more analogous to 2D-LVEF, and is also influenced by the interactions between ventricles through the septum. Whereas, strain is angle independent and can measure the longitudinal myocardial fibres that constitute the majority of RV myocardium. Longitudinal shortening in the LV measured by GLS is a sensitive marker of cardiotoxicity, while LVEF drops later and represent more progressive dysfunction.[27] Similarly, RV strain has been shown to be superior in detecting subtle changes in RV function.[28 29]

Our findings showed reduced RV function to be strongly associated with progressive LVSD and elevated NT-pro-BNP. This finding is in consensus with statements that proclaim reduced RV function as a good prognostic marker.[9 10] Therefore, allo-HSCT survivors identified with reduced RV function may profit from frequent surveillance and assertive cardioprotective interventions. However, due to the infrequency and subclinical severity of RV impairments, dedicated echocardiography of the RV is most beneficial in survivors with LV dysfunction, pre-existing structural cardiac disease, respiratory complications or arrhythmias.

CONCLUSION

Long-term survivors of allo-HSCT who were treated in their youth had significantly reduced RV function as compared with a control group. This was associated with progressive left ventricle systolic dysfunction. The occurrence of RVSD was less frequent and was milder than coexisting LVSD in this cohort.

Limitations

This cross-sectional study can only describe associations. Reasons for death in individuals deceased prior to study inclusion are not available. Registry data on non-participants was restricted. The controls were recruited without known cardiovascular disease, introducing potential selection bias. Larger prospective studies are required to determine the prognostic effects in survivors with RV impairment.

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Supplement Table 1: Pearsons bivariate correlations between echocardiographical parameters of left and right ventricle function in 103 allo-HSCT survivors.

		Left ventricle			
		2D-LVEF	3D-LVEF	GLS	E/e'
Right ventricle	FAC	0.405*	0.338‡	-0.359*	-0.016
	TAPSE	0.320‡	0.352‡	-0.442*	0.078
	RV-s'	0.281‡	0.349‡	-0.277†	0.141
	RVFWS	-0.259†	-0.224†	0.477*	-0.124
	RIMP	-0.187	-0.089	-0.367*	0.137
	TRP	-0.065	-0.015	0.140	0.357‡
	E/e'	-0.087	-0.125	0.241†	-

Pearson's bivariate correlation significance: *p<0.001, ‡p<0.005, †p<0.05. Parameters of strain are negative values. 3D-LVEF (n=87), GLS (n=99), RV-s' (n=102), RIMP (n=99), RVFWS (n=95), TRP (n=76). **Abbreviations**: E/e': ratio of early-diastolic velocity to mean early-diastolic myocardial velocity, FAC: Fractional Area Change, GLS: Global Longitudinal Strain, LVEF: Left Ventricular Ejection Fraction, RIMP: Right Ventricular Index of Myocardial Performance, RVFWS: Right Ventricular Free-Wall Strain, RV-s': Right Ventricular peak systolic velocity, TAPSE: Tricuspid Annular Plane Systolic Excursion, TRP: Tricuspid Regurgitation Pressure.

Supplement Table 2: Right ventricular function in survivors and controls, excluding individuals with pericardial abnormalities (n=8).

Variable	Allo-HSCT (n=95)	Control (n=55)	p-value	Adjusted p-value*
RA morphology				
RA area (cm/m^2)	8.1 ±1.5	8.3 ±1.4	0.340	0.557
RA-EDV-index (ml/m^2)	22.4 ±6.8	22.8 ±4.9	0.729	0.978
Dilated RA-EDV [¤]	*13(13.5)*	*5(9.1)*	0.404	-
RV morphology				
RVIDd basal (cm/m^2)	2.0 ±0.3	2.0 ±0.2	0.085	0.043
RVIDd mid-wall (cm/m^2)	1.8 ±0.3	1.6 ±0.2	**0.002**	**<0.001**
RVIDs basal (cm/m^2)	1.6 ±0.2	1.5 ±0.2	0.255	0.097
RVIDs mid-wall (cm/m^2)	1.3 ±0.2	1.1 ±0.2	**<0.001**	**<0.001**
RV length (cm/m^2)	4.1 ±0.5	3.9 ±0.4	0.101	0.128
RV wall-thickness (mm)	39 ±4 (n=82)	38 ±4 (n=48)	0.132	0.198
RV-EDA (cm/m^2)	11.1 ±2.3	10.8 ±1.0	0.230	0.216
Dilated RV-EDA [§]	*31(32.6)*	*9(16.4)*	**0.030**	-
RV-ESA (cm/m^2)	6.6 ±1.4	6.2 ±1.1	0.119	**0.028**
RV function				
Fractional area change (%)	40.0 ±5.3	42.2 ±5.1	0.173	**0.049**
FAC <35%	*11(11.6)*	*2(3.6)*	0.134	-
TAPSE (mm)	20.9 ±3.8	24.6 ±3.8	**<0.001**	**<0.001**
TAPSE <17mm	*13(14.0)*	*1(1.8)*	**0.015**	-
RV s' (cm/s)	11.2 ±2.2	12.3 ±2.3	**0.007**	**0.002**
RV-s' <9.5cm/s	*23(24.5)*	*8(14.8)*	0.165	-
RV-e' (cm/s)	10.2 ±2.7	11.7 ±2.5	**0.002**	**<0.001**
RV-e' <7.8cm/s	*14(14.9)*	*1(1.9)*	**0.011**	-
RIMP	0.45 ±0.09	0.45 ±0.07	0.897	0.932
RIMP >0.54	*15(16.5)*	*3(5.6)*	0.054	-
RVFWS (%)	-27.3 ±4.2	-28.5 ±3.3	0.089	0.082
RVFWS >-20%	*3(3.4)*	*0(0)*	0.289	-
TRP (mmHg)	18.2 ±4.1	16.7 ±2.6	0.061	0.101
PASP >35mmHg	*3(3.1)*	*0(0)*	0.299	-
Tricuspid valve regurgitation	82(86.3)	37(67.3)	**0.006**	-
Mild	*77(81.1)*	*37(67.3)*	0.057	-
Moderate	*5(5.3)*	*0(0)*	0.159	-

Severe	*0(0)*	*0(0)*	-	-
Pulmonary valve regurgitation	80(85.1)	42(76.4)	0.181	-
Mild	*75(79.8)*	*40(72.7)*	0.322	-
Moderate	*5(5.3)*	*2(3.6)*	1.000	-
Severe	*0(0)*	*0(0)*	-	-
LV function				
2D-LVEF	55.1 ±6.0	59.0 ±2.9	**<0.001**	**<0.001**
GLS	-17.5 ±2.2	-19.7 ±1.4	**<0.001**	**<0.001**
E/e'	6.6 ±2.2	5.7 ±1.4	**0.006**	**0.023**

Data presented as mean ±SD or n(%),Student t-tests, Chi-square / Fishers exact tests,.* Adjusted p-values by propensity scoring. Statistically significant values in boldface ($p<0.05$). [¤] Dilated RA in males if >32ml/m^2 and in females if >27ml/m^2. [§] Dilated RV-EDA in males if >12.6cm^2/m^2 and in females if >11.5cm^2/m^2. **Abbreviations:** E/e': ratio of early-diastolic velocity to mean early-diastolic myocardial velocity, FAC: Fractional Area Change, GLS: Global Longitudinal Strain, LVEF: Left Ventricular Ejection Fraction, PASP: Pulmonary Arterial Systolic Pressure**,** RA: Right Atrium, RA-EDV: RA-End-Diastolic Volume, RIMP: Right Ventricular Index of Myocardial Performance, RV: Right Ventricular, RV-e': RV early-diastolic velocity, RV-EDA: RV-End-Diastolic Area, RV-ESA: RV-End-Systolic Area, RVFWS: RV-Free-Wall Strain, RVIDd: RV-Internal Dimension in diastole, RVIDs: RV-Internal Dimension in systole, RV-s': RV peak systolic velocity, TAPSE: Tricuspid Annular Plane Systolic Excursion, TRP: Tricuspid Regurgitation Pressure.

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RESEARCH ARTICLE

Reduced exercise capacity is associated with left ventricular systolic dysfunction in long-term survivors of allogeneic hematopoietic stem-cell transplantation

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Abstract

Purpose: Exercise intolerance is a common complication in survivors of allogeneic hematopoietic stem-cell transplantation (allo-HSCT). The aim of this study was to determine if cardiac function measured with echocardiography is associated with exercise capacity measured with cardio-pulmonary exercise tests in long-term survivors treated in their youth with allo-HSCT.

Methods: The study included 96 patients, of which 54.2% were female, aged 34.9 ± 11.6 years and 17.7 ± 9.3 years after allo-HSCT. Reduced exercise capacity was defined as <85% of predicted-peak oxygen uptake (VO_{2peak}). Linear regression was used in the prediction of VO_{2peak} (ml/kg/min). Receiver operating characteristic evaluated the accuracy of predicting reduced exercise capacity.

Abbreviations: Allo-HSCT, allogeneic hematopoietic stem-cell transplantation; ANOVA/ANCOVA, analysis of variance/covariance; AUC, area under curve; BMI, body mass index; BOS, bronchiolitis obliterans syndrome; CPET, cardio-pulmonary exercise test; CAYA, children, adolescents and young adults; FAC, fractional area change; FEV_1, forced expiratory volume in 1 s; GLS, global longitudinal strain; GVHD, graft versus-host-disease (aGVHD: acute, cGVHD: chronic); HDL, high-density lipoprotein; HR, heart rate; LV, left ventricular; LV-e', mean myocardial early diastolic velocity; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; LV-s', mean systolic myocardial velocity (average of septum and lateral annulus); LVSD, left ventricular systolic dysfunction; MAPSE, mitral annular plane systolic excursion (average of septum and lateral annulus); MV, mitral valve; MV_A, mitral valve late-diastolic wave velocity; MV_{DT}, mitral valve deceleration-time; $MV_{E/A}$, ratio of mitral valve early diastolic wave velocity (MV_E) to MV_A; PASP, pulmonary artery systolic pressure; NT-ProBNP, N-terminal pro-b-type natriuretic peptide; NYHA, New York Heart Association; RER, respiratory exchange ratio; ROC, receiver operating characteristic; RV, right ventricular; RV-s', right ventricular systolic velocity (average of septum and lateral annulus); RVFWS, right ventricular free-wall strain; RV-GLS, right ventricular global longitudinal strain; RVSD, right ventricular systolic dysfunction; SBP, systolic blood pressure; STE, speckle tracking echocardiography; TAPSE, tricuspid annulus plane systolic excursion; TRP, tricuspid regurgitation pressure; VIF, variance inflation factor.

Results: VO_{2peak} was 36.2 ± 7.7 ml/kg/min and 43 (44.8%) had reduced exercise capacity. Left ventricular ejection fraction was 55.4 ± 5.9% and global longitudinal strain (GLS) was −17.6% ± 2.0%. Left and right ventricular functions were significantly lower in survivors with reduced exercise capacity. Increased body mass index, lower physical activity score, reduced pulmonary function (by forced expiratory volume in 1-s) and reduced left ventricular systolic function (by GLS) were significant independent predictors for reduced VO_{2peak}. GLS was superior to other echocardiographical indices for identifying reduced exercise capacity (area under curve = 0.64, $p = 0.014$).

Conclusions: Left ventricular systolic dysfunction measured by GLS is associated with reduced exercise capacity in long-term allo-HSCT survivors.

KEYWORDS

cardio-pulmonary exercise test, echocardiography, global longitudinal strain, left ventricular ejection fraction, peak ventilatory oxygen-uptake, three-dimensional echocardiography

1 | INTRODUCTION

Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is increasingly being selected as a potentially curative therapy for young recipients with malignant and non-malignant disease.[1] In parallel with advances in treatment, more recipients are surviving the initial years after transplantation.[2] Consequently, increased focus has been directed towards improving quality of life by reducing therapy related complications.

A common complication in survivors of cancer therapies is exercise intolerance. The main limiting factors for exercise capacity are cardiac and pulmonary function, hematological capacity and metabolism in skeletal muscle. In long-term survivors of allo-HSCT, the risk of myocardial and pulmonary disease is elevated due to chemotherapy, high rates of cardiovascular risk factors, graft-versus-host disease (GVHD) and physical de-conditioning. A challenge for the clinician is to identify cardiac dysfunction as the cause and to promptly initiate treatment to prevent irreversible heart failure. Cardio-pulmonary exercise tests (CPET) is a widely used method to differentiate reasons for dyspnea. In addition, the acquirement of peak oxygen uptake (VO_{2peak}) provides valuable prognostic information on cardiovascular related and all-cause mortality.[3–5]

Echocardiography provides confirmation of cardiac dysfunction and insight into mechanisms for reduced exercise capacity. However, relationships between VO_{2peak} and left ventricular (LV) systolic function by echocardiography have been inconsistent. This is in part due to inadequacies with traditional measurements of left ventricular ejection fraction (LVEF). Three-dimensional (3D) imaging and global longitudinal strain (GLS) from speckle tracking echocardiography (STE) have shown to improve sensitivity in detecting subtle effects of cardiotoxicity.[6–10] As such, these methods may align better with observations of oxygen uptake. In particular, is the reported ability of GLS to predict functional capacity in patients with myocardial dysfunction.[11,12]

Previous examinations in this cohort have shown a high prevalence of left ventricular systolic dysfunction (LVSD) that is strongly associated with first-line anthracycline therapies.[13] The present study aims to determine if cardiac function measured with echocardiography is associated with exercise capacity measured with CPET, in long-term survivors of allo-HSCT treated in childhood, as adolescents or young adults (CAYA). We hypothesize that modern techniques such as 3D-LVEF or GLS are more accurate in determining relationships with the prognostic marker of VO_{2peak} (L/min/kg).

2 | METHODS

2.1 | Study design

This nationwide cross-sectional study was designed to include all survivors of allo-HSCT conducted at our institution in a multidisciplinary study investigating the long-term effects of allo-HSCT. Eligibility criteria were: Treatment at our national center for allo-HSCT, age <30 years at transplantation, age >16 years at study inclusion and >5 years follow-up time. Indications for allo-HSCT were malignant and non-malignant diseases. Survivors with Hurler syndrome were excluded due to the possibility of multi-organ pathology as part of their primary disease. Written informed consent was obtained from all participants, and the study was approved by the Regional Committee for Medical and Health Research Ethics.

2.2 | Clinical assessment

All participants underwent a medical examinations, questionnaires and blood sampling from June 2014 to February 2016. Dyspnea was classified according to the New York Heart Association (NYHA).[14] Anthracycline cumulative dosage was converted to isotoxic doses of doxorubicin.[15] Blood pressures were acquired after echocardiography (>30 min), in the supine position as the average of three measurements. Blood samples were collected after overnight fasting and analyzed at the hospital laboratory. N-terminal pro-brain-type

natriuretic peptide (NT-proBNP) concentrations were determined by an electrochemiluminescence immunoassay (Roche Diagnostics, Basel, Switzerland). The lowest detectable level was 5n/L and manufacturer's recommendations were used for classifying elevated NT-proBNP according to the age and sex specific cutoffs. Anemia was defined as reduced hemoglobin (males: <13.5 g/dL and females: <12 g/dL). Hypertension was defined as use of anti-hypertensive drugs and/or systolic blood pressure (SBP) >140 mmHg or diastolic blood pressure (DBP) >90 mmHg. Hypercholesterolemia was defined as low-density lipoprotein (LDL) >4.1 mmol/L (160 mg/dl) or use of lipid lowering medication. Diabetes mellitus was identified by hemoglobin HBA1c >6.5% (48 mmol/mol), fasting glucose ≥7.0 mmol/L or current use of glucose-lowering medication. Obesity was classified as body mass index (BMI) ≥30 kg/m^2. Acute graft-versus-host disease (aGVHD) was graded by Glucksberg scales and chronic GVHD (cGVHD) was graded by Shulman scales.[16,17]

2.3 | Physical activity

Physical activity was quantified from a self-reported questionnaire (HUNT 2/3) that has been validated against measurements of VO_{2peak}, METS calculations and international physical activity questionnaires in a comparative population.[18] This calculated physical activity during a week as the product of weighted scores for the categories of frequency (scores of 0, 0.5, 1, 2.5, 5.0 ranging from 'never' to 'almost every day'), intensity (scores of 1.0, 2.0, 3.0 ranging from 'light exercise' to 'near-exhaustion') and duration (scores of 0.1, 0.38, 0.75, 1.0 ranging for '<15 min' to '>60 min').[18] The range is 0 to 15 and higher values reflect greater weekly physical activity. An example: Exercising 2–3 times a week, for a total 60–180 min at a moderate intensity gives a physical activity score of 3.75. This scoring system generates a numerical scale that can be used to quantify the level of physical activity.

2.4 | Pulmonary function

Spirometry was conducted as recommended by European Respiratory Society.[19] Pulmonary function in this cohort has previously been described.[20] For the present study, we used forced expiratory volume in 1-s (FEV_1) to represent lung function. The rational was two-fold; FEV_1 is readily attainable, and is strongly associated with bronchiolitis obliterans syndrome (BOS), which is the most clinically relevant respiratory disorder in this cohort. Percent of predicted-FEV_1 was calculated for each individual using recommended equations that adjust for race, ethnicity, sex and height.[21] BOS was defined according to the National Institutes of Health (NIH) Consensus Criteria.[22]

2.5 | Cardio-pulmonary exercise test

CPET was conducted after echocardiography by experienced personnel at our institution. The test was performed on a treadmill (TechnoGym Runrace) using the modified Balke protocol.[23] Incremental changes in ventilatory parameters were measured at regular intervals with Vyntus-CPX (CareFusion). Predicted-VO_{2peak} and percent of predicted-VO_{2peak} were calculated for each individual based on equations that adjust VO_{2peak} by age and sex in a healthy control population.[24] Reduced exercise capacity was defined as VO_{2peak} < 85% of predicted as recommended.[25] Oxygen-pulse was calculated by dividing VO_{2peak} by maximal heart rate, and predicted oxygen-pulse by dividing percent of predicted-VO_{2peak} by maximal heart rate.[25]

2.6 | Echocardiography

Transthoracic echocardiograms were performed using Vivid-E9 scanners, M5S-D/M5Sc-D (1.5–4.6 MHz) and 4 V-D (1.5–4.0 MHz) probes and dedicated software (Echo-PAC v113.1.3; GE-Healthcare). The study followed current guidelines set by European Association of Cardiovascular Imaging and American Society of Echocardiography for the evaluation of LV and RV function, categorization of diastolic dysfunction and elevated filling pressures.[26–28] Scanner settings were optimized and measurements were averaged from a minimum of three consecutive heart cycles. All examinations were conducted and analyzed by the same experienced investigator (R.J.M), en-bloc and in random order after the last inclusion. After acquisition and prior to analyses, all echocardiograms were de-identified and the investigator was blinded to medical treatments and CPET results. Parameters of systolic, diastolic and RV function were measured at separate occasions to reduce bias. Detailed technical description of echocardiographical methods, including variability tests and findings from analyses of LV and RV function have previously been published.[13,29] Measures of diastolic function included transmitral Doppler, myocardial tissue velocities (TVI), tricuspid regurgitation peak (TRP) and left atrium volume. Longitudinal strain of the RV by STE was calculated by two methods: RV-global longitudinal strain (RV-GLS) as the average value from six segments including free-wall and septum, and RV free-wall strain (RVFWS) as the average of the three free-wall segments. Left ventricular systolic dysfunction (LVSD) was defined as reduced 2D-LVEF (male: <52% and female: <54%) and/or GLS ≥−17%.[13] Right ventricular systolic dysfunction (RVSD) was defined as reduction of at least two of the parameters: Fractional area change (FAC) <35%, tricuspid annular plane systolic excursion (TAPSE) <17 mm, RV systolic myocardial velocity (RV-s') <9.5 cm/s, RV free-wall strain (RVFWS) >−20%, and RV index of myocardial performance (RIMP) >0.54.[26] Pulmonary artery systolic pressure (PASP) was calculated from TRP using the Bernoulli equation plus right atrium pressure estimated by size and respiratory variation of the inferior vena cava.[30]

2.7 | Statistical analysis

Statistical analysis was conducted with SPSS version-25 (SPSS, Inc.) and $p < 0.05$ was considered significant. Histograms and Shapiro–Wilk test were used to assess normality. Continuous data are reported as

mean ± standard deviation or as median (25th, 75th percentile), and categorical data as numbers and percentages. Student's *t* test, One-way analysis of covariance (ANCOVA) and Mann Whitney *U* were used to compare continuous data. Chi-square and Fisher's exact test were used for comparisons of categorical data.

Cardiac function was compared in participants with normal (VO_{2peak} > 85% of predicted) verse reduced (VO_{2peak} < 85% of predicted) exercise capacity. ANCOVA was used to control for potential confounders to cardiac function created by this sub-grouping. Covariates included age at examination, BMI, heart rate (HR) and SBP. An additional supplementary analysis with ANCOVA, Kruskal–Wallis test and with Bonferroni correction was used to compare cardiac function between participants with mildly reduced (75%–85% of predicted-VO_{2peak}), moderately reduced (<75% of predicted-VO_{2peak}) and normal exercise capacity (>85% of predicted-VO_{2peak}) (Table S1).

Pearson bivariate correlations determined the presence of linear relationships. Univariable and multivariable linear regressions were used to determine significant explanatory variables for VO_{2peak} (ml/kg/min). The multivariable analysis included a priori selected variables considered as central determinants for exercise capacity, and /or variables with $p < 0.2$ in the univariable analysis. All continuous variables were standardized and values presented with beta, confidence intervals and *p* value. Assumption testing included histograms, residual plots and assessment of multi-collinearity by Pearson correlations, tolerance and variance inflation factor (VIF). Considerations were made to avoid over-fitting. Pairwise omission was chosen to handle missing data. Receiver operating characteristics (ROC) were performed and areas under the curve (AUC) calculated to test the parameter's ability to identify patients with reduced exercise capacity.

3 | RESULTS

Two-hundred and ninety patients received allo-HSCT in the time frame specified for this study. Of these, 131 (45.2%) died prior to study start and two were excluded due to incomplete patient files (Figure 1). One-hundred and fifty-seven were eligible for inclusion, of which 104 (66.2%) were examined with echocardiography, spirometry and blood tests, and 96 (61.1%) examined with CPET. Eight participants were excluded from with CPET due to musculoskeletal disorders ($n = 3$), significant systemic hypertension and reduced cardiac function ($n = 2$), suspected coronary artery disease or abnormalities ($n = 2$) and congenital aortic stenosis ($n = 1$).

3.1 | Patient characteristics

Table 1 shows patient characteristics and treatments. In the 96 survivors who completed both echocardiography and CPET: 52 (54.2%) were female, 34.9 ± 11.6 years of age at examination and with a follow-up time of 17.7 ± 9.3 years. Malignant diseases were the most common (74.0%, of which 56.3% were female) indications for allo-HSCT. In patients with malignant diseases, 43 (44.8%, 60.5% were female) received first-line therapies with anthracyclines and two (2.1%) were treated with mediastinal radiotherapy. The median isotoxic cumulative anthracycline dosage was 270 mg/m^2 and values ranged from 45 to 585 mg/m^2. The majority received standardized myeloablative conditioning regimes consisting of busulfan and cyclophosphamide. Seven survivors had mild anemia (hemoglobin >10.9 g/dL). Thirteen (13.5%) were obese (≥30 kg/m^2). Hypertension was found in 37 (38.5%) and 30 (31.3%) used anti-hypertensive medication.

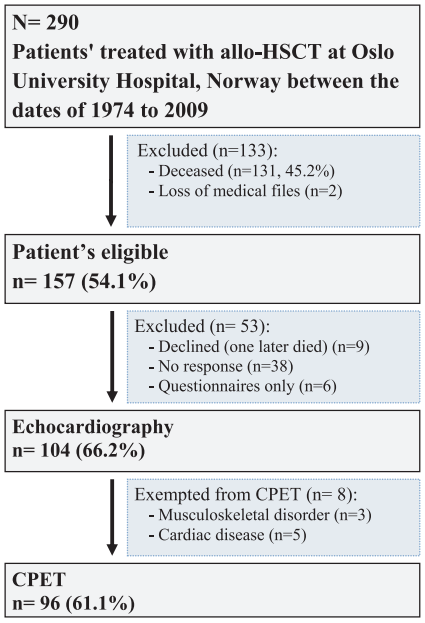

FIGURE 1 Flow chart of patient inclusion.

3.2 | Pulmonary function

FEV_1 was 3.27 ± 0.81 L/s, and percent of predicted-FEV_1 was 88.4% ± 17.8%. Percent of predicted-FEV_1 was significantly lower in survivors with BOS (56.0% ± 15.2% vs. 92.1% ± 13.9%, $p < 0.001$), anthracycline exposure (83.3% ± 18.2% vs. 92.6 ± 16.5%, $p = 0.010$) and LVSD (83.6% ± 20.1% vs. 92.2% ± 14.9%, $p = 0.018$). BOS was diagnosed in 10 (10.4%), of which five were female, eight (80%, $p = 0.012$) had cGVHD and seven had co-existing LVSD (70%, $p = 0.098$).

3.3 | CPET and exercise capacity

The main findings from CPET are summarized in Table 2. All individuals were exercised to peak effort: Borg scale ≥18 and/or respiratory exchange ratio (RER) ≥1.10. VO_{2peak} was significantly higher in males (male: 39.5 ± 6.7 ml/kg/min vs. female: 33.4 ± 7.5 ml/kg/min, $p < 0.001$). Percent of predicted-VO_{2peak} was similar between sexes (male: 85.5% ± 13.2% vs. female: 91.5% ± 21.1%, $p = 0.096$), although

TABLE 1 Survivor characteristics

Variable	All survivors ($n = 96$)
Age at allo-HSCT (years)	17.7 ± 9.3
Years to follow-up (years)	17.2 ± 5.6
Age at examination (years)	34.9 ± 11.6
Female gender	52 (54.2)
Body mass index (BMI) (kg/m^2)[a]	24.2 ± 5.1
BMI ≤18 kg/m^2	8 (8.3)
BMI ≥30 kg/m^2	13 (13.5)
Systolic blood pressure (mm Hg)[a]	121 ± 17
Diastolic blood pressure (mm Hg)[a]	71 ± 12
Heart rate (bpm)[a]	68 ± 11
Malignant/non-malignant disease	71 (74.0) / 25 (26.0)
Mediastinal radiotherapy	2 (2.1)
Anthracyclines	43 (44.8)
Cum. Anthracycline dosage (mg/m^2)	270 (140, 435)
Dosage >300 mg/m^2	20 (20.8)
Myeloablative conditioning:	94 (97.9)
Chemotherapy (Bu/Cy)	88 (91.7)
Chemotherapy + TBI	6 (6.3)
No conditioning required	2 (2.1)
Graft-versus-host disease (GVHD)	62 (64.6)
Acute GVHD	25 (26.0)
Chronic GVHD	11 (11.5)
Both	26 (27.1)
New York Heart Association (NYHA)	
Class-I	71 (74.0)
Class-II	15 (15.6)
Class-III	10 (10.4)
Class-IV	0 (0)
Physical activity	
Physical activity score	3.8 (1.5, 5.0)
Risk factors	
Hypertension	37 (38.5)
Diabetes mellitus	2 (2.1)
Hypothyroidism	8 (8.3)
Hypercholesterolemia	13 (13.7)[b]
Smoking	26 (27.1)[b]
Current	10 (10.4)
Previous	16 (16.7)
Anemia[b]	7 (7.3)
Bronchiolitis obliterans syndrome (BOS)	10 (10.4)
Left ventricular systolic dysfunction (LVSD)	42 (43.8)
Laboratory parameters	
NT-proBNP (ng/L)	48 (22, 83)
Elevated NT-proBNP[c]	14 (14.6)
High-density lipoprotein (mmol/L)	1.5 ± 0.4
Low-density lipoprotein (mmol/L)	3.0 ± 0.8[b]
Hemoglobin (g/ml)[d]	14.3 ± 1.3
P-CK-MB (μg/L)	1.5 (1.1, 2.4)
Glomerular filtration rate (<60 ml/min/1.73 m^2)	5 (5.2)
Creatinine (μmol/L)	76 (68, 89)
C-reactive protein (mg/l)[e]	1.6 (0.6, 3.1)

Note: Data presented as mean ± SD, median (25th, 75th percentiles) or number (%).
Abbreviations: NT-proBNP, N-terminal pro-brain-type natriuretic peptide; P-CK-MB, plasma-creatine kinase-myocardial band; TBI, total body irradiation.
[a]Measured at echocardiography.
[b]$n = 95$.
[c]Elevated NT-proBNP = age 18–44 years male >86 ng/L, female 18–44 ng/L > 130 ng/L; age 45–54 years male >121 ng/L, female >249 ng/L.
[d]Hemoglobin in males <13.5 g/dL and females <12 g/dL.
[e]CRP lowest recordable value was 0.6 mg/L.

significantly lower in survivors who were obese (78.4% ± 13.5% vs. 90.4% ± 18.2%, $p = 0.026$), had BOS (76.5% ± 15.0% vs. 90.2% ± 18.0%, $p = 0.023$) and treated with anthracyclines (84.7% ± 15.6% vs. 92.0% ± 19.4%, $p = 0.049$).

Forty-three (45.8%) survivors had reduced exercise capacity (VO_{2peak} < 85% of predicted). Collectively, survivors with reduced exercise capacity differed from survivors with normal exercise capacity by younger age (31.2 ± 11.8 years vs. 37.9 ± 10.6 years, $p = 0.005$), shorter follow-up time (15.2 ± 5.9 years vs. 18.8 ± 4.7 years, $p = 0.001$), sex (male: 55.8% vs. female: 44.2%, $p = 0.077$) and larger BMI (25.4 ± 6.1 kg/m^2 vs. 23.3 ± 3.9 kg/m^2, $p = 0.052$). In addition, a higher prevalence of BOS (18.6% vs. 3.8%, $p = 0.039$), LVSD (55.8% vs. 34.0%, $p = 0.032$), higher frequency of dyspnea (NYHA ≥II, 41.9% vs. 13.2%, $p = 0.001$) and trend towards lower levels of physical activity (median scores: 2.0 vs. 3.8, $p = 0.056$) were found in the group with reduced exercise capacity. Oxygen-pulse was significantly higher in males (male: 16.3 ± 4.0 ml/beat vs. female: 12.3 ± 2.4 ml/beat, $p < 0.001$), although percent of predicted equivalents of oxygen-pulse did not significantly differ (male: 84.1% ± 19.8% vs. female: 91.5% ± 19.1%, $p = 0.065$). Forty-four (44.8%) survivors had reduced oxygen-pulse (<85% of predicted oxygen-pulse).

3.4 | Echocardiography and cardiac function

Good image quality allowed high reproducibility.[13,29] 2D-LVEF was 55.4% ± 5.9% (range: 35% to 69%) and GLS was −17.6% ± 2.0% (range: −11.3% to −20.8%). LVSD was present in 42 (43.8%). Twelve (12.5% in total) had symptomatic LVSD (NYHA ≥II), of which 11 also had reduced exercise capacity. Elevated filling pressures were categorized in 21 (21.9% of total) with LVSD. RVSD occurred in 13 (11.5% of total, 11 had co-existing LVSD) and diastolic dysfunction in the absence of LVSD was present in six (6.3%). Valvular heart disease was rare and no lesions were severe. Estimated PASP was less than 37 mmHg for all individuals.

TABLE 2 Summary of CPET results for allo-HSCT survivors ($n = 96$)

Variable	Value ($n = 96$)
Peak heart rate (bpm)	181 ± 15
Oxygen-pulse (ml/beat)	14.1 ± 3.8
Percent of predicted oxygen-pulse (%)[a]	88.1 ± 19.7
<85% predicted oxygen-pulse (%)	44 (45.8%)
VO_{2peak} (l/min)	2.6 ± 6.7
VO_{2peak} (ml/kg/min)	36.2 ± 7.7
VO_{2peak} < 40 ml/kg/min	69 (66.3%)
VO_{2peak} < 30 ml/kg/min	25 (26.0%)
Percentage of predicted-VO_{2peak} (%)[a]	88.8 ± 18.1
<85% of predicted-VO_{2peak} (%)	43 (44.8%)

Note: Data presented as number (%), mean ± SD.
[a]Values adjusted by age and sex.

Tables 3 and S1 shows cardiac function by echocardiography in all participants and in the sub-groups with normal and reduced exercise capacity. The greatest levels of cardiac dysfunction corresponded to lowest levels of absolute or predicted values of oxygen uptake. Left- and right ventricular function remained significantly reduced after controlling for possible confounders. Significant group differences for LV systolic function remained after the removal of individuals with BOS (seven with co-existing LVSD). Survivors with LVSD had lower values of percent of predicted-VO_{2peak} (LVSD: 86.1% ± 18.5% vs. non-LVSD: 90.8% ± 17.7%, $p = 0.207$) and predicted oxygen-pulse (LVSD: 83.9 ± 15.3% vs. non-LVSD: 91.4% ± 22.1%, $p = 0.050$). A diagnosis of LVSD increased the likelihood of reduced VO_{2peak} (OR 2.5, 95% CI 1.0 to 5.2, $p = 0.032$). Survivors with 2D-LVEF <50% had lower percent of predicted-VO_{2peak} (80.0% ± 11.7% vs. 90.2% ± 18.7%, $p = 0.050$) and trend towards reduced percent of predicted oxygen-pulse (79.3% ± 14.0% vs. 89.6% ± 20.2%, $p = 0.071$).

3.5 | Predictors of VO_{2peak}

VO_{2peak} (ml/kg/min) significantly correlated with BMI ($R = -0.45$, $p < 0.001$), oxygen-pulse ($R = 0.43$, $p < 0.001$), mean myocardial early-diastolic velocity (LV-e', $R = 0.37$, $p < 0.001$), FEV_1 ($R = 0.35$, $p < 0.001$), physical activity score ($R = 0.32$, $p < 0.001$), age at examination ($R = -0.32$, $p = 0.002$), transmitral deceleration-time (MV_{DT}, $R = -0.31$, $p = 0.002$), ratio of early and late transmitral velocity ($MV_{E/A}$, $R = 0.27$, $p = 0.007$) and ratio of early diastolic filling velocity and *e*' (*E*/*e*', $R = 0.25$, $p = 0.016$). Regression analysis for the prediction of VO_{2peak} is presented in Table 4. Assumptions for regression were satisfied: Pearson's correlations <0.5, tolerance >0.6 and VIF <1.5 indicating limited risk of multi-collinearity. Anthracyclines were negatively correlated with both cardiac and pulmonary function and hence not included in the multivariable model. *E*/*e*' was considered the most representative parameter for elevated filling pressures. Permutations with different indices of LV systolic and RV function were tested. LV systolic function represented by GLS ($\beta = -0.18$, $p = 0.030$), but not 3D-LVEF ($\beta = 0.14$, $p = 0.122$) or 2D-LVEF ($\beta = 0.09$, $p = 0.305$) was found to predict VO_{2peak}. Unadjusted FEV_1 and BOS as a categorical variable (also significant) were tested separately to control for effects of pulmonary diseases. In the final prediction model, the variables of sex, BMI, physical activity score, GLS and FEV_1 were found to be significant independent predictors for VO_{2peak}.

ROC analysis showed percent of predicted-FEV_1 (but not unadjusted values of FEV_1) and GLS to have similar and fair abilities to correctly identify survivors with reduced exercise capacity: Percent of predicted-FEV_1 (AUC 0.66, 95% CI: 0.55–0.77, $p = 0.007$) and GLS (AUC 0.64, 95% CI: 0.53–0.75, $p = 0.014$). Other parameters of cardiac function failed to significantly predict survivors with reduced exercise capacity. A GLS cut-off value of −18% gave a sensitivity of 67% and specificity of 62% for correctly identifying survivors with reduced exercise capacity. The prediction abilities increased mildly when tested with the combined probabilities of GLS and percent of predicted-FEV_1 (AUC 0.70, 95% CI: 0.59 to 0.81, $p = 0.001$).

3.6 | N-terminal pro-brain-type natriuretic peptide

Median value of NT-proBNP was 48 ng/L (range 6–668 ng/L). Elevated NT-proBNP was found in 14 (14.6%), including nine (64.3%) with impaired LV and/or RV function identified by echocardiography. NT-proBNP significantly correlated with multiple parameters related to cardiac function, most prominently: 2D-LVEF ($R = -0.38$, $p < 0.001$), TRP ($R = 0.37$, $p = 0.002$), MAPSE ($R = -0.36$, $p < 0.001$), LV-s' ($R = -0.34$, $p = 0.001$), 3D-LVEF ($R = -0.34$, $p = 0.002$, $p = 0.005$), oxygen-pulse ($R = -0.30$, $p = 0.003$), RV-GLS ($R = 0.29$, $p = 0.007$), GLS ($R = 0.26$, $p = 0.011$), *E*/*e*' ($R = 0.26$, $p = 0.012$), cardiac output ($R = -0.24$, $p = 0.022$), RV-s' ($R = -0.23$, $p = 0.024$) and FAC ($R = -0.23$, $p = 0.024$). NT-proBNP was not significantly ($p = 0.429$) associated with VO_{2peak} in the multivariable regression.

4 | DISCUSSION

In this cohort, 46% of long-term survivors of allo-HSCT who were treated as children, adolescents and young adults (CAYA) were found to have reduced exercise capacity. With modern echocardiographical techniques such as GLS, we showed that LV systolic function was a significant and independent predictor for VO_{2peak} (ml/kg/min). We are not aware of previous literature reporting similar findings in survivors of allo-HSCT.

Recently, we reported on pulmonary function in relation to cardio-respiratory fitness in this cohort.[20] We found that reduced gas diffusion (DL_{CO}), physical de-conditioning (low VO_{2peak} in absence of cardiac (LVEF) or pulmonary limitations) and low 2D-LVEF as factors associated with reduced percent of predicted-VO_{2peak}.[20] This

TABLE 3 Cardiac function by echocardiography in survivors with normal (VO_{2peak} > 85% of predicted) and reduced exercise capacity (VO_{2peak} < 85% of predicted)

Variable	All survivors	Normal exercise capacity	Reduced exercise capacity	*p* value[a]	Adjusted *p* value[b]
Number	96	53 (55.2)	43 (44.8)	–	–
VO_{2peak} (ml/kg/min) (range)	36.2 ± 7.7 (19–54)	39.4 ± 6.6 (28–54)	32.3 ± 7.2 (19–44)	**<0.001**	–
Peak heart rate (bpm)	181 ± 15	179 ± 15	183 ± 15	**0.198**	–
Oxygen-pulse (ml/beat)	14.1 ± 3.8	15.1 ± 4.1	12.9 ± 2.9	**0.003**	–
Predicted oxygen-pulse (%)	88.1 ± 19.7	96.4 ± 17.9	77.9 ± 16.8	**<0.001**	–
Anthracycline exposure	43 (44.8)	21 (39.2)	22 (51.2)	0.258	–
Anthracycline dosage (mg/m^2)	0 (0, 219)	0 (0, 207)	45 (0, 219)	0.477	–
NT-proBNP (ng/l)	48 (22, 83)	47 (27, 82)	50 (18, 93)	0.583	–
NYHA (grade ≥ II)	25 (26.0)	7 (13.2)	18 (41.9)	**0.001**	–
Systolic function					
Cardiac output (l/min)	4.77 ± 1.13 ($n = 95$)	4.70 ± 1.16 ($n = 52$)	4.86 ± 1.11	0.502	0.140
2D-LVEF (%)	55.4 ± 5.9	56.3 ± 5.5	54.3 ± 6.3	0.095	0.052
3D-LVEF (%)	54.1 ± 4.9 ($n = 83$)	55.0 ± 4.4 ($n = 49$)	52.9 ± 5.4 ($n = 34$)	0.057	**0.046**
GLS (%)	−17.6 ± 2.0 ($n = 94$)	−18.0 ± 1.9 ($n = 52$)	−17.1 ± 2.0	**0.022**	**0.043**
MAPSE (mm)	13.1 ± 2.0	13.2 ± 2.1	12.9 ± 1.9	0.373	0.124
LV-s' (cm/s)	8.1 ± 1.7	8.0 ± 1.8	8.3 ± 1.5	0.357	0.605
Diastolic function					
MV_{DT} (ms)	160 ± 39	160 ± 33	160 ± 46	0.949	0.199
$MV_{E/A}$ ratio	1.6 ± 0.8	1.7 ± 0.9	1.6 ± 0.7	0.671	0.387
e' (cm/s)	11.1 ± 3.1	11.0 ± 3.2	11.2 ± 3.0	0.695	0.299
E/*e*'	6.4 ± 2.1	6.5 ± 2.4	6.4 ± 1.7	0.870	0.867
Right ventricular function					
FAC (%)	41.1 ± 5.3	40.9 ± 5.0	41.5 ± 5.6	0.576	0.759
RVFWS (%)	−27.0 ± 4.4 ($n = 89$)	−27.4 ± 4.5 (n = 51)	−26.4 ± 4.1 ($n = 38$)	0.319	**0.041**
RV-GLS (%)	−21.8 ± 3.2 ($n = 89$)	−22.1 ± 3.3 ($n = 51$)	−21.4 ± 3.1 ($n = 38$)	0.268	**0.021**
TAPSE (mm)	20.8 ± 3.7	20.9 ± 3.8	20.7 ± 3.6	0.876	0.734
RV-s' (cm/s)	11.1 ± 2.2 ($n = 95$)	10.9 ± 2.2 ($n = 52$)	11.3 ± 2.3	0.392	0.633
TRP (mmHg)	17.7 ± 3.4 ($n = 72$)	17.8 ± 3.6 ($n = 41$)	17.6 ± 3.1 ($n = 31$)	0.749	0.822

Note: Values presented as number (%), mean ± SD (and range) or median (25th, 75th percentiles). Significant *p* values (<0.05) are in boldface.
Abbreviations: *e*', Mean myocardial early-diastolic velocity; *E*/*e*', MV_E:e' ratio; FAC, fractional area shortening; GLS, global longitudinal strain; LV, left ventricular; LVEF, LV ejection fraction; LV-s', LV systolic myocardial velocity (average of septum and lateral annulus); MAPSE, mitral annular plane systolic excursion (average of septum and lateral annulus); MV, mitral valve; MV_{DT}, MV deceleration-time; $MV_{E/A}$, ratio of MV early-diastolic wave velocity (MV_E) to MV late-diastolic wave velocity (MV_A); NT-ProBNP, N-terminal pro-b-type natriuretic peptide; RV, right ventricular; RVFWS, RV-free-wall strain; RV-GLS, RV-global longitudinal strain; RV-s', RV systolic velocity (average of septum and lateral annulus); TAPSE: tricuspid annulus plane systolic excursion; TRP, tricuspid regurgitation pressure.
[a]Comparison between survivors with normal and reduced exercise capacity made with Student's *t* test, Mann–Whitney *U* test, Chi-square and Fisher's exact test.
[b]ANCOVA with covariates of age at examination, BMI, heart rate at echocardiography and systolic blood pressure at echocardiography.

complimentary study differs by describing in detail cardiac function (beyond 2D-LVEF) in survivors and in those individuals found to have reduced exercise capacity.

Exercise capacity (VO_{2peak}) is affected by many non-cardiac factors that are taken into consideration in calculations of predicted values. In common with previous studies, we found sex, BMI and physical activity to be significantly associated with VO_{2peak}. Other potential factors to consider in this cohort are the temporal aspects of age at transplantation and follow-up time. Treatment at a young age may disrupt growth and development, while the long follow-up time

TABLE 4 Linear regression for the prediction of VO_{2peak} (ml/kg/min)

	Univariable			Multivariable $R = 0.752$ $r^2 = 0.565$		
Variable	***β***	**95% CI**	***p* value**	***β***	**95% CI**	***p* value**
Gender (female)	0.79	0.41–1.16	**<0.001**	0.69	0.34–1.04	**<0.001**
Age at examination (years)	−0.32	−0.51 to −0.12	**0.002**	−0.07	−0.23 to 0.10	0.427
Body mass index (kg/m^2)	−0.45	−0.64 to −0.27	**<0.001**	−0.49	−0.66 to −0.32	**<0.001**
Physical activity score	0.32	0.13–0.52	**<0.001**	0.23	0.08–0.39	**0.003**
NT-proBNP (ng/L)	−0.19	−0.39 to 0.01	0.065	−0.07	−0.24 to 0.10	0.429
Pulmonary function						
FEV_1 (l)	0.35	0.16–0.55	**<0.001**	0.22	0.06–0.39	**0.010**
Predicted-FEV_1 (%)	0.13	−0.08 to 0.33	0.215			
BOS (yes/no)	−0.51	−1.17 to 0.15	0.128			
Cardiac function						
2D-LVEF (%)	0.03	−0.17 to 0.24	0.748			
3D-LVEF (%)	0.08	−0.14 to 0.30	0.449			
GLS (%)	−0.17	−0.37 to 0.04	0.113	−0.18	−0.33 to −0.02	**0.030**
$MV_{E/A}$	0.27	0.08–0.47	**0.007**			
E/*e*'	−0.25	−0.44 to −0.05	**0.016**	0.03	−0.13 to 0.20	0.704
RV-s' (cm/s)	0.13	−0.07 to 0.34	0.199			
RVFWS (%)	−0.04	−0.26 to 0.17	0.696			
PASP (mmHg)	−0.01	−0.21 to 0.20	0.946			

Notes: All continuous variables (including dependent and independent) are standardized. GLS and RVFWS are fitted as negative values. Significant *p* values (<0.05) are in boldface.

Abbreviations: BOS, bronchiolitis obliterans syndrome; FEV_1, forced expiratory volume in 1 s; GLS, global longitudinal strain; LVEF, LV ejection fraction; $MV_{E/A}$, ratio of early-diastolic wave velocity (MV_E) to late-diastolic wave velocity (MV_A); PASP: peak pulmonary artery systolic pressure; RVFWS, right ventricular free-wall strain. RV-s', right ventricular systolic myocardial velocity (average of septum and lateral annulus).

prolongs de-conditioning, increases the risk of accumulating cardiovascular risk factors and promotes sedentary lifestyles. However, it should be recognized that this cohort represents survivors that have evaded the most serious short-term complications and have benefited from long recovery times. Hence, follow-up regimes in young recipients of allo-HSCT should encourage healthy lifestyles and include exercise rehabilitation.

A distinct feature of this cohort was the complex and unique set of risk factors for reduced oxygen uptake. Deciphering the effects related to cardiac dysfunction was compounded by co-existing pulmonary disorders. The results showed cardiac and pulmonary dysfunction to have similar levels of association with reduced exercise capacity. Cardiac dysfunction as an explanatory factor was supported by corresponding reductions in oxygen-pulse. The presumed causes for cardiac dysfunction are myocardial impairment and reduced contractility induced by anthracyclines, and the secondary effects from cardiovascular risk factors such as increased afterload due to hypertension. Pulmonary dysfunction may have arisen from complications from chemotherapy, and/or the effects of BOS secondary to chronic GVHD. These findings illustrate the importance of considering both heart and lung dysfunction as reasons for exercise intolerance in survivors of allo-HSCT.

Echocardiography performed at rest is a surrogate measure of actual myocardial function during exercise. However, multiple studies (mostly unrelated to cardiotoxic therapies) have shown relationships between functional capacity and echocardiographical measurements of systolic function,[11,12,31] diastolic function[32,33] and RV function.[34,35] In this cohort, we found multiple and overlapping phenotypes of cardiac dysfunction. Most common (44%) were cases of mild to moderate LVSD that were strongly associated with RVSD.[13] A categorization of LVSD gave a significant increase in risk (OR 2.5) for reduced exercise capacity. Arguably, clinically relevant reductions in oxygen uptake were reserved to cases with readily identifiable cardiac dysfunction (2D-LVEF <50%). Comparisons also showed RV function (RV strain) to be lower in survivors with reduced exercise capacity. However, the effects of reduced RV function were conditional to the presence of co-existing LVSD and were not significant enough to independently predict VO_{2peak} alone. Elevated filling pressures were found in approximately half of the cases with LVSD. The consequences of elevated filling pressures in this study are not entirely known. Parameters of diastolic function (measured at rest) were significantly correlated with VO_{2peak}, however were not significant in multivariable regressions. This may be due to interactions with factors of BMI, age and blood pressure.

In similarity with the larger St. Jude Lifetime cohort by Ness et al., that examined long-term (≥10 years) adult survivors of childhood cancer, we also found GLS to be superior to 2D-LVEF (and also 3D-LVEF in our study) for predicting exercise capacity.[12] A possible explanation for 2D-LVEF inability to establish associations with VO_{2peak} may in part be due to geometric assumptions used in its calculation. The higher sensitivity of GLS to detect mild reductions due to cardiotoxicity, better reproducibility and lower variability compared to 2D or 3D-LVEF are other possible reasons.[6–9] It is also likely that the level of association between parameters of systolic function and exercise capacity is underestimated in our study due to exclusion of several patients with heart disease for CPET.

This study also included the biomarker NT-proBNP owing to its moderate ability in predicting VO_{2peak} in patients with heart failure.[36] Most instances of elevated NT-proBNP in this cohort were found in survivors with more pronounced cardiac dysfunction. It was notable that NT-proBNP was within normal limits in many participants with cardiac dysfunction by imaging, and was not associated with VO_{2peak}.

CPET is considered the gold standard for assessing functional capacity in patients with heart disease and the assessment of VO_{2peak} provides valuable prognostic information, including all-cause mortality.[3–5] In childhood survivors of cancer with reduced exercise capacity (VO_{2peak} < 85% of predicted) the hazard rate for death increases by approximately fourfold.[12] While, a finding of VO_{2peak} > 20 ml/kg/min in patients with heart failure is considered to correspond to better short-term prognosis.[37] To our knowledge, there are no other data documenting the prognostic impact of mild or moderate reduction in exercise capacity in long-term survivors of HSCT. However, the ability to identify cardiac dysfunction as a cause for reduced exercise capacity (even if mild) has obvious medical value and potential prognostic benefits.

A limitation with CPET is its inability to distinguish specific cardiac mechanisms for reduced oxygen uptake. Thus, identifying tools that explain exercise intolerance is important for clinical decision-making. This is especially relevant in survivors of allo-HSCT with multiple comorbidities and with uncertain origins of functional dyspnea. We recommend GLS in screening of allo-HSCT survivors to confirm cardiac dysfunction (irrespective of symptoms) and to provide explanation for reduced oxygen uptake. As demonstrated in this study, GLS when measured at rest had superior ability to predict VO_{2peak} compared with NT-proBNP and other tested echocardiographical parameters. Lower measurement variability for GLS is one possible explanation for this occurrence. Moreover, GLS has been shown to have valuable prognostic capacity.[6,7,10] Our study was not designed to address mortality, although based on our findings we are supportive. Finally, the use of GLS is endorsed by experts in cardio-oncology for early detection of cardiotoxicity, which assists in earlier therapeutic interventions to hinder progressive heart disease associated with reductions in LVEF.[28,38]

5 | CONCLUSION

Reduced exercise capacity in long-term survivors of allo-HSCT treated in their youth was found to be associated with left ventricular systolic dysfunction by GLS, impaired pulmonary function by FEV_1, increased BMI and lower levels of physical activity. In conclusion, we recommend GLS for identifying and monitoring of left ventricular systolic dysfunction, and for providing an explanation for exercise intolerance in recipients of cancer related therapies.

6 | STRENGTHS AND LIMITATIONS

The strengths of this study are the nationwide inclusion, completeness in the cohort and comprehensive clinical evaluation of the participants. Interpretations from cross-sectional studies are limited by the absence of longitudinal data and the prognostic significance of findings is unknown. Echocardiography was performed at rest and considerations need to be taken when evaluating correlations with VO_{2peak}. For patient safety reasons, several participants with cardiovascular disease were exempted from CPET. This introduced potential selection bias and likely led to an underestimation of the predictive power of echocardiography and NT-proBNP on exercise capacity.

AUTHOR CONTRIBUTIONS

All stated co-authors have fulfilled the ICMJE criteria for authorship. Design and administration of the 'Norwegian allo-HSCT survivorship study' was by Ellen Ruud. Svend Aakhus made subsequent protocols for evaluation of cardiac function. Richard John Massey, Ole Henrik Myrdal, Phoi Phoi Diep, and Marta Maria Burman were responsible for data collection. Echocardiograms were performed by Richard John Massey and analyzed by Richard John Massey with guidance from Svend Aakhus. Professional advice of study methodology and interpretation of results was given by Ellen Ruud, Phoi Phoi Diep, Lars Lysgaard Gullestad, Svend Aakhus, and Jan Otto Beitnes. Jan Otto Beitnes gave supervision to study conduct. All authors contributed to reviewing and editing of the manuscript.

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DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due restrictions set by Norwegian Regional Committee for Medical Research Ethics, but are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The Norwegian Regional Committee for Medical and Health Research Ethics (2014/370) approved this study. Informed consent was obtained from all participants. All authors have read and approved the final manuscript for publication. There are no conflicts of interest and nothing to declare.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplement Table 1: Echocardiography in survivors with normal ($V0_{2peak}$ >85% of predicted) and mildly reduced ($V0_{2peak}$: 75% to 85% of predicted) and moderately reduced ($V0_{2peak}$ <75% of predicted).

Variable	All survivors	VO_{2peak} <75% of predicted	VO_{2peak} 75-85% of predicted	VO_{2peak} >85% of predicted	p-value *	Adjusted p-value †
Number	96	20	23	53	-	-
VO_{2peak} (ml/kg/min) (range)	36.2 ± 7.7 (19 - 54)	28.0 ± 5.5 (19 - 39)	36.0 ± 6.4 (26 - 44)	39.4 ± 6.6 (28 - 54)	**<0.001 a,b**	**<0.001 a,b,c**
Peak heart rate (bpm)	181 ± 15	182 ± 16	185 ± 15	179 ± 15	0.359	-
Oxygen pulse (ml/beat)	14.1 ± 3.8	12.1 ± 3.0	13.6 ± 2.6	15.1 ± 4.1	**0.007 a**	**<0.001 a,c**
FEV_1 (l/sec)	3.3 ± 0.8	2.9 ± 0.9	3.3 ± 0.7	3.4 ± 0.8	0.088	**0.007 a**
Anthracycline exposure	43 (44.8)	12 (60.0)	10 (43.5)	21 (39.2)	0.292	-
Anthracycline dosage (mg/m^2)	0 (0, 219)	75 (0, 405)	0 (0, 205)	0 (0, 207)	0.276	-
NT-proBNP (ng/l)	48 (22,83)	71 (21, 142)	45 (14, 82)	47 (27, 82)	0.300	-
NYHA class ≥ grade II	25 (26.0)	12 (60.0)	6 (26.1)	7 (13.2)	**<0.001 a,b**	-
Physical activity score	3.8 (1.5, 5.0)	1.5 (0.2, 3.8)	3.8 (1.5, 5.6)	3.8 (1.9, 6.6)	**0.009 a,b**	-
SYSTOLIC FUNCTION						
Cardiac Output (l/min)	4.77 ± 1.13 (n=95)	4.73 ± 1.10	4.97 ± 1.13	4.70 ± 1.16 (n= 52)	0.637	0.082
Fraction Shortening (%)	31.0 ± 5.3	28.5 ± 5.6	31.9 ± 5.2	31.5 ± 5.1	0.058	0.054
2D-LVEF (%)	55.4 ± 5.9	52.3 ± 6.6	56.1 ± 5.5	56.3 ± 5.5	**0.025 a**	**0.010 a**
3D-LVEF (%)	54.1 ± 4.9 (n= 83)	50.5 ± 6.9 (n= 13)	54.3 ± 3.7 (n= 21)	55.0 ± 4.4 (n= 49)	**0.013 a**	**0.015 a**
GLS (%)	-17.6 ± 2.0 (n= 94)	-16.4 ± 2.1 (n= 19)	-17.7 ± 1.7	-18.0 ±1.9 (n= 52)	**0.006 a**	**0.010 a**
MAPSE (mm)	13.1 ± 2.0	12.2 ± 2.1	13.4 ± 1.5	13.2 ± 2.1	0.078	**0.025 a**
LV-s' (cm/s)	8.1 ± 1.7	8.1 ± 1.4	8.4 ± 1.6	8.0 ±1.8	0.525	0.540
DIASTOLIC FUNCTION						
MV_{DT} (ms)	160 ± 39	168 ± 38	154 ± 52	160 ± 33	0.465	0.147
$MV_{E/A}$ ratio	1.6 ± 0.8	1.5 ± 0.5	1.7 ± 0.8	1.7 ± 0.9	0.475	0.272
e' (cm/s)	11.1 ± 3.0	11.0 ± 3.0	11.4 ± 3.0	11.0 ± 3.1	0.822	0.454
E/e'	6.4 ± 2.1	6.0 ± 1.5	6.8 ± 1.9	6.5 ± 2.4	0.454	0.298
RIGHT VENTRICULAR FUNCTION						
FAC (%)	41.1 ± 5.3	40.0 ± 6.6	42.8 ± 4.5	40.9 ± 5.0	0.197	0.290
RVFWS (%)	-27.0 ± 4.4 (n= 89)	-25.3 ± 4.6 (n= 17)	-27.4 ± 3.6 (n= 21)	-27.4 ± 4.5 (n= 51)	0.199	**0.045 a**
RV-GLS (%)	-21.8 ± 3.2 (n= 89)	-20.5 ± 3.2 (n= 17)	-22.1 ± 2.9 (n= 21)	-22.1 ± 3.3 (n= 51)	0.184	**0.030 a**
TAPSE (mm)	20.8 ± 3.7	19.8 ± 3.8	21.6 ± 3.2	20.9 ± 3.8	0.274	0.243
RV-s' (cm/s)	11.1 ± 2.2	10.3 ± 2.6	12.2 ± 1.6	10.9 ± 2.2 (n= 52)	**0.015 b**	**0.007 b**
TRP (mmHg)	17.7 ± 3.4 (n= 72)	16.0 ± 1.9 (n= 13)	18.7 ± 3.3 (n= 18)	17.8 ± 3.6 (n= 41)	0.073	0.089

Values presented as n (% in group), mean ± SD or median (25^{th}, 75^{th} percentiles). Significant p-values (<0.05) are in boldface. * ANOVA, Kruskal-Wallis test, Chi-square test. † ANCOVA with covariates of age at examination, BMI, heart rate at echocardiography and systolic blood pressure at echocardiography.

a: Bonferroni correction: Significant difference ($p<0.05$) between (<75% of predicted-VO_{2peak}) with normal (>85% of predicted-VO_{2peak}).

b: Bonferroni correction: Significant difference ($p<0.05$) between (<75% of predicted-VO_{2peak}) and (75% to 85% of predicted-VO_{2peak}).

c: Bonferroni correction: Significant difference ($p<0.05$) between (75% to 85% of predicted-VO_{2peak}) and normal (>85% of predicted-VO_{2peak}).

Abbreviations: e': Mean myocardial early-diastolic velocity, E/e': MV_E: e' ratio, FAC: Fractional area shortening. GLS: Global longitudinal strain, LV: Left ventricular, LV-s': LV systolic myocardial velocity (average of septum and lateral annulus), LVEF: LV ejection fraction, MAPSE: Mitral annular plane systolic excursion (average of septum and lateral annulus), MV: Mitral valve, MV_{DT}: MV deceleration-time, $MV_{E/A}$: Ratio of MV early-diastolic wave velocity (MV_E) to MV late-diastolic wave velocity (MV_A), NT-ProBNP: N-terminal pro-b-type natriuretic peptide, RV: Right Ventricular, RV-s': RV systolic velocity (average of septum and lateral annulus), RVFWS: RV-free-wall strain, RV-GLS: RV-global longitudinal strain, TAPSE: Tricuspid annulus plane systolic excursion and TRP: Tricuspid regurgitation pressure.