Cardiovascular status in long term survivors of lymphomas and malignant ovarian germ cell tumors

Thesis for degree of philosophiae doctor (Ph.D.)

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LIST OF PAPERS

I  Heart Failure and asymptomatic left ventricular dysfunction in Lymphoma survivors treated with Autologous Stem Cell Transplantation – Results from a National Cross-Sectional Study

II  Valvular Dysfunction in Lymphoma Survivors treated with Autologous Stem Cell Transplantation – Results from a National Cross-Sectional Study

III  Impaired Right Ventricular Function in Long-Term Lymphoma Survivors.

IV  The impact of cisplatinum-based chemotherapy on right ventricular function and cardiovascular risk factors in female survivors after malignant germ cell cancer.
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SELECTED ABBREVIATIONS

Auto-HCT = High dose chemotherapy with autologous stem cell transplantation
HF = heart failure
HL = Hodgkins lymphoma
LVEF = left ventricular ejection fraction
LVSD = left ventricular systolic dysfunction
NHL = Non Hodgkins lymphoma
RT = radiation therapy
TAPSE = tricuspid annular plane systolic excursion
VD = valvular dysfunction
1 INTRODUCTION

1.1 General background

The disciplines of cardiology and oncology have increasingly recognized the benefits of collaboration in caring for patients exposed to cardiotoxic treatment. As a result, the medical field of cardiooncology has developed, and especially during the last decade, there has been an increased focus on research in this field. This thesis aims to expand our knowledge of late effects of cardiotoxic treatment.

With improved cancer survival and, therefore, a growing number of cancer survivors, long-term cardiovascular side effects of cancer treatment are a growing concern. Among agents used in cancer treatment, the anthracyclines (i.e doxorubicin, epirubicin, daunorubicin) used to treat hematological malignancies including lymphomas, breast cancer and sarcomas; thoracic radiation therapy used for lymphomas and breast cancer; and cisplatinum-based chemotherapy for the treatment of germ cell tumors, testicular cancer and ovarian cancer, all have a recognised potential for cardiotoxicity.

High dose chemotherapy with autologous stem cell transplantation (auto-HCT) has been a treatment option with curative intent since the mid-1980s for patients with relapsed or refractory malignant lymphomas. It is also a part of the initial treatment for selected patients at particular high-risk for relapse [1-5]. Transplantation strategies and supportive care have improved since the start of the HCT-era, resulting in increased survival rates [6]. Lymphoma patients who remain in complete remission for at least 2 years after HCT have a favourable long term prognosis, with 10 years survival rates exceeding 80 % for certain subgroups [7]. Consequently, there is a growing population of long-term lymphoma survivors after HCT and an increasing concern about long-term complications. However, knowledge of long-term adverse effects of this highly intensive treatment is sparse.
In survivors treated for testicular cancer, cisplatinum has been associated with development of premature cardiovascular disease [8-10]. In general, women have lower risk of cardiovascular disease than males. The findings from testicular cancer survivors cannot automatically be extrapolated to females, and the load of late cardiovascular effects of cisplatinum based chemotherapy in female long-term cancer survivors remains largely unknown.

On this background, we designed two national studies focusing on a broad set of late effects in lymphoma survivors after auto-HCT and in malignant ovarian germ cell tumor survivors. Eligible survivors were invited to participate in a follow-up study including clinical examinations and questionnaires, performed between 2012 and 2014.

1.2 Auto-HCT for lymphomas in Norway

In Norway, auto-HCT has been used in the treatment of lymphomas since 1987 at the Norwegian Radium Hospital and from 1996 at the four other Norwegian university hospitals [5]. Auto-HCT was initially an experimental treatment performed according to study protocols, but has gradually become the standard therapy for patients with Hodgkin’s (HL) and non-Hodgkin’s lymphoma (NHL) fulfilling specific criteria. The conditioning regimens for auto-HCT comprised fractionated total body irradiation (1.3 Gy x 2 x 5, total 13 Gy) combined with high-dose cyclophosphamide until 1995, and thereafter chemotherapy only (carmustine, etoposide, cytarabine and cyclophosmamide or carmustine, etoposide, cytarabine and melphalan). Auto-HCT involves a long period with highly intensive treatment, several days in isolation, high risk for acute toxicity, and for many patients a long recovery-period lasting months. These factors raise the question of whether survivors after auto-HCT are more prone to late adverse effects than lymphoma survivors treated with conventional chemotherapy.
1.3 Non-cancer related mortality in lymphoma survivors

Previous studies have shown that mortality is increased in HL survivors compared with the general population [11-14]. However, the mortality in HL patients more than 10 years after treatment has only been addressed in a few studies [11, 12]. These studies indicate that, contrary to common beliefs, the increased mortality rate does not subside with time. The most common causes of death in this population are relapse of HL, second cancers, cardiovascular disease and infections [11, 12]. Our group has previously demonstrated that HL survivors treated at the Norwegian Radium Hospital in the period 1971 - 1991 had a 5-fold increased risk for cardiovascular death compared with age- and gender-matched controls from the general Norwegian population [15]. Mortality after treatment for NHL has been studied to a lesser degree, but data from the Childhood Cancer Survivors Study indicated that these patients also had higher death rates from solid tumors, leukemia, cardiovascular disease and pneumonia than the general US population [16].

1.4 Cardiovascular morbidity and associated risk factors in lymphoma survivors

Studies have reported that cardiovascular morbidity is the leading non-malignant cause of death in long-term survivors after HL [7]. Data regarding late cardiovascular effects after treatment for NHL are scarcer. Lymphoma survivors are at increased risk of cardiovascular complications. The best documented risk-factors are mediastinal irradiation and chemotherapy regimens comprising anthracyclines [17, 18].

1.4.1 Heart failure (HF) and left ventricular systolic dysfunction (LVSD)

HF is a serious complication of lymphoma treatment that may occur many years after completed therapy [19]. Anthracyclines and radiation therapy (RT) involving the heart, including direct and/or scattered irradiation, are important determinants for the development
Anthracyclines may induce cardiomyopathy and may further elevate the risk of coronary heart disease when combined with mediastinal radiotherapy [17, 18, 22]. Left ventricular function, assessed by ejection fraction and regional shortening parameters (strain), was found to be reduced in long-term HL survivors compared with healthy controls, and this was more pronounced in those treated with anthracyclines [23]. After treatment for HL and NHL, there is a dose-dependent risk of anthracycline-associated cardiomyopathy and congestive heart failure [18, 24]. Adult patients receiving cumulative doses of anthracyclines not exceeding 500-550 mg/m² of body surface are considered to have a relatively low risk for developing cardiomyopathy [18, 24]. However, even after treatment with anthracyclines in lower doses (median dose 300 mg/m²) for NHL, subclinical cardiomyopathy has been reported in almost one third of the patients five years or more after treatment [25].

The different conditioning regimens for auto-HCT have not been shown to be associated with the risk of subsequent HF [19, 26]. After auto-HCT, left ventricular function was found to be reduced shortly after therapy in patients treated for NHL with chemotherapy only as conditioning regimen, but had almost normalized at 3 months’ follow-up [27]. In another study, survivors of auto-HCT reported a higher prevalence of congestive HF and exercise induced shortness of breath than their siblings. The prevalence of other cardiovascular diseases, however, was comparable with that reported by the control population (siblings) [28]. Among survivors of auto-HCT, previous exposure to chest irradiation and the presence of co-morbidities were responsible for the long-term association with cardiovascular disease after auto-HCT [29]. In the same population, exposure to antracyclines and the presence of co-morbidities were associated with congestive HF occurring years (median 7.3 years) after exposure [19]. However, these studies assessed a heterogeneous patient population comprising patients with either malignant lymphomas or hematological malignancies, where 67 % were treated with auto-HCT and 33 % with
allogeneic HCT. In addition to treatment with anthracyclines and RT involving the heart, female gender, hypertension, diabetes mellitus type II, and a higher number of cycles of chemotherapy prior to HCT have been identified as risk factors for the subsequent development of HF [19, 26, 30].

Depending on the observation period from auto-HCT, the reported prevalence of overt HF in lymphoma survivors has ranged from 2 - 9 % [19, 26, 31], but the prevalence of asymptomatic left ventricular systolic dysfunction (LVSD) is unknown. Asymptomatic LVSD is found to be more prevalent than HF in the general population [32, 33]. Asymptomatic LVSD precedes the development of overt HF and is associated with increased cardiac morbidity and mortality [34]. Echocardiography may detect LVSD, and identification and early treatment of asymptomatic LVSD may prevent the development of HF.

1.4.2 Valvular dysfunction (VD) in lymphoma survivors

VD is a well known complication of mediastinal RT [35, 36]. The prevalence of VD after mediastinal RT for lymphoma varies, and ranges from 30 % to > 50 % in studies among HL-survivors observed 10 to more than 20 years after primary treatment [35, 37]. With increasing observation time, VD becomes increasingly more prevalent in HL survivors treated with mediastinal RT than in age-matched controls. For instance, aortic or mitral valve regurgitation was found in 24 % of HL survivors 10 years after mediastinal RT, and more than mild aortic regurgitation was found in 17 % [37]. In addition, thickened pericardium was found in 15 % of the HL survivors [37]. Our group has previously described the slowly evolving process where fibrosis, valve retraction and calcification due to mediastinal RT causes mainly left-sided valvular regurgitations and stenoses in HL survivors, underscoring the importance of time in VD development [38]. After 22 years of follow-up, 37 % of those with mild aortic or mitral valve regurgitation at 10-years follow-up had developed significant valvular
regurgitation [38]. Risk factors for developing VD other than mediastinal RT have not consistently been demonstrated. However, female gender and anthracycline treatment have been identified as additional risk factors for VD in HL survivors [17, 37].

Anthracyclines can induce cardiomyopathy and could potentially be associated with altered valvular function through ventricular remodelling. However, there is a paucity of data regarding valvular function in cancer survivors treated with anthracyclines without RT involving the heart. Thus it remains unclear if there is an association between treatment with anthracyclines per se and the development of VD.

1.4.3 Right ventricular function in lymphoma survivors

Multiple studies have assessed left ventricular (LV) function in lymphoma survivors after cardiotoxic treatment, showing that LV function is reduced during or shortly after treatment [39-41] as well as in long-term survivors [23]. However, less is known regarding the effects on right ventricular (RV) function. A subclinical decline in RV function has been observed shortly after completion of anthracycline-containing chemotherapy for breast cancer [42], but no available data describe RV function in adult lymphoma survivors. The lack of data regarding RV function, and the fact that RV function has not been adequately studied in cancer survivors, were acknowledged in a recent expert consensus rapport on cardiac imaging in adult patients during and after cancer therapy [43]. In the general population, RV systolic dysfunction is associated with increased mortality from systolic HF [44], but its prognostic value in cancer survivors is unknown.

1.5 Cisplatinum based chemotherapy

Cisplatinum-based chemotherapy is curative for the majority of patients with germ cell malignancies, even in the face of metastatic disease [45-48]. As this cancer most often affects
young adults, research has increasingly focused on the long-term effects of cisplatinum-based treatment. Such effects have been most thoroughly investigated in testicular cancer survivors, with data showing increased risks of metabolic syndrome [9, 49], cardiovascular morbidity [8, 10, 50] and mortality [51]. Additionally, cardiovascular dysfunction, evaluated based on LV diastolic dysfunction, has been demonstrated as early as one year after cisplatinum-based chemotherapy [52] as well as at long-term follow-up [53].

Little is known about the cardiovascular effects of cisplatinum-based chemotherapy in females treated for malignant ovarian germ cell tumors. The lack of data is probably related to the low incidence of this malignancy (0.5 cases per 100,000 womenyears) [54]. A 2004 report by de Vos et al. [55] indicated that malignant ovarian germ cell tumors survivors who had received cisplatinum-based treatment had increased risks of cardiovascular morbidity and metabolic syndrome. However, their study included only 17 patients with malignant ovarian germ cell tumor, underscoring the need for additional research within this field.
2 AIMS OF THE THESIS

2.1 General Aims:

To characterise the cardiovascular late effects of cardiotoxic treatment in lymphoma survivors treated with auto-HCT and in malignant ovarian germ cell tumor survivors.

2.2 Specific Aims:

Paper I  To determine the prevalence of LVSD, including overt HF and asymptomatic LVSD in adult lymphoma survivors after auto-HCT and to identify risk factors for LVSD in this population.

Paper II To assess the prevalence and associated risk factors for VD observed in adult lymphoma survivors after auto-HCT, and to determine whether anthracycline-containing chemotherapy alone in these patients is associated with VD.

Paper III To assess the long-term effect of cardiotoxic treatment on RV systolic function in adult lymphoma survivors treated with auto-HCT.

Paper IV To investigate the impact of cisplatinum-based chemotherapy on cardiac function and cardiovascular risk factors in female survivors after malignant ovarian germ cell tumor.
3 PATIENTS AND CONTROLS

3.1 Paper I, II and III

3.1.1 Study design

Paper I-III is based on a cross-sectional study including lymphoma survivors treated with auto-HCT in Norway. All these papers have a case-control design, with a patient:control ratio of 1:1. The median observation time from the primary diagnosis was 13 (range, 4 - 34) years.

3.1.2 Patients

Eligibility criteria for the survey were: treatment with auto-HCT for HL or NHL from 1987 until 2008; age > 18 years at auto-HCT; and being alive as of 31.12.2011. The only exclusion criterion was current treatment for relapse of lymphoma (n = 4). Other reasons for not participating in the study were emigration (n = 1), lack of present postal address (n = 3) and death between initiation of study and reception of invitation letter (n = 8). In total, 399 lymphoma survivors were invited to take part in the study. Of these, 274 (69 % of all eligible) participated in the present substudies. All examinations were performed between March 2012 and March 2014.
Figure 1. Chart of recruitment of eligible lymphoma survivors after autologous hematopoietic stem cell transplantation (auto-HCT) in Norway

728 auto-HCT for lymphoma 1987-2008

- 321 deceased
  - 12 died of cardiac disease

407 patients alive at survey (2012)

- 8 excluded
  - 3 no address
  - 1 emigrated
  - 4 relapsed disease

399 survivors invited to participate

- 124 declined/no response /did not attend
  - 1 died prior to examination

274 completed examinations
  - 188 in Oslo
  - 41 in Trondheim
  - 24 in Bergen
  - 21 in Tromsø

3.1.3 Lymphoma treatment

Information about prior cancer treatment was collected from patient’s medical records, the lymphoma registry at the Norwegian Radium Hospital and radiotherapy registries. The use of anthracyclines (i.e. doxorubicin) was registered and the total cumulative dose was calculated. Some patients also received daunorubicin, and cumulative doses were converted to
doxorubicin isotoxic doses using a conventional conversion factor of 0.83 [56]. The majority of lymphoma survivors had also received cyclofosfamide, and total cumulative doses were calculated. Furthermore, treatment with cisplatinum-containing chemotherapy and bleomycine was registered. We also registered the number of treatment lines of chemotherapy administered prior to auto-HCT. In addition, some lymphoma survivors received a mini-allogenic stem-cell transplantation post-HCT due to relapse, and these were registered.

Conditioning regimen for auto-HCT consisted of total body irradiation and high dose cyclofosfamide from 1987 until 1995. Thereafter, patients received chemotherapy conditioning only, including carmustine, etoposide, cytarabine and melphalan.

Subgroups received additional RT, and for the purpose of the present studies, RT including the mediastinum / the heart was registered (mediastinal fields, mantle field radiation and total body irradiation (13 Gy, estimated RT dose to the heart equivalent to approximately 20 Gy)). All mediastinal radiation fields were reevaluated to confirm heart involvement.

In paper I and III, the lymphoma survivors were categorized into four groups based on the median cumulative anthracycline dose and a known cut-off for high dose cardiac RT [57]: a) low dose anthracyclines (< 300mg/m²), b) higher dose anthracyclines (≥ 300mg/m²), c) anthracyclines and low dose cardiac RT (equivalent to ≤ 30Gy) and d) anthracyclines and high dose cardiac-RT (> 30Gy). Patients treated with chemotherapy only were compared with the low- and high dose cardiac RT groups in paper II.

3.1.4 Non-participating lymphoma survivors

Of all eligible lymphoma survivors, 124 (31 %) did not participate in these studies. However, the primary diagnosis (NHL or HL), gender, mean cumulative doxorubicin dose, proportion
receiving RT, age at survey or time from auto-HCT did not differ between non-participants and participants.

3.1.5 Controls

The control subjects were recruited from the third wave of the Norwegian Health Study in Nord-Trøndelag (HUNT-study) [58, 59], which includes an echocardiographic database consisting of 1266 healthy participants. Individuals already diagnosed with cardiovascular disease, hypertension or diabetes mellitus prior to inclusion were not invited to participate. Controls were matched in a 1:1 fashion based on age, gender, systolic blood pressure and body mass index. The matching variables were highly congruent between patients and controls (gender, $p = 1.0$; age, $p = 1.0$; systolic blood pressure, $p = 0.8$; and body mass index, $p = 0.26$).

3.2 Paper IV

3.2.1 Study design

This is a cross-sectional, case control study in survivors of malignant ovarian germ cell tumors treated with cisplatinum based chemotherapy in Norway. The median observation time since diagnosis was 14 years (range 5 - 31 years).

3.2.2 Patients

Eligibility criteria for the survey were: treatment for malignant ovarian germ cell tumors between 1980 and 2009; being alive and living in Norway as of June 2012; age > 18 years at survey; and a continuously disease free period of at least 3 years. Patients were identified by the Cancer Registry of Norway. All eligible patients were invited to complete a questionnaire and attend an out-patient consultation at Oslo University Hospital, performed between March
2013 and March 2014 including blood sampling and a comprehensive echocardiographic examination.

In total, 74 malignant ovarian germ cell tumors survivors (48 % of all eligible) consented to participate and met to the out-patient examination, of which 41 had received cisplatinum-based chemotherapy (62 % of eligible cisplatinum-based chemotherapy survivors). These patients were categorized into a low- and high dose cisplatinum based chemotherapy group, based on median cumulative doses of cisplatinum.

Figure 2. Flow chart of recruitment of eligible survivors after malignant ovarian germ cell tumor in Norway

MOCGT: malignant ovarian germ cell tumor; CBCT: cisplatinum-based chemotherapy
3.2.3 Cancer treatment for the malignant ovarian germ cell tumors

Surgery was the initial treatment in all cases, and we categorized surgery as fertility sparing or radical. If possible young patients (< 40 years at diagnosis) underwent fertility sparing surgery. Depending on the stage of the disease, patients received 3 or 4 cycles of standard CVB (cisplatinum, vinblastine, bleomycin), BEP (bleomycin, etoposide, cisplatinum), or EP (etoposide, cisplatinum), with cisplatin 20 mg/m² administered daily during 5-day cycles every 3rd week [60]. The total cumulative doses of cisplatinum and bleomycin for each patient were calculated.

3.2.4 Non-participating survivors

In total, 79 malignant ovarian germ cell tumor survivors (52 %) did not participate in the study. Participants differed from non-participants in terms of more often having received adjuvant chemotherapy (Figure 2), but age at survey and time since the diagnosis did not differ.

3.2.5 Controls

As a reference population for both echocardiographic parameters and laboratory measurements, 37 age-matched females without known hypertension or diabetes mellitus living in Oslo or Akershus were randomly recruited from the general Norwegian Population Register.
4 METHODS

4.1 Echocardiography

4.1.1 Echocardiographic examination

The patients in all papers were examined in the left lateral decubitant position after a minimum of 5 minutes rest, using parasternal and apical projections as recommended [61]. Ultrasound recordings were obtained using digital high-end echocardiographic scanners (Vivid 7 or e9, GE Vingmed Ultrasound, Horten, Norway) with standard settings with 2. harmonic imaging, and optimal gain and contrast. The frame rates were > 90 and > 40 frames per second during tissue Doppler recordings and grey scale imaging, respectively. We obtained at least three consecutive cine loops (> 5 in arrhythmia), which were stored for off-line analysis using dedicated software (EchoPAC version 112; GE Ultrasound).

4.1.2 Echocardiographic analyses

Left ventricular dimensions were measured by using parasternal M-mode registrations. Left ventricular mass was calculated according to the Devereux formula [62]. Left ventricular systolic function was reported as left ventricular ejection fraction (LVEF) by Simpson’s biplane rule [63].

In paper I, we defined LVSD, including asymptomatic (stage B HF) [64] as well as symptomatic HF, as an LVEF < 50 % [33], and HF was defined as recommended by the American College of Cardiology and the American Heart Association (corresponding to stage C–D HF) [64].

Left ventricular systolic function was also estimated using two dimensional speckle tracking echocardiography in the three standard apical image planes to obtain LV global peak longitudinal strain (GLS) in a 16-segment model. In paper III LV GLS above −17.0 % was taken to indicate abnormal LV systolic function [65].
Global longitudinal strain by speckle tracking was used to measure left ventricular systolic function. The upper left panel shows an apical 4 chamber view with the region of interest demarked with different colours and the strain curves for each corresponding segment are displayed in the lower panel. A bull’s-eye plot depicting left ventricular averaged peak global longitudinal strain (-19.8 % indicating normal global left ventricular systolic function) in 16 myocardial segments is shown in the upper right panel.
Left atrial volumes were measured using the area length method and the biplane rule as recommended by guidelines [63]. Diastolic parameters were recorded using conventional Doppler techniques according to current recommendations [66].

Right ventricular dimensions and functional measurements were obtained as recommended [67]. We measured tricuspid annular plane systolic excursion (TAPSE), RV fractional area change (FAC), RV peak systolic velocity at the lateral tricuspid annular plane (RV S’) by pulsed tissue Doppler imaging (TDI), and RV index of myocardial performance (RIMP) by pulsed TDI at the lateral tricuspid annulus. Peak systolic longitudinal RV strain was measured using two-dimensional speckle-tracking echocardiography [68, 69]. We estimated the RV strain (average of six segments, including the interventricular septum) and the RV free-wall strain (average of three segments, excluding the interventricular septum) in a dedicated four-chamber view.

Figure 4 RV Strain
Longitudinal strain by speckle tracking was used to measure right ventricular systolic function. The upper left panel shows an apical 4 chamber view with the region of interest demarked with different colours. The lower left panel displays the peak negative strain in each segment, whereas the right panel shows the corresponding strain curves and a time-dependent color-map of the strain throughout the right ventricular wall in this view. In this example, a study patient with pathological right ventricular function displays impaired right ventricular longitudinal strain at -14.2 %.

Based on previous studies in healthy individuals, we defined the following cut-off values as indicators for abnormality: TAPSE < 17 mm, RV FAC < 35 %, RV S’ < 9.5 cm/s, RIMP > 0.54, and absolute RV free-wall strain < 20 % [67]. Lacking a validated global definition of impaired RV systolic function, we considered RV systolic dysfunction to be present when ≥ 2 of these parameters were below the cut-points. We considered a peak velocity of tricuspid valve regurgitation of > 2.8 m/s to indicate elevated pulmonary arterial pressure [70]. The right atrium was considered enlarged when the indexed right atrial area was >10.4 cm²/m² [71]. When comparing the numbers of patients with abnormal RV and LV function, in addition to the aforementioned definitions, we also used cut-off values derived from the matched healthy control population. For the latter analyses, abnormality was defined as values below the lower limits of normal (< 2 SD) for TAPSE and LV GLS.

Valvular regurgitations were defined by visual assessment [72] in combination with Doppler echocardiography [73], and the magnitude of regurgitation graded on a scale of 0 to 3, with 0 denoting none, ≤ 1 mild, 1.5 – 2 moderate, and 2.5 – 3 severe regurgitation. The degree of valvular stenosis was evaluated in compliance with current recommendations [74]. Valvular dysfunction was defined as either of regurgitations more than mild, any stenosis or
prior valve replacement. The presence of valvular degenerative changes (i.e fibrosis and/or calcification) was registered in all valves.

A single experienced echocardiographer (Klaus Murbræch), who was blinded to patient treatment, performed all echocardiographic analyses in the cancer survivors. The control subjects in papers I-III were all examined by using a Vivid 7 scanner, whereas Vivid E9 in paper IV. All of the original recordings in the controls were re-assessed by one investigator (Klaus Murbræch).

4.1.3 Reproducibility

Based on reassessment of images in 25 randomly chosen lymphoma survivors, and expressed as intraclass correlation coefficient (ICC) with 95 % confidence intervals, TAPSE, RV GLS, RV FAC, LVEF, LV GLS and E/e’-ratio had an intraobserver variability of 0.96 (0.92 - 0.99), 0.97 (0.92 - 0.99), 0.87 (0.72 - 0.95), 0.94 (0.87 - 0.97), 0.95 (0.90 - 0.98) and 0.94 (0.86 - 0.98) (all p < 0.001), respectively.

Further, expressed as ICC (95 % confidence intervals), RV basal diameter, RV mid diameter, RV end-diastolic area, TAPSE, RV FAC, RV strain and RV free-wall strain had an interobserver variability of 0.95 (0.89 - 0.98), 0.91 (0.78 - 0.96), 0.98 (0.96 - 0.99), 0.96 (0.91 - 0.99), 0.88 (0.73 - 0.95), 0.93 (0.82 - 0.97) and 0.91 (0.79 - 0.96) (p < 0.001 for all), respectively.

4.2 Cardiopulmonary exercise test in lymphoma survivors

Maximal, upright, symptom-limited exercise testing was performed in a subset of participants (all patients in Oslo and in Trondheim) using an electrically braked ergo meter bicycle at a constant cadence of 60 rpm. The test employed an individualized, stepwise protocol, with the load incrementally increased every minute, starting at 25 or 50 Watts. Based on the patient’s
age, gender, size and physical fitness, an expected maximum load was estimated for each patient, and the protocol was chosen as to reach this load after approximately 10 minutes. The test was discontinued at patient exhaustion (defined as an inability to keep the pedaling rate steady at 60 rpm).

Simultaneous hemodynamic monitoring and gas exchange analysis were performed (Cardiovit CS-200, Schiller, Baar, Switzerland and Ganshorn PowerCube, Ganshorn, Niederlauer, Germany). Peak oxygen uptake (VO_2) was defined as the VO_2 achieved at maximum load at the end of the exercise over a period of 20 seconds. We calculated the oxygen consumption per kg/min, and then expressed the peak result as a percentage of the age, weight and gender adjusted reference values as recommended by Hansen/Wasserman et al and others [75-77].

4.3 Laboratory parameters and comorbidities

Following an overnight fast, blood samples were drawn at approximately 8 a.m prior to the echocardiographic examination in all papers. We measured blood levels of total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, glycated hemoglobin (HbA1c), N-terminal pro-brain natriuretic peptide (NT-proBNP), creatinine, hemoglobin and estradiol (only paper IV).

In the high-cisplatinum-based chemotherapy group in paper IV, one patient was diagnosed with diabetes mellitus prior to being diagnosed with malignant ovarian germ cell tumor and excluded from statistical analyses assessing the effect of treatment on HbA1c and serum glucose.
4.4 Blood pressure measurements

In all papers, systolic blood pressure and diastolic blood pressure were measured in the supine position using an oscillometric technique (Dinamap ProCare 300-Monitor, Criterion, GE Medical Systems, USA), after a minimum of 5 minutes rest. We used the mean of the three most consistent measures for analysis. We defined hypertension as either systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg or current treatment with antihypertensive agents for hypertension.

4.5 Comorbidities

In paper IV, hypercholesterolemia was defined as low-density lipoprotein ≥ 3.6 mmol/L or the use of lipid-lowering agents without known cardiovascular disease. In papers I-III, hypercholesterolemia was defined more conservatively as low-density lipoprotein ≥ 4.1 mmol/L or the use of lipid-lowering agents in patients without known cardiovascular disease. In all papers, diabetes mellitus was defined as HbA1c ≥ 6.5 %, fasting glucose ≥ 7.0 mmol/L or the use of antidiabetic medication, and thyroid disease was defined as abnormal thyroid function tests according to local laboratory cut-points, or the use of thyroid hormone replacement therapy. In paper IV, renal dysfunction was defined as estimated glomerular filtration rate < 60 ml/min/1.73m², and metabolic syndrome was defined according to the 2005 guidelines of the American Heart Association/National Heart, Lung, and Blood Institute (paper IV). [78]

4.6 Clinical assessments

All study participants underwent clinical examination and blood sampling. Their previous medical history was assessed by a structured interview, and present medication (i.e. angiotensin-converting enzyme-inhibitors, angiotensin II receptor-blockers, betablockers,
calcium channel blockers, diuretics, statins and aspirin/warfarin), was registered during the same interview performed by an experienced clinician.

4.7 Statistics

Data are presented as mean ± SD, median (ranges), or numbers (%). We used independent Student’s t-tests and one-way ANOVA to compare normally distributed data, and Mann-Whitney U test and Kruskal-Wallis tests to compare skewed data. Chi-square tests or Fischer’s exact test were used for categorical data. We used Pearson’s correlation coefficient as a measure of the relationship between patient characteristics and LV systolic function (LV GLS) and RV systolic function, respectively, among the lymphoma survivors (paper III).

Papers I and II: We used multivariable logistic regression to identify variables associated with LVSD and VD (dependent variable) in all lymphoma survivors, and also to estimate the overall risk for LVSD and VD in lymphoma survivors compared with controls.

Paper III: Linear regression analyses were used to assess differences in parameters of RV systolic function between the treatment groups and their matched controls, and also to evaluate the effects of RT involving the heart on RV systolic function, and to assess the association between peak VO$_2$ (dependent variable) and RV systolic function parameters.

Paper IV: We used linear regression analyses to assess the differences in tricuspid annular plane systolic excursion (TAPSE) among survivors. Factors that were associated with TAPSE in univariate regression analysis at a p-level < 0.15 were included in the multivariable regression model to identify independent factors associated with TAPSE.

In the control group used in papers I-III, individuals previously diagnosed with cardiovascular disease, hypertension or diabetes were not included. For methodological reasons, we applied the same criteria in the lymphoma survivors. Consequently, when
comparing patients and controls in these papers, we excluded the lymphoma survivors (n = 52) who had been diagnosed with any of these comorbidities prior to the survey.

All statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, USA). P-values \( \leq 0.05 \) were considered statistically significant.

### 4.8 Ethical considerations

The studies were approved by the South East Regional Committee for Medical and Health Research Ethics. Written informed consent was provided by all participants. Both studies were conducted according to the Declaration of Helsinki.
5 SUMMARY OF RESULTS

5.1 Paper I

We found the prevalence of LVSD in long-term lymphoma survivors to be 15.7 % (95 % confidence interval 11 – 20 %); 18.2 % in males vs. 11.5 % in females (p = 0.17 for gender difference). Twenty-nine (10.6 %) of the lymphoma survivors had overt HF, whereas 14 (5.1 %) had asymptomatic LVSD (defined as LVEF < 50 %). The lymphoma survivors had a significantly higher prevalence of LVSD than the control population (odds ratio = 6.6, 95 % confidence interval 2.5 - 17.6, p < 0.001) (Figure 5). Apart from the low anthracycline-group (< 300 mg/m²), all treatment groups had increased risk of LVSD compared with controls. The highest risk was observed in survivors treated with anthracyclines and high dose cardiac-RT (> 30 Gy) (Figure 5). We identified cardiac-RT > 30 Gy and a cumulative doxorubicin dose ≥ 300 mg/m² as independent risk factors for LVSD.
Figure 5 Odds ratios (ORs) for estimated risk of left ventricular systolic dysfunction (LVSD) in treatment groups compared with controls

ORs for LVSD according to cancer-related treatment groups, adjusted for age and gender. Controls are used as reference. Bars indicate OR. AC, anthracycline (mg/m²); CI, confidence interval; RT, radiation therapy involving the heart in Gray units. *p < 0.01 compared with controls. Lymphoma survivors already diagnosed with cardiovascular disease (i.e. heart failure, myocardial infarction or valvular replacement), hypertension or diabetes mellitus at time of survey are excluded from the comparisons with controls.

5.2 Paper II.

In paper II we focused on valvular dysfunction in the lymphoma survivors and observed a total of 82 dysfunctional valves in 61 survivors (22.3 %). The majority of dysfunctional
valves were left–sided (72%). Valvular regurgitations constituted 87 % of all valvular dysfunction. All treatment groups had significantly increased risk for valvular dysfunction compared with controls, with higher cardiotoxic burden leading to increased risk of valvular disease. In the group treated with anthracyclines without RT 16.9 % had valvular dysfunction, corresponding to a three-fold increased risk compared with controls (odds ratio = 3.3, 95 % confidence interval 1.7 - 6.2, p < 0.001). Similar results were obtained in subsequent analysis excluding lymphoma survivors priorly diagnosed with cardiovascular disease, hypertension or diabetes mellitus (odds ratio = 2.9, 95 % confidence interval 1.5 - 5.8, p = 0.002; Figure 6). In addition, degenerative changes in the aortic valve were more prevalent in the anthracycline-
group without additional RT compared with controls. Female gender, age ≥ 50 years at primary diagnosis, ≥ 3 lines of chemotherapy prior to auto-HCT, and cardiac-RT > 30 Gy were identified as independent risk factors for valvular dysfunction, whereas low dose cardiac-RT did not increase risk for valvular dysfunction.
Figure 6 Odds Ratios (ORs) for estimated risk of Valvular Dysfunction (VD) in Treatment Groups compared with Controls

ORs 95% CI                      2.4–8.0     1.5–5.8     2.4–14      6.3–41
% VD        5.9            22.3         16.9           23.7           44.7

ORs for VD according to cancer-related treatment groups. Controls are used as reference, and lymphoma survivors already diagnosed with cardiovascular disease (i.e. heart failure, myocardial infarction or valvular replacement), hypertension or diabetes mellitus at time of survey are excluded from the comparisons with controls. All comparisons are adjusted for gender and age. The presented prevalence of VD includes all the lymphoma survivors. Bars indicate OR. ACCT = anthracycline containing chemotherapy; CI = confidence interval; high RT = radiation therapy involving the heart >30 Gray; low RT = radiotherapy involving the heart ≤30 Gray. * p < 0.001 compared with controls.
5.3 Paper III

Paper III focused on RV function in lymphoma survivors. On average, all parameters of RV systolic function were impaired in lymphoma survivors compared with controls. The most pronounced difference was observed for TAPSE (22.8 ± 4.3 mm vs. 27.1 ± 4.2 mm, p < 0.001). In general, the more severe cardiotoxic treatment burden, the larger was the impairment in RV functional parameters.

After multivariable adjustments, all parameters of RV systolic function were impaired in patients treated with high-dose cardiac RT compared with patients receiving chemotherapy only. There were only minor differences between patients treated with chemotherapy only and the subgroup receiving low-dose RT.

For all indices of RV systolic function, there were larger percentages of individuals with pathological values among lymphoma survivors than among healthy controls. However, only modest proportions of patients had parameters below cut-off: ranging from 3.6 % to 12.3 %. The group treated with high-dose cardiac RT had the highest proportion of pathological values (Figure 7).
Proportions of LSs, controls, and treatment subgroups with RV systolic dysfunction, defined by ≥ 2 pathological RV systolic functional parameters (TAPSE, RV FAC, RV S', RIMP, and RV free-wall strain). Bars indicate proportions (%) with RV systolic dysfunction in all LSs with associated prevalence. For methodological reasons, comparisons between controls and the LSs are performed after excluding the LSs (n = 52) with known cardiovascular disease, hypertension or diabetes prior to study participation, and corresponding prevelences for the LSs are given in brackets. †p < 0.01 compared with controls. AC, anthracycline (in mg/m²)

Among the lymphoma survivors, LV dysfunction was more prevalent than RV dysfunction (30.8 % vs. 6.2 %, respectively; p < 0.001). Of the 17 survivors with RV systolic dysfunction, 15 (88 %) also had LV systolic dysfunction. When using cut-off values derived from matched healthy controls to define RV dysfunction (TAPSE < 19 mm) and LV dysfunction (GLS above −16.8 %), abnormal LV function remained more prevalent than abnormal RV function (27.8 % vs. 12.5 %, respectively; p < 0.001).
5.4 Paper IV

In paper IV in survivors of malignant ovarian germ cell tumors treated with cisplatinum based chemotherapy, RV function significantly differed between the high-cisplatinum based chemotherapy group and both the low-cisplatinum group and the healthy controls. Compared with the low-cisplatinum group, the patients treated with high doses of cisplatinum-based chemotherapy had significant reductions of most parameters of RV systolic function. This phenomenon was observed to an even larger extent when comparing patients treated with high cisplatinum doses and healthy controls. However, no patients had truly abnormal RV function or pulmonary hypertension.

Univariate regression analysis showed that TAPSE was significantly associated with LV myocardial contraction by GLS (p = 0.03) and cumulative dose of cisplatinum (p = 0.004). We observed a trend towards an association with current smoking (p = 0.08). These factors were included in a multivariable linear regression model, and only cumulative cisplatinum dose remained significantly associated with TAPSE (p = 0.01).

**Figure 8 Tricuspid annular plane systolic excursion (TAPSE) in patients and controls**
Representative recordings of TAPSE (mm) in patients from the high-cisplatinum based chemotherapy group (CBCT) (left), low-CBTC (middle), and controls (right). *p < 0.001 compared with both other groups.

Additionally, LV diastolic function was reduced in patients treated with high-dose cisplatinum-based chemotherapy in agreement with observations made in male cancer survivors. None of the patients had renal dysfunction or known cardiovascular disease. The prevalence of metabolic syndrome were similar in patients (15%) and controls (14%) (p = 0.89), in contrast to prior findings in men. The frequency of hypercholesterolemia and obesity were equal in patients and controls.
6 DISCUSSION

In this thesis, I have evaluated the cardiac function in lymphoma survivors and female survivors of malignant ovarian germ cell tumors. We assessed the long term effects of anthracyclines and concomitant RT and cisplatinum based chemotherapy, respectively. In both instances, we observed long term adverse effects, particularly in the lymphoma survivors, suggesting a need for targeted surveillance strategies in high risk patients.

6.1 Heart failure and left ventricular systolic dysfunction in lymphoma survivors

HF is a well known and feared complication of treatment with anthracycline containing chemotherapy. For decades, physicians have known that anthracyclines induce HF in a dose dependant manner, but uncertainties have existed with regard to the prevalence of this complication. The reported prevalence of HF in lymphoma survivors varies between studies, partly due to differences in cardiotoxic exposure, observation time and methodology [19, 26, 31, 79]. The participants in our studies did not seem to differ from those in previous reports with regard to cumulative doxorubicin doses (considered moderate), but the proportion of patients who also received cardiac radiation therapy (35 %), was higher than in previous studies [19, 26]. In addition to the longer follow-up and possibly methodological differences, this may explain why we observed a somewhat higher prevalence of HF (11 %) in our population.

In paper I, we identified both doxorubicin and radiotherapy involving the heart as risk factors for developing LVSD. Both of these factors seemed to affect the risk in a dose dependant manner with the risk being higher with doses above ≥ 300 mg/m² and > 30 Gy, respectively.

As previously mentioned, the cardiotoxic effect of doxorubicin is dose-dependent [20, 21, 26], but the pathophysiologic mechanism behind this cardiotoxicity still remains unclear.
With modern imaging techniques, the estimated cumulative incidences of HF were 5 % and 26 % after treatment with doxorubicin doses of 400 mg/m² and 550 mg/m², respectively [21]. This dose-related, as well as the timely difference in the development of HF after doxorubicin exposure [19], suggest that humans have a large biological variation in the vulnerability for doxorubicin-induced cardiotoxicity. Despite a low to moderate lifetime doxorubicin exposure, LVSD was prevalent in our cohort of lymphoma survivors. In a series of experimental studies in mice, Zhang et al. showed that topoisomerase-IIβ, a mitochondrial enzyme important for DNA metabolism, played an essential role in the doxorubin-induced cascade of reactions leading to cellular apoptosis [80]. The huge variation observed in the cardiotoxic effect of doxorubicin may be related to the natural biological variation of topoisomerase-IIβ expression in humans. This theory could explain the pathophysiologic mechanism for doxorubicin induced cardiotoxicity, but needs to be verified in future prospective studies.

LVSD comprises symptomatic HF as well as asymptomatic myocardial dysfunction. In previous literature, there is a paucity of information about the prevalence and significance of the latter category in lymphoma survivors. In our cohort, we observed a prevalence of LVSD of 15.7 % of whom one third were asymptomatic. However, there are no historical data with which to compare these numbers, and there are several considerations to be aware of when interpreting these results. We used left ventricular ejection fraction (LVEF) by echocardiography to define LVSD and set the cut-off to < 50 %. First, however, the interobserver variability in LVEF by echocardiography is known to be as high as 10 %. Secondly, in the literature there is no universally agreed-upon limit of LVEF which defines LVSD. To overcome the problem of variability in measurements of LVEF, we could have used a lower threshold, f.ex LVEF < 40 %. However, by this approach, we would probably have underestimated the true prevalence of LVSD. Furthermore, in the latest guidelines the normal values for LVEF by echocardiography have been reassessed, and the lower limit of
normal is now considered to be $LVEF = 53\%$ [67]. All in all, it seemed reasonable to use a cut-point for $LVEF < 50\%$ to define LVSD. In addition the intraobserver variability for LVEF was good.

Global longitudinal strain (GLS) has emerged as a promising and more sensitive method to describe LV systolic function. Using GLS (paper III), we observed a prevalence of LVSD of more than 30\% in the same lymphoma patients. Cut-offs to define LVSD by GLS were based on normal values reported in the literature (-17.0\%) [65], and also derived from the 1:1 matched control group (-16.8\%). Furthermore, the corresponding number of survivors with asymptomatic LVSD was above 20\% compared with 5\% when based on LVEF. Most likely, the prevalence of LVSD is somewhere in between, nevertheless, this exemplifies how important it is to consider the method used when interpreting prevalences of LVSD.

6.2 Valvular function in lymphoma survivors

The association between anthracyclines and HF is well documented, on the other hand, any association between anthracyclines and VD is less well studied. In paper II we observed that anthracyclines was associated with VD also in the absence of concomitant cardiac RT. Compared with controls, the anthracycline-group had a three-fold increased risk of VD and indications of increased aortic valve degeneration.

Although this was a cross-sectional study, without having the possibility to compare findings with echocardiograms prior to cancer treatment, our findings suggest an anthracycline-associated risk of VD independently of treatment with cardiac-RT in lymphoma survivors. There are several aspects that support this hypothesis, however, any direct causality cannot be drawn due to the cross-sectional nature of the survey. First, there was no difference between patients and controls with respect to general risk factors, such as smoking habits and hypertension. Secondly, in VD-cases, we observed a modest increase in LV volumes, whereas
LV inner dimension was borderline increased compared with patients without VD. Furthermore, we noted that degenerative changes of dysfunctional valves in the anthracyclines group was infrequent with respect to the mitral valve (n = 2). These findings indicate a mild left ventricular remodeling, a phenomenon previously observed in HL survivors [38]. However, the remodeling was probably not of clinical significance in terms of being the sole cause of VD, as demonstrated by comparable prevalence of mitral regurgitation in anthracycline-patients and controls. This supports an understanding of myocardial toxicity not being the only reason for increased valvular regurgitations in the anthracycline-group. Finally, this notion was further supported by a highly significant association between reduced LV systolic function (i.e. indicating anthracycline related cardiac dysfunction) and degenerative changes in the aortic valve in the anthracycline-group. This observation could indicate that myocardial and aortic valve injury parallel each other.

A possible underlying pathological mechanism for valvular injury by anthracyclines has not previously been reported. Chemotherapy causes direct cellular toxicity eventually leading to apoptosis in the myocardium, and maybe valvular endothelium can be affected as well causing degenerative changes. Nevertheless, future experimental and clinical research is needed to gain more knowledge on this issue.

Although it is an intriguing concept of chemotherapy induced valvular dysfunction, it still remains open what clinical significance it may impose on lymphoma survivors. All regurgitations observed in the anthracycline-group were graded as moderate. None were considered severe. Furthermore, only 3 of 23 patients in the anthracycline-group had developed aortic stenosis despite degenerative changes in the aortic valve, of whom one patient had severe stenosis. Therefore, these findings are mainly of subclinical significance at this point. Time is a pivotal factor for development of VD in HL survivors after cardiac-RT [35], as also reported in a 13 years follow-up study where one out of three moderate
regurgitations evolved into severe regurgitation [38]. However, one can not directly extrapolate data obtained from HL survivors treated with cardiac-RT into lymphoma survivors without cardiac-RT, due to possibly different pathophysiological mechanisms for the VD. Consequently, only further prospective long term follow-up can elucidate this even further.

The reported cumulative incidence of VD after cardiac-RT in lymphoma survivors varies due to differences in study design, cardiotoxic treatment burden, classification of VD and observation time [17, 35-37]. In the subgroup of lymphoma survivors treated with high dose cardiac-RT, we observed a VD-prevalence of 44.7 % at a mean follow-up of 15 years from primary treatment. Lund et al reported a VD-prevalence of 31 % in HL survivors after high dose cardiac-RT using a similar definition of VD as in the present study with a mean observation time of 9 years [37]. Our observed prevalence is somewhat higher, most likely due to longer follow-up, a more comprehensive use of anthracyclines, and participants being nearly 10 years older at survey. Further, aortic stenosis was observed in 16 % in this subgroup and highly comparable to previous findings [35].

6.3 Reasonable thresholds for adverse cardiac effects of anthracyclines and cardiac RT

There seems to be a threshold of doxorubicin exposure below which heart failure rarely occurs. Armenian et al. found that doxorubicin $\geq 250$ mg/m$^2$ was associated with a more than three-fold increased risk of development of heart failure after HCT for hematological malignancies compared with lower doses [19]. We found that the risk of left ventricular systolic dysfunction was increased to a similar degree in lymphoma survivors who had received $\geq 300$ mg/m$^2$ of doxorubicin. In concordance with previous reports [21], our results seem to indicate that a cumulative dose of 300 mg/m$^2$ can be used as a threshold when estimating the risk of left ventricular systolic dysfunction in lymphoma survivors treated with
auto-HCT. However, in our statistical model, we dichotomized doses of doxorubicin based on median values, and we did not perform a formal estimation of the optimal cut-point. Consequently, we can not claim this limit as the best threshold for evaluating risk for left ventricular systolic dysfunction after doxorubicin exposure.

In HL survivors after cardiac-RT, former reports have advocated a threshold of approximately 30 Gy, both in children [81] and adults [35, 82, 83], to avoid subsequent VD. Our observations are in accordance with this threshold, further supported by the comparable degree of degenerative valvular changes after no/low dose cardiac-RT during 18 years of follow-up, whereas significantly increased in the high dose cardiac-RT group compared with both other groups. However, our study have no evidence that supports a threshold of 30 Gy as the most robust and reliable limit when considering risk for VD. It was merely chosen as cut-off based on being defined as high dose therapy in the literature. Therefore, we can not rule out a possibility for increased risk for VD also at a lower threshold for cardiac RT.

### 6.4 Right ventricular function in lymphoma survivors

We observed impaired RV systolic function in 6.2 % of lymphoma survivors compared with 0.7 % in controls. Anthracycline-treated patients receiving high-dose cardiac RT most commonly had impaired RV systolic function, whereas not different from controls in the other subgroups. The consistent observations in all predefined treatment subgroups and across a wide range of RV function parameters strengthen the validity of our results. Previous studies have demonstrated impaired RV function during and shortly after cardiotoxic treatment, among adults as well as children. Tanidi et al. performed an echocardiographic study in anthracycline-treated breast cancer patients, and reported a subclinical decrease in RV function shortly after treatment [42]. Ylanen et al. examined teenager survivors of childhood
cancer who had received anthracycline treatment, and found that 27% had RV dysfunction as judged by decreased RV ejection fraction on magnetic resonance imaging [84].

Due to its anatomical position, the RV is susceptible to cardiotoxic effects from RT. It is well known that cardiac RT can impair LV function [35, 85]. Another study in HL survivors revealed a more reduced LV systolic function in patients receiving anthracyclines and concomitant RT than in patients receiving cardiac RT alone [23], suggesting an additive detrimental effect of anthracyclines and cardiac RT. Our results also showed negative effects of cardiac RT on all parameters of RV systolic function. Further, RV systolic function after low-dose cardiac RT was comparable with that observed after treatment with chemotherapy alone, implying a dose-related effect of cardiac RT on RV function. However, even after relevant adjustments, we can not completely rule out that differences in the cardiovascular risk profile could have influenced these results, and interpretations must be done with caution. Also, this study did not include a subgroup solely treated with cardiac RT, which would have enabled us to compare the isolated effects of cardiac RT without concomitant anthracyclines.

Overall, the proportion of patients with RV dysfunction was modest, but increased with the burden of cardiotoxic treatment. No study has to the best of our knowledge, evaluated the clinical significance of RV dysfunction in cancer survivors after cardiotoxic treatment. The majority of lymphoma survivors with RV dysfunction were asymptomatic and RV dysfunction was only observed in two patients without associated LV dysfunction. Furthermore, only a minor proportion with symptomatic heart failure also had indications of RV dysfunction. On the other hand, RV systolic function was weakly associated with peak VO₂, suggesting that echocardiographic detection of RV dysfunction among these patients may have clinical implications. However, this association disappeared when LV systolic function was introduced in the statistical model. As a result, the observation of RV dysfunction is most likely of subclinical importance, although indicating cardiotoxicity and
may precede future clinical deterioration. Further clarification of this assumption, however, demands prospective studies with long-term follow up.

6.5 Differences in right- and left ventricular function in response to cardiotoxic treatment in lymphoma survivors

There are several anatomical differences between the RV and LV. The RV consists of two muscle layers instead of three, has a larger volume and a considerably lower mass, and works against a low afterload [86]. Thus, one could expect that the RV and LV might be differently affected by cardiotoxic treatment. This was supported by observations in an experimental study that demonstrated marked asymmetry in the degree of molecular remodeling in response to chronic anthracycline treatment, with LV myocytes being more profoundly affected than RV myocytes [87]. The finding in paper III indicating that the left- and right ventricle respond differently to cardiotoxic treatment is a novel observation in a clinical setting.

While our observations suggest a global cardiotoxic effect of anthracyclines and cardiac RT as evidence for RV systolic dysfunction rather than caused by LV dysfunction, they also indicate that RV function is less influenced by these effects, even with its exposed position in the chest. Among the lymphoma survivors in our study, a higher proportion showed abnormal LV function (as judged by LV GLS) than abnormal RV function across all treatment subgroups. This observation was highly significant whether using cut-off values for LV/RV dysfunction from recommended guidelines, or the more liberal cut-off values derived from our cohort of healthy control subjects. In this perspective, it is conceivable that parameters of RV systolic function is less reliable compared with parameters of LV systolic function, and results must be interpreted with this in mind. However, considering the multitude of LV and RV functional parameters pointing concordantly in the same direction,
our findings support that the LV seems more vulnerable to cardiotoxicity than the RV, rather than it being a methodological problem.

A possible explanation to the ventricles’ discrepant responses to cardiotoxic treatment could be the differences in anatomical structure, unequal distribution of topoisomerase-IIß in myocytes in RV/LV, in combination with the LV being exposed to a much higher mechanical workload. As this remains speculative, future investigations are needed to clarify this issue.

6.6 Cardiovascular effects of cisplatinum based chemotherapy in long term female cancer survivors

In paper IV in this thesis, we introduced cisplatinum, which is another chemotherapeutic agent with a cardiotoxic potential. In female long term survivors after malignant ovarian germ cell tumor treated with cisplatinum based chemotherapy, we observed a cisplatinum-dose-dependent reduction in LV diastolic function. We also found differences in LV morphology between the high-cisplatinum group and healthy controls, with increased wall thicknesses and consequently higher LV mass index. Furthermore, we observed an impaired RV systolic function in the high-cisplatinum group, this will be discussed further separately.

Reduced LV diastolic function has been observed in several studies of male survivors after cisplatinum based chemotherapy [53, 88, 89]. In the present study, we report similar results among survivors of malignant ovarian germ cell tumor, indicating that the unfavorable effect of cisplatinum on LV diastolic function is equal across genders. Even if we observed an impaired LV diastolic function in the high-cisplatinum group compared with controls, the overall differences were but minor, and likely without significant clinical implications.

Multiple studies have shown that testicular cancer survivors are at increased risk of developing metabolic syndrome [8, 49, 90]. Similar results were observed in a study of 21 long-term female cancer survivors treated with cisplatinum based chemotherapy [55]. In
contrast, we found lipid profiles; glucose and HbA1c levels; and the frequency of metabolic syndrome, hypercholesterolemia, and obesity that were highly comparable to those in healthy controls, indicating no increased risk of metabolic syndrome in females after cisplatinum based chemotherapy. The present study used slightly different definitions of these conditions than de Vos et al. [55], making direct comparison difficult. The increased prevalence of metabolic syndrome after cisplatinum therapy in males is often discussed as a consequence of reduced testosterone levels [49, 90, 91]. Thus, the presently observed low frequency of metabolic syndrome could be related to gender differences in testosterone levels.

Male survivors after cisplatinum based chemotherapy have been reported to have increased cardiovascular morbidity [8, 50]. In females, we observed a reduction in LV diastolic function as previously reported in males. On the other hand, we did not observe an increased risk of metabolic syndrome in cisplatinum-treated survivors from malignant ovarian germ cell tumor. Consequently, women may have a lower risk of developing cardiovascular disease after cisplatinum treatment. However, the women in our survey had a median age of less than 40 years, and much longer follow-up is required to estimate their risk of clinically significant cardiovascular disease.

6.7 Impaired RV systolic function after cisplatinum based chemotherapy in females

Little is known about RV function in cancer survivors following cisplatinum treatment. We found no previous studies with which to compare our present findings of cisplatinum-dose-related impairment in RV systolic function. One recent publication evaluated RV function by magnetic resonance imaging 3 months after initiation of cisplatinum for testicular cancer, and reported a minor and statistically insignificant decrease in RV ejection fraction compared with baseline values [89]. Our present multivariate regression analysis indicates that related to the cumulative dose, cisplatinum based chemotherapy has an independent effect on RV function,
as evaluated based on TAPSE (a well-validated measure of RV function) even after adjusting for possible confounders. Nevertheless, the significance of our observed reduction in RV function is currently unclear and presumably of subclinical importance, since RV function parameters were within normal range and no patients had RV dysfunction.

The pathophysiology for the observed cisplatin-related impairment of RV function remains unclear. In contrast to observations of the effects of antracyclines in paper I and III, cisplatinum seems to affect RV function rather than LV function in females. An experimental study focusing on RV function demonstrated that RV function indices were associated with right-sided ventricular-arterial coupling rather than with RV contractility [92]. Several studies of testicular cancer survivors have reported direct vascular injury and consequent endothelial dysfunction secondary to cisplatinum treatment [88, 93]. Therefore, cisplatinum-induced alternation in arterial properties of the pulmonary vasculature could contribute to the subclinical impairment of RV systolic function in long-term survivors of malignant ovarian germ cell tumor. Bleomycin, widely used in relation to malignant germ cell tumors, has a potential for pulmonary toxicity and further development into pulmonary fibrosis, although occurring infrequently [94]. Consequently, bleomycin may have a negative effect on RV function through reduced pulmonary function. However, in a long term follow-up study in testicular cancer survivors, bleomycin did not lead to any impairment in pulmonary function [95], making it less likely to explain our findings.

Of course, we can not rule out the possibility that the findings of impaired RV function were observed by chance due to a large number of parameters analysed. On the other hand, reductions in RV parameters were demonstrated across a wide range of indices of RV systolic function, making it less likely to be random. However, it can not be completely excluded. Nevertheless, these observations warrant further studies investigating RV function in survivors exposed to cisplatinum based chemotherapy.
6.8 Methodological considerations

In papers I-III we had 69 % participation of all eligible patients, which is considered satisfactory for these types of studies. The non-participants did not differ from study subjects in terms of cardiotoxic treatment burden or relevant patient characteristics. Thus, this support the relevance of observations made in these papers, and strengthens the validity of our conclusions. In paper IV, the participation of females treated with cisplatinum based chemotherapy was 62 % of all eligible and also considered adequate. Furthermore, neither age nor observation time did differ from non-participants. In addition, both studies were nationwide and recruited patients from all regions, minimizing the risk of selection bias due to differences in populations from different geographical areas. Lack of information from all the deceased lymphoma patients (n = 321) since 1987 until 2008 impose another possible bias. However, only 12 of the 321 (3.7 %) deaths in this period were registered as caused by heart disease (Norwegian cause of death registry). We consider these numbers too low to have a relevant impact on the observations made in papers I-III. Overall, the internal validity, referring to which extent the results from both studies can be generalized to all eligible patients, was therefore considered good.

Both studies in this thesis are based on a cross-sectional design, which is suitable to estimate prevelences and detect associations. However, causality is difficult to prove due to uncertainty of the timing of different exposures and specific features/events of interest, and the fact that information is gathered at only one time point. Echocardiography was not routinely performed at lymphoma diagnosis, pre auto-HCT or at diagnosis of malignant ovarian germ cell tumor. Consequently, we can not completely exclude that pathological features involved in this thesis like LVSD, VD or RV dysfunction was already present before cardiotoxic treatment was initiated. However, the fact that cardiovascular disease is
considered a contraindication for treatment with auto-HCT, and none had been diagnosed with heart failure pre-auto-HCT, makes this unlikely.

The control group used in all the papers introduces a potential limitation due to the selection criteria used in the HUNT study (i.e. excluding those already diagnosed with cardiovascular disease (papers I-III), hypertension (all papers) or diabetes (all papers)). Consequently, in all comparisons between the lymphoma survivors and controls we excluded patients that would not have been included in the control group, securing that both patients and controls are selected under as equal criteria as possible. In this way, comparisons can be performed in a more appropriate manner, and most importantly not exaggerating the effects of cardiotoxic treatment. However, by doing this we should be aware of a potential for "inversed bias" by excluding patients who may have developed these conditions because of cancer treatment. In paper IV, we did not leave any patients out from comparisons with controls.

The small sample size in paper IV may be considered as a limitation, however the incidence of malignant ovarian germ cell tumor is very low and, thus, study samples are inherently small. We present the largest sample of long-term survivors of malignant ovarian germ cell tumor to date for investigation of the long-term impact of cisplatinum based chemotherapy on cardiac function in females. Additionally, the study was nationwide, and thus not affected by the potential biases of single-center studies. Therefore, we think these data are robust, and provide a unique insight into the long-term cardiovascular effects of cisplatinum treatment in females.

The gold standard for evaluating the RV systolic function is ejection fraction by magnetic resonance imaging, however RV ejection fraction is not possible to measure by echocardiography due to the geometrical shape of the right ventricle. Instead, echocardiography offers a set of single parameters measuring different RV functional...
characteristics, in which none are considered to define global RV dysfunction. Consequently, echocardiographic evaluation of the right ventricle is challenging. In this thesis, we observed impairment in RV systolic function in both lymphoma survivors and in survivors of malignant ovarian germ cell tumor compared with controls. In both groups of survivors, there was a consistency in the decline in multiple parameters of RV systolic function, which supports the finding of a cardiotoxic effect on the right ventricle.

Even though images were obtained by different echocardiographers, all echocardiographic images in patients and controls were obtained with the same equipment. Furthermore, all images in patients and controls were analyzed by the same investigator (Klaus Murbræch), eliminating the risk of interobserver variability affecting the results. Overall, reproducibility was good.
7 CONCLUSIONS

1. In lymphoma survivors the prevalence of HF (11 %) was more prevalent than previously reported, however, the survivors with HF were mainly mildly affected symptomatically. Severe HF was rare. In addition, asymptomatic LVSD was observed in 5 % of patients. Doxorubicin exposure > 300 mg/m² and cardiac RT > 30 Gy were identified as risk factors for LVSD. This may be helpful in the development of intensified surveillance strategies in these long term lymphoma survivors.

2. The prevalence of VD in the lymphoma survivors was 22 %, and female gender, age ≥ 50 years at primary diagnosis, ≥ 3 lines of chemotherapy prior to auto-HCT, and cardiac-RT > 30 Gy were identified as independent risk factors for VD. Anthracycline containing chemotherapy was associated with VD without concomitant cardiac RT. Furthermore, there was a highly significant association between reduced LV systolic function (i.e. indicating anthracycline related cardiac dysfunction) and degenerative changes in the aortic valve in the group treated with anthracycline containing chemotherapy without concomitant cardiac RT, implying that myocardial and aortic valve injury may parallel each other.

3. RV function was impaired in adult lymphoma survivors compared with the general population. The proportion of patients with RV systolic dysfunction was modest, but increased with the burden of cardiotoxic treatment, however most likely of subclinical importance. The right ventricle seemed less susceptible to developing systolic dysfunction than the left ventricle, implying a different response to cardiotoxic treatment.

4. A subclinical impairment in LV diastolic function and RV systolic function were observed in malignant ovarian germ cell tumor survivors exposed to high dose cisplatinum treatment.
In contrast to what have been observed in males after cisplatinum treatment, the prevalence of metabolic syndrome in females was comparable with controls.
8 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

In total, pathology in terms of HF, VD, LV or RV dysfunction was observed in 42% of the lymphoma survivors. Observations done in papers I-III are summerized in figure 9, showing a considerale amount of overlap between the pathological features observed. As demonstrated in this thesis and further illustrated in figure 9, in a subgroup of lymphoma survivors there is a large burden of cardiac adverse late effects. This especially holds true for survivors exposed to high dose radiation therapy combined with anthracyclines. Based on this thesis, intensified surveillance strategies for high risk patients should be strongly considered. These patients should attend regular controls at a cardiologist, preferably with interest within the field of cardiooncology.

An interesting observation was made when identifying a possible link between anthracycline containing chemotherapy and VD in lymphoma survivors. However, the VD observed in these patients was of subclinical importance, and even further follow-up is necessary to elucidate if it has a potential to develop into symptomatic valvular disease.

The clinical relevance of being identified with LV or RV systolic dysfunction in terms of the proportion that will develop overt HF after cardiotoxic treatment still remains an open question. This is a very important issue which hopefully can be answered in future prospective studies, both in studies starting from the time of diagnosis but also in long-term survivors. Thus, in this regard, a follow-up study in 5-10 years time in the lymphoma survivors involved in this thesis would be interesting.
Figure 9 Summary of pathological observations in the lymphoma survivors in papers I-III

The blue circle represents all the lymphoma survivors, whereas the other circles (LVSD = red, 30.8 % - VD = yellow, 22.3 % - HF = purple, 10.6 % and RVSD = green, 6.2 %) are in proportion with the whole cohort as well as with each other in terms of overlapping pathological features.

(Illustration by Øystein H. Horgmo, University in Oslo)
This thesis also showed that there seem to be no need for development of specific surveillance strategies in females exposed to cisplatinum with respect to cardiovascular function. However, even further long-term follow up is necessary to clarify this issue, especially considering the females studied being at a low risk age for cardiovascular disease.

Nevertheless, the field of cardiooncology is growing and the amount of patients exposed to cardiotoxic treatment will supposedly continue to increase in the future due to enhanced cancer survival rates. In this perspective, one could also argue for the creation of specialized cardiooncologic units in Norway, to secure optimized surveillance both during cancer treatment and at long-term follow up.
9 REFERENCES


[63] Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. 2005;18:1440-63.


