Pulmonary function and cardiorespiratory fitness in young long-term survivors of severe blood disorders treated with or without allogeneic hematopoietic stem cell transplantation

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

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Summary in Norwegian

Alvorlig hematologisk sykdom omfatter både benigne og maligne tilstander, og rammer ofte yngre personer. Akutt lymfatisk leukemi (ALL) er den hyppigst forekommende kreftsykdommen hos barn og unge, og står for 1/3 av alle krefttilfeller hos barn. I løpet av de siste tiårene har behandlingen av slike tilstander blitt dramatisk forbedret. Blodsykdommer med tidligere dårlig prognose kan helbredes, og dødelig utfall er erstattet med utsikter til langtids overlevelse. For barn og unge med leukemi er 15-års overlevelse nå mer enn 80%. Kjemoterapi, gitt som kombinasjonsregimer med ulike typer cytostatika, er hjørnesteenen i behandlingen av maligne blodsykdommer. Men kjemoterapi har bivirkninger; medikamentene er generelt lungetoxiske og kan gi både akutte, forbigående lungekomplikasjoner og permanente langtidsskader av lungene. Et stort fremskritt i behandlingen av alvorlige blodsykdommer er allogene stamcelletransplantasjon (allo-HSCT). Etter myeloablative kondisjonering (benmargsutryddende forbehandling) med cytostatika blir stamceller fra benmargen til en donor (ofte en nær slektning) tilført pasienten.


To grupper unge voksne langtidsoverlevere etter alvorlig hematologisk sykdom ble undersøkt som ledd i to store multidisiplinære prosjekter. Den ene gruppen var kurativt behandlet med cytostatika for ALL som barn, og ble identifisert via Kreftregisteret. Den andre gruppen hadde gjennomgått allo-HSCT, en behandling som i Norge er sentralisert til Oslo Universitetssykehus, Rikshospitalet. ALL-gruppen inkluderte 116 langtidsoverlevere med median alder 29 år, 5 år ved diagnose, oppfølgingsstid 23 år, 53% kvinner. Gruppen som hadde gjennomgått allo-HSCT inkluderte 103 langtidsoverlevere med median alder 35 år, 20 år ved diagnose, observasjonstid 17 år, 56% kvinner. I denne gruppen var det 75% som hadde underliggende malign blodsykdom, 45% var tidligere behandlet med ulike kjemoterapiregimer.
For begge studiegruppene med langtidsoverlevere ble det registrert demografiske og kliniske opplysninger, inkludert informasjon om livsstilsvaner som røyking og fysisk aktivitet. Deretter gjennomgikk de undersøkelser med lungefunksjonstester og kondisjonstesting. Lungefunksjonstestene omfattet spirometri, måling av statiske lungevolum og gassdiffusjonskapasitet (DL\textsubscript{CO}). Kondisjonstesting for å måle maksimalt O\textsubscript{2}-opptak (VO\textsubscript{2}peak) ble utført på ergometersykkel (ALL studien) eller tredemølle (allo-HSCT studien). Gruppen allo-HSCT gjennomgikk også høyoppløselig CT-undersøkelse av lungene (HRCT). Begge studiegruppene (ALL og allo-HSCT) ble i tillegg undersøkt med ekkokardiografi.

Gjennomsnittsverdier for lungefunksjon og kondisjonsnivå var i nedre del av normalområde for begge gruppe, sammenliknet med friske med samme alder og kjønn. I ALL-gruppen hadde 22% nedsatt (% forventet) DL\textsubscript{CO} og 42% nedsatt VO\textsubscript{2}peak. Kvinner hadde lavere DL\textsubscript{CO} enn menn, og andel kvinner med nedsatt DL\textsubscript{CO} var 34% mot 7% menn. I en multippel lineær regresjonsanalyse var redusert DL\textsubscript{CO} assosiert med hunkjønn, høy BMI og røyking. Nedsatt kondisjonsnivå var assosiert med røyking og høy BMI.

I gruppen allo-HSCT hadde 17% redusert DL\textsubscript{CO}, men her var det ingen overhyppighet hos kvinner. De som hadde fått kjemoterapi for underliggende blodsykdom før allo-HSCT hadde lavere DL\textsubscript{CO} enn de som ikke hadde fått slik behandling. En tredjedel hadde utviklet kronisk transplantat-mot-vert sykdom (cGVHD), av disse hadde 40% cGVHD i lungene, en tilstand kalt bronchiolitis obliterans syndrom (BOS). BOS defineres og graderes ut fra luftveisobstruksjon, basert på spirometri, statiske lungevolum og HRCT. Nær halvparten av langtidsoverleverne var overvektige og mer enn 2/3 oppfylte ikke kriteriene for fysisk aktivitet i dagliglivet (i henhold til definisjon og anbefalinger fra WHO). Hos 43% ble det påvist nedsatt kondisjonsnivå, signifikant assosiert med lav DL\textsubscript{CO}, lav venstre ventrikkel ejeksjonsfraksjon (EF), etablert BOS, overvekt og fysisk inaktivitet. Halvparten av de med nedsatt VO\textsubscript{2}peak var begrenset av nedsatt hjerte- og/eller lungefunksjon, mens den andre halvparten var begrenset av dårlig fysisk form (dekondisjonert). Halvparten av langtidsoverleverne hadde patologiske forandringer på HRCT. Det vanligste funnet på HRCT var luftveissykdom, men ulike interstitielle forandringer ble også hyppig påvist.

Studiene (artikkel I, II og III) har vist at nedsatt lungefunksjon og kondisjonsnivå er en vanlig senfølge hos unge langtidsoverlevere som er behandlet for alvorlig blodsykdom som barn, ungdom eller ung voksen. Reduksjon i lungefunksjon er for det meste subklinisk, men kan bli klinisk relevant hos personer som senere i livet vil trenge behandling med andre
potensielt lungetoksiske midler. I tillegg er det uvisst om en subklinisk reduksjon vil forbli uendret eller progrediere videre gjennom voksenlivet. Røyking og annen lungeskadelig eksponering (som kan unngås) må derfor sterkt frarådes. Vi anbefaler individuelt tilpasset oppfølging ved både subklinisk og klinisk manifest reduksjon i lungefunksjon. Langtidsoverlever har også økt risiko for kardiovaskulære sykdommer og sekundærcancer. En stor andel av studiedeltagerne var overvektige, fysisk inakte og hadde nedsatt kondisjonsnivå, en del var også røykere. De har også økt risiko for kardiovaskulære sykdommer og sekundærcancer. Målrettet informasjon og rådgivning om modifiserbare livsstilsfaktorer bør inngå i kontrollprogrammet på et tidlig tidspunkt, og gjentas regelmessig i den videre langtidsoppfølgingen for å forebygge utvikling av behandlingsrelaterte tilleggssykdommer.
Summary

Severe hematological diseases include benign and malignant diseases that often affect young individuals. Acute lymphoblastic leukemia (ALL) is the most common malignant disease in children and adolescents and represents 1/3 of all childhood cancers. During the last decades, the treatment of such diseases has evolved and dramatically improved survival. Hematological diseases with previously dismal prognosis have become treatable and largely curable, and fatal outcome has been replaced with the prospect of long-term survival. 15 years after diagnosis, relative survival for children and adolescents with leukemia now exceeds 80%. Multiagent chemotherapy regimen is the basis for treatment of malignant hematological diseases. However, treatment-related pulmonary complications are common after chemotherapy. Pulmonary toxicity is frequent, often serious and may include acute, transient complications as well as late permanent adverse effects. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents a major improvement in the treatment of severe hematological diseases. Myeloablative conditioning, which is the first step in allo-HSCT, includes chemotherapy that eradicates the bone marrow. Following the myeloablative treatment, stem cells from a matched donor are transferred to the patient.

Since the numbers of young, long-term survivors are increasing, treatment-related, late occurring effects have become a challenge. Several organs may be affected, and pulmonary adverse effects are among the most serious. The primary aim of the present project was to determine the extent and degree of late, treatment-related pulmonary effects in young, long-term survivors of severe hematological diseases, treated in childhood, adolescence, or young adulthood. Additional aims were to identify risk factors for impairment in pulmonary function and in cardiorespiratory fitness.

Two cohorts of young adult long-term survivors of severe hematological diseases were included in interdisciplinary studies. The first cohort had been treated for childhood ALL with chemotherapy only. The study participants were identified through the Cancer Registry of Norway. The second cohort had been treated with allo-HSCT, a treatment that in Norway is centralized to Oslo University Hospital. The ALL cohort included 116 long-term survivors (median age 29 years, at diagnosis 5 years, follow-up 23 years, 53% females) and the allo-HSCT cohort included 103 long-term survivors (median age 35 years, at diagnosis 20 years, follow-up 17 years, 56% females). In the allo-HSCT cohort, 75% had primary malignant hematological disorders of whom 45% had received chemotherapy prior to allo-HSCT.
In both study cohorts, demographic and clinical characteristics were registered, including lifestyle factors like smoking habits. In the allo-HSCT cohort, also physical activity levels were recorded. The survivors were examined with pulmonary function tests and cardiopulmonary exercise tests. The pulmonary function tests included spirometry, measurement of static lung volumes and gas diffusing capacity (DL\textsubscript{CO}). Cardiorespiratory fitness was determined by maximum oxygen uptake (VO\textsubscript{2} peak) on a bicycle ergometer in the ALL survivors and on a treadmill in the allo-HSCT survivors. The allo-HSCT survivors were also examined with high-resolution computed tomography (HRCT) of the lungs. In both study cohorts, an echocardiographic examination was done, with estimation of left ventricle ejection fraction (LVEF).

Mean pulmonary function and cardiorespiratory fitness level were above lower limit of normal in both cohorts, compared to healthy subjects with the same age and gender. In the ALL-cohort, 22% had impairments in DL\textsubscript{CO} % of predicted and 42% had impaired VO\textsubscript{2} peak. Females had lower DL\textsubscript{CO} % of predicted compared to males, and the proportion of females with impaired DL\textsubscript{CO} was 34% versus 7% in males. In a multiple linear regression model, female gender, elevated BMI and smoking were risk factors for reduced DL\textsubscript{CO}. Impaired cardiopulmonary fitness was associated with smoking and BMI. In the allo-HSCT cohort, 17% of the subjects had impaired DL\textsubscript{CO}, without any female predisposition. Survivors who had received chemotherapy prior to allo-HSCT had lower DL\textsubscript{CO} than those who had not received such treatment. Approximately one-third had developed chronic graft-versus-host disease (cGVHD), of whom 40% had pulmonary involvement, known as bronchiolitis obliterans syndrome (BOS). The diagnosis of BOS is based on an obstructive impairment determined by spirometry, static lung volumes and HRCT. The extent of BOS is determined by the degree of obstructive impairment. Almost half of the long-term survivors were overweight, and more than two-third did not fulfill the criteria for physical activity in daily life, according to the definition and recommendation of WHO. Impaired cardiorespiratory fitness was found in 43%, and associated with reduced DL\textsubscript{CO}, reduced LVEF, BOS, overweight and physical inactivity. Deconditioning and cardiopulmonary factors were equally common limitations to impairment in cardiorespiratory fitness. Nearly half of the survivors had pathological changes on HRCT. The most common finding on HRCT was airways disease, but different patterns of interstitial changes were also found.

The present studies show that impairments in pulmonary function and cardiorespiratory fitness are common in long-term survivors of severe hematological disease,
treated in childhood, adolescence, or as young adults. Although the impairments in pulmonary function are predominantly of a subclinical nature, they may progress in the survivors’ further adulthood, which is particularly important in individuals who may need new treatment with potentially lung-toxic agents. Since it is not known how pulmonary function will develop in the future - if the impairments will progress or remain unchanged – we recommend individually adapted, appropriate follow-up plans for survivors with clinical as well as subclinical impairments in pulmonary function. Several of the long-term survivors in the studies were overweight, physical inactive and had reduced cardiorespiratory fitness, and many were smokers. These factors increase the risk for cardiovascular disease and secondary cancers. We therefore recommend that targeted advice on modifiable lifestyle factors should be included in follow-up programs for long-term survivors. In order to prevent treatment-related additional disorders, advice should be given at an early stage and be repeated throughout the follow-up period.
List of papers


All publishers (Taylor & Francis, Springer, Karger) permit use of papers in a work that is not to be published commercially.
### Selected abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ALL</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Allo-HSCT</td>
<td>Allogeneic hematological stem cell transplantation</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myeloblastic leukemia</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BOS</td>
<td>Bronchiolitis obliterans syndrome</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic myelogenous leukemia</td>
</tr>
<tr>
<td>CPET</td>
<td>Cardiopulmonary exercise test</td>
</tr>
<tr>
<td>DL\textsubscript{CO}</td>
<td>Gas diffusing capacity for carbon monoxide</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ECSC</td>
<td>European Community for Steel and Coal, reference values</td>
</tr>
<tr>
<td>ERS</td>
<td>European Respiratory Society</td>
</tr>
<tr>
<td>FEV\textsubscript{1}</td>
<td>Forced expiratory volume in 1 sec</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GLI</td>
<td>Global Lung Initiative</td>
</tr>
<tr>
<td>GOLD</td>
<td>Global Initiative for Chronic Obstructive Lung Disease</td>
</tr>
<tr>
<td>GVHD</td>
<td>Graft versus host disease</td>
</tr>
<tr>
<td>HRCT</td>
<td>High-resolution computed tomography</td>
</tr>
<tr>
<td>ILD</td>
<td>Interstitial lung disease</td>
</tr>
<tr>
<td>LONIPC</td>
<td>Late-onset non-infectious pulmonary complication</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricle ejection fraction</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institute of Health</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>TBI</td>
<td>Total body irradiation</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>VE</td>
<td>Minute ventilation</td>
</tr>
<tr>
<td>VO\textsub{2} max</td>
<td>Maximum oxygen uptake</td>
</tr>
<tr>
<td>VO\textsub{2} peak</td>
<td>Peak oxygen uptake</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</tbody>
</table>
1. Introduction

1.1. General background

Severe hematological diseases, such as leukemias, may often affect young individuals. Over the last decades, treatment of these disorders has evolved and dramatically improved survival. Diseases with previously dismal prognosis have become treatable and largely curable, and fatal outcome has been replaced with the prospect of long-term survival [1]. Figure 1 shows the increase in 5-year relative survival rates in leukemia in Norway from 1965 to 2019. Since 1965, survival has increased from approximately 5% for both genders to almost 70% for males and exceeding 70% for females [2]. From 2002, the Cancer Registry of Norway included polycythaemia vera and myelodysplastic syndrome in the statistical analysis for leukemia, which explains the steep increase in incidence that year.

![Figure 1](image-url)


The development and progress in radiation therapy, multidrug combination chemotherapy and allogeneic hematopoietic stem cell transplantation (allo-HSCT) have improved the cure rate in severe hematological diseases [3], so that a large proportion of the patients may look forward to resume active and productive lives. However, the therapeutic progress has also involved substantial toxicity and hence the risk of various short-term and long-term adverse effects. Young individuals may tolerate aggressive multimodal therapy better than older individuals, which may partly explain the huge difference in survival by age (Figure 2). The figure shows that 15 years after the diagnosis of leukemia, relative survival in children, adolescents and young adults (0-44 years) is more than 80%, for adults 45-60 years 70%, for the age group 60-75 years 40%.
1.2. Severe hematological diseases

Hematological diseases may be divided into malignant and non-malignant. Malignant hematological diseases include a collection of heterogeneous conditions, which may be further divided into myeloid-, lymphoid- and histiocytic/dendritic neoplasms, according to the 2016 WHO classification [4]. The WHO classification is based on cell-of-origin and its genetic expression, immunophenotype and morphology, rather than the anatomic location of the lesion. The classification takes into account that any lymphoma may evolve to, or present with, leukemia, as well and vice a versa. Lymphoid malignancies are divided according to their B-, T- lymphoid or natural killer cell origin. Leukemia is divided into two main groups: Acute and chronic. Acute forms comprise acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL), and chronic forms include chronic myelogenous leukemia (CML) and chronic lymphoblastic leukemia (CLL). In 2019, 734 males and 545 females were diagnosed with leukemia in Norway [2]. The number of cases per 100,000 was 27 for males and 18 for females. In the age group 0-44 years, the average annual number of new cases of leukemia was 88 for males and 74 for females [2]. The group of non-malignant severe hematological diseases is heterogeneous and comprises different acquired and inherited disorders, such as severe aplastic anaemia, beta-thalassemia and other anaemias.
1.2.1. Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and adolescents [5], accounting for approximately 1/3 of all cancers in children [2]. Since 2016, the WHO classification categorizes ALL together with acute lymphoblastic lymphoma, and the two disorders are overlapping in clinical presentation. ALL is a clonal disease of the early lymphoid cell precursor that proliferates, either as solid tumor(s) or leukemia.

1.2.2. Acute myeloid leukemia (AML)

Acute myeloid leukemia (AML) consists of neoplasms involving precursor cells form the myeloid cellular line. AML is the most frequent acute leukemia in adults, while in children less than 10 years of age, AML occurs in fewer than 10% of all with acute leukemia. Incidence in Europe is estimated to be 4 per 100 000 [6]. The choice of treatment is according to medical fitness of the patient and the subtype of AML i.e., its cytogenetic or molecular features.

1.2.3. Chronic myelogenous leukemia (CML)

Chronic myelogenous leukemia (CML) occurs primarily in adults, only 2-3% of leukemias in patients <20 years are CML [7]. CML differs from the other leukemias, since it often appears in a chronic stable state, before evolving to an accelerated state (acute leukemia or ‘blast crises’).

1.2.4. Chronic lymphocytic leukemia (CLL)

CLL is the most common leukemia in older adults, the disease usually occurs in individuals over the age of 65. In general, CLL grows very slowly. Early-stage disease is often asymptomatic and many patients live with CLL for several years without treatment. However, as the cancer progresses, symptoms develop and treatment becomes an option. At that stage, the benefits of treatment must be carefully weighed against potential risks and adverse effects. Both immunotherapy and chemotherapy may be helpful, but the risk of complications must be considered in light of the patient’s age and general health. No patients with CLL as underlying diagnosis were included in our studies.

1.3. Principles for treatment of severe blood disorders

The modern goal of therapy in hematological diseases is to obtain cure, often using aggressive multimodal therapy, with as few side-effects as possible. For ALL, regimens of multiagent chemotherapy have been the main treatment throughout the years, resulting in increased survival rates during the last five decades [5]. In the early 1970s ALL was treated with mainly vincristine, steroids, methotrexate (oral and intravenous) and 6-mercaptopurine. In the 1990s, patients received methotrexate in doses according to risk group and high-risk patients also received cytarabine. In the same period, allo-HSCT became a therapeutic option for selected patients with very high-risk disease.
or disease relapse. Today, the strategy often involves tailored doses of chemotherapy, sometimes in combination with radiation, comprehensive supportive measures and various forms of prophylaxis. The treatment regimens often include vincristine, methotrexate, cyclophosphamide and anthracyclines (i.e., doxorubicin, epirubicin, daunorubicin). The myeloablative conditioning regimen prior to allo-HSCT usually consists of busulfan in combination with cyclophosphamide, or cyclophosphamide alone, and in some cases also total body irradiation. For patients with CML, the treatment is different from other leukemias. Intravenous traditional chemotherapy is not first treatment of choice, but a tyrosine kinase inhibitor or a cytotoxic agent, like hydroxyurea. Also, the various phases of CML have specific treatment options. The goal in AML treatment is reducing the leukemic burden and restore normal bone marrow function. In patients where age and fitness indicate an advantage of treatment, a typical induction therapy may include cytarabine and anthracyclines [8].

1.3.1. Allogeneic hematopoietic stem cell transplantation (allo-HSCT)

During the last decades, improved therapy regimens and increasing use of allogeneic hematopoietic stem transplantation have resulted in a higher number of survivors [9]. Allo-HSCT has a potential to cure malignant and severe non-malignant blood diseases such as leukemia and severe aplastic anaemia. Allo-HSCT is the choice when regular treatment is failing to cure the patient. In allogeneic transplantation, stem cells from a matched donor are transferred to the patient after myeloablative treatment. This may trigger an immunological response by the graft called graft-vs-host disease (GVHD) which may affect both short-term and long-term mortality and morbidity [3]. Another aspect of the immunological response from the graft is the graft-vs-tumor effect, which is beneficial since it helps to eliminate residual malignant cells and thereby contribute to prevent disease recurrence [10]. After allo-HSCT, the patients need immunosuppressive therapy for a limited time (usually a few months), and during that period they are at increased risk for opportunistic infections.

1.4. Treatment-related complications

Treatment related complications are common after cancer treatment. Chemotherapy, radiation or a combination of the two may induce organ-specific toxicity. The focus of this project was to investigate late pulmonary adverse effects in patients who had received curative treatment with various regimens of multidrug chemotherapy for hematological disorders.

1.4.1. Organ-specific chemotherapy toxicity

Pulmonary

Pulmonary adverse effects are frequent and often serious after multimodal treatment of hematological diseases [11]. Multimodal treatment may consist of chemotherapy with or without radiation. Radiation is known to cause mainly interstitial lung disease, but may also cause airways disease. [11], [12]. Total
radiation dose and fractions are important factors for long-term pulmonary toxicity [13]. Several chemotherapeutic agents, such as bleomycin, busulfan, cyclophosphamide, nitrosoureas and methotrexate have been linked to pulmonary toxicity [11]. Bleomycin is the model drug for studying cytotoxic lung injury. A threshold cumulative dose of $\geq 400$ units/m$^2$ has generally been suggested, but pulmonary injury in association with doses less than 150 units/m$^2$ has been reported [14]. For the various other agents, fixed thresholds are yet to be established. Myeloablative conditioning prior to allo-HSCT consists of busulphan and cyclophosphamide; both drugs may induce lung injury. In fact, busulphan was the first chemotherapeutic agent associated with pulmonary toxicity [15], but no clear-cut relationship between dose and pulmonary toxicity has been established. Pulmonary toxicity caused by cyclophosphamide has been described in several case reports, and can be divided into early onset (<6 month after treatment) and late onset (>6 month), where the latter does not seem to be dose-dependent and is unresponsive to glucocorticoid therapy [16]. However, since historical reports on drug toxicity often refer to the use of single agents, they may not be directly applicable to modern multi-drug regimens [17] since concomitant use of two (or more) agents may generate a synergy effect [13]. The proposed harmful mechanisms of chemotherapeutic agents are oxidative effects, toxic effects and hypersensitivity reactions [13].

**Cardiac**

Chemotherapy-related cardiac toxicity is an important cause of mortality and morbidity in survivors of severe hematological disease [18]. Anthracyclines and related agents are associated with an increased risk for left ventricular heart failure with reduced LVEF, especially seen as a late manifestation. High doses of anthracycline chemotherapy ($\geq 250$ mg/m$^2$), chest radiation therapy ($\geq 35$ Gy) or a combination of anthracyclines (any doses) and chest radiation ($\geq 15$ Gy) are known to increase the risk for cardiac adverse effects and follow-up is recommended [17]. The total dose of anthracyclines must be converted to doxorubicin isotoxic doses in order to calculate the total dose received for each patient [19]. The prevalence of cardiac toxicity varies extensively, depending on prevalence of cardiovascular risk factors in the study population, treatment regimens, length of follow-up, investigative methods and definitions of heart injury.

1.4.2. **Pulmonary complications to allo-HSCT**

A diversity of pulmonary complications, both infectious and non-infectious, may occur after allo-HSCT (Table 1). Only late and very late occurring non-infectious complications are within the scope of the present project, and will be further discussed.
1.4.2.1. **Graft-vs-host disease (GVHD)**

GVHD is an immunological response after allo-HSCT, when immunocompetent T-cells from the donor recognize antigens from the recipient as foreign. The recipient’s T-cells activate donor T-cells and destroy tissues of the recipient [20]. GVHD is the most frequent and potentially fatal complication following allo-HSCT [21]. The clinical picture of the immune response has traditionally been divided into acute, i.e. occurring before 100 days post allo-HSCT, and chronic GVHD, i.e. occurring later than 100 days. This conventional division has been questioned [22], since signs of both acute and chronic GVHD may occur outside these periods (often referred to as overlap syndrome) [23].

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| Table 1 Non-infectious and infectious pulmonary complications to allo-HSCT |
|-----------------|------------------|------------------|------------------|------------------|
| **Pre-engraftment** | **Early post-transplantation** | **Late post-transplantation** | **Very late post-transplantation** |
| 0-30 days | 31-100 days | >100 days | >1 year |
| **Non-infectious** | | | |
| Diffuse alveolar hemorrhage | Acute graft-versus-host disease | Air-leak syndrome | Interstitial lung disease |
| Engraftment syndrome | Acute radiation pneumonitis | Bronchiolitis obliterans syndrome | |
| Idiopathic pneumonia syndrome | Idiopathic pneumonia syndrome | Chronic graft-versus-host disease | |
| Pulmonary edema | | Pleuroparenchymal fibroelastosis | |
| | | Post-transplant lymphoproliferative disease | |
| | | Venoocclusive disease | |
| **Infectious** | | | |
| Bacterial pneumonia | Cytomegalovirus pneumonia | | |
| Invasive fungal pneumonia | Fungal pneumonia | | |
| Respiratory syncytial virus | Herpes simplex/varicella zoster | | |
| | | Pneumocystis jiroveci pneumonia | |

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Acute GVHD usually affects the skin, liver and gastrointestinal tract; it rarely affects the lungs. Chronic GVHD may often resemble autoimmune syndromes in its clinical presentation. In the lungs, the main manifestation of cGVHD is obliterative bronchiolitis. The National Institute of Health (NIH) has established criteria for the diagnosis of chronic GVHD. For the different organs, the criteria are divided into diagnostic and distinctive [24], the latter are insufficient alone to establish the diagnosis and need evidence of other organ involvement. For example: In the setting of pulmonary chronic GVHD, biopsy-proven bronchiolitis obliterans is diagnostic, while bronchiolitis obliterans syndrome (BOS) based on pulmonary function tests and HRCT is distinctive. However, if distinctive signs of chronic GVHD are present in other organ systems, BOS in addition to extra-pulmonal features are sufficient to be diagnostic for lung chronic GVHD [24]. To ease inclusion in clinical trials, the guideline states that if all distinctive criteria for BOS are fulfilled, the diagnosis of chronic GVHD is established [24].

1.4.2.2. Late-onset non-infectious pulmonary complications (LONIPCs)
LONIPCs occur more than one year after allo-HSCT, and are non-specific in clinical and radiological manifestations, since principally all anatomic structures of the lung may be affected [25]. Two clinical situations may lead to the diagnosis of LONIPCs: Deterioration in pulmonary function or development of respiratory symptoms, both dependent on excluding infection as a cause [26]. In essence, bronchiolitis obliterans syndrome and different histological entities of interstitial lung disease (ILD) comprise LONIPCs in most clinical cases. Best described ILDs are organizing pneumonia and pleuroparenchymal fibroelastosis. Further, different histological patterns of ILD include usual interstitial pneumonia, non-specific interstitial pneumonia, diffuse alveolar damage, lymphoid interstitial pneumonia, eosinophilic pneumonia and acute fibrinous organizing pneumonia [27]. LONIPCs are associated with mortality and morbidity in allo-HSCT survivors [28].

1.4.2.3. Bronchiolitis obliterans syndrome (BOS)
Bronchiolitis obliterans (BO) is a non-specific inflammatory injury, affecting mainly the small airways. It is clinically recognized by airflow limitation, and histologically by intraluminal fibrosis narrowing the airway lumen. Pathogenesis is not well defined, but immune reactions by T-cells from the donor and the innate immune system of the recipient are thought to contribute to the development of BO [29]. There is a clear association between BO and cGVHD. To circumvent the need for invasive procedures to obtain a lung biopsy, BO syndrome (BOS) is diagnosed clinically. The National Institute for Health (NIH) has defined consensus criteria for post-HSCT BOS [24], based on pulmonary function tests with a new-onset, non-reversible obstructive ventilatory pattern, when infectious causes are ruled out. The spirometric criteria are FEV₁/FVC <0.7 and FEV₁<75% of predicted value, or >10% decline in FEV₁ % predicted during a 2-year period. Additionally, one or two supporting features must be present: Residual volume (RV) > 120% of predicted by measurement of static lung volumes or
signs of bronchiectasis on HRCT or air trapping on the expiratory scans. If BOS is the only manifestation of cGVHD without any other affected organ, a biopsy is mandatory to establish the diagnosis of cGVHD [24].

1.5. National centralized treatment policy

Centralized treatment of childhood cancer is a well-established national policy in Norway. The University hospitals within the four geographical health regions (located in Oslo, Bergen, Trondheim and Tromsø) have been assigned responsibility for treating patients with pediatric cancers. Oslo University Hospital, Rikshospitalet has been responsible for treating patients with ALL from the South-Eastern health region, which comprises approximately half of the population of Norway (4.9 million inhabitants at the time of the study). Rikshospitalet is the national single-centre for allo-HSCT (5.4 million inhabitants).

1.6. Methods used to detect pulmonary and cardiopulmonary late effects after chemotherapy

1.6.1. Pulmonary function tests

The principal method for detecting pulmonary sequelae after multiagent chemotherapy, is pulmonary function testing. The methods include spirometry, whole body plethysmography and single breath determination of carbon monoxide uptake in the lung. By these methods, dynamic lung volumes, static lung volumes and gas diffusing capacity can be measured. Pulmonary function tests are cost-effective, non-invasive and well standardized. The tests are reliable, given that the subject is compliant and able to co-operate, and that the criteria for acceptability and repeatability of the tests are met.

1.6.2. Cardiopulmonary exercise test (CPET)

Determining maximum exercise capacity during a symptom-limited cardiopulmonary incremental exercise test is a valuable tool in clinical settings. The test is performed on a treadmill or a stationary, electrically braked cycle ergometer. CPET with estimation of maximum oxygen uptake (VO₂max) is considered by The World Health Organization to be the single best indicator of aerobic physical fitness[30]. The VO₂max is often expressed as the volume of oxygen consumed per unit of time relative to body mass (ml/kg/min), and reflects aerobic power. The parameter is an important predictor for all-cause mortality[31], cancer mortality in a general population [32, 33], and for prognosis after cardiovascular disease in males [34]. CPET is the only standardized method by which the global effect of pulmonary and cardiac impairments may be assessed. In real-life CPET, VO₂max is not always obtained in reaching a stable plateau. Since the plateau sometimes is missing, VO₂peak is used instead, which denotes the highest O₂ uptake during the test [35]. A phase with a plateau is preferable, since a plateau may be more precise than two - three single measurements to determine the subjects’ correct
fitness level. In this work, VO$_2$max is applied when maximum oxygen uptake is described in general terms, while the highest O$_2$ uptake achieved in the study subjects is referred to as VO$_2$peak.

**1.6.3. Echocardiography**

Since cardiorespiratory exercise capacity depends on both pulmonary and cardiac function, pulmonary function tests must be supplemented by assessment of cardiac function. Echocardiography is a reliable, cost-effective, non-invasive method, which is well established. The method is, however, user-dependent and both image acquisition and measurements rely on skilled and well-trained sonographers and cardiologists. Measurement of left ventricle ejection fraction (LVEF) is a well-established parameter for assessing left ventricular systolic function. The common threshold for distinguishing between normal systolic function and left heart dysfunction is 50% [36], later updated with different thresholds for males and females [37].

**1.6.4. High Resolution Computed Tomography (HRCT)**

HRCT is performed using a conventional CT scanner. High resolution refers to a technique in which thin-slice (0.5-1.5 mm) images of the lung are obtained and then processed in a high-spatial-frequency reconstruction algorithm. By HRCT, images with fine details of lung tissue can be obtained, thereby enabling detection of subtle pathology. HRCT is a well-established and important tool in pulmonary medicine. The first report on the use of HRCT in Norway was published in 1998 [38]. HRCT may be performed in both inspiration and expiration. Expiratory scans are particularly useful in detecting airways disease. The patient may also lie prone (face down) rather than in the usual supine (face up) position. This is useful for detecting pathology at the bases of the lungs. HRCT is used mainly for diagnosis and assessment of interstitial lung disease, emphysema and airways disease. Intravenous contrast agents are not used for HRCT and the radiation dose to the patient is considerably less than for conventional CT.
2. Aims of the project

2.1. General aims

The general aims of this project were to determine the extent and degree of late treatment-related pulmonary effects in young, long-term survivors of severe hematological diseases treated with chemotherapy only or with allogeneic hematopoietic stem cell transplantation. We also aimed to identify risk factors for impairments in pulmonary function and in cardiorespiratory fitness.

2.1.1. Specific aims

Paper I – To assess very long-term effects of chemotherapy on pulmonary function and cardiorespiratory fitness in young long-term adult survivors of childhood acute lymphoblastic leukemia, and to identify possible predictors of impairments.

Paper II – To investigate cardiorespiratory fitness in young adult long-term allo-HSCT survivors who had received myeloablative conditioning with chemotherapy only, and to identify associations between cardiorespiratory fitness and cardiac- and pulmonary limiting factors and modifiable lifestyle factors.

Paper III – To determine the occurrence and degree of late pulmonary sequelae in young long-term survivors of allo-HSCT based on pulmonary function tests and HRCT, and to identify associations between radiological- and physiological findings and clinical characteristics.

2.2. General hypothesis and paper-specific hypotheses

We hypothesized that survivors of severe hematologic disease in childhood, adolescence and young adulthood, treated with chemotherapy only or with allogeneic hematopoietic stem cell transplantation, may develop late-onset non-infectious pulmonary complications that may affect physical fitness.

Paper I - Long-term survivors of childhood ALL may develop impaired pulmonary- and cardiac function that – along with lifestyle factors - will contribute to reduced cardiorespiratory fitness in early adulthood.

Paper II - Long-term survivors of allo-HSCT performed in childhood, adolescence and young adulthood will develop impairments in pulmonary- and cardiac function that – along with life-style factors - will cause reduced cardiorespiratory fitness compared to a healthy reference population.

Paper III - Long-term survivors of allo-HSCT performed in childhood, adolescence and young adulthood will have reduced pulmonary function compared to healthy age- and gender matched controls, and pathological findings on HRCT that will reflect and correspond to the various types of impairment in pulmonary function.
3. Patients and controls

3.1. Study design

The three studies included in this project are sub-studies performed within the context of two large multidisciplinary projects, examining a wide range of late health conditions in young long-term survivors of severe blood disorders, treated in childhood, adolescence or early adulthood. The studies were cross-sectional in design and included two cohorts: Survivors of ALL (paper I) and survivors of allo-HSCT (paper II and III). Figure 2 shows the flowcharts of patients eligible for recruitment in the two cohort studies.

**Figure 3** Flow chart of survivors in cohort I (paper I) and cohort II (papers II and III).

### 3.1.1. Survivors of childhood ALL (paper I)

This study, which was part of a large cross-sectional survey of late effects after childhood ALL, included 116 long-term survivors (fig 3). The Cancer Registry of Norway identified all individuals < 16 years old who had been diagnosed with ALL during the period 1970 to 2009 in the South-Eastern Region of Norway (covering approximately half of the Norwegian population, approximately 4.9 mill at the time of the survey). Patients > 18 years old and alive in 2009 were eligible for the survey. The Cancer Registry identified 538 treated patients, of whom 182 (34%) were dead, 94 (17%) had not
turned 18 years, 32 (5%) had been treated in other hospitals and 20 (4%) were lost to follow-up. Of the remaining 210 (39%) survivors who fulfilled the eligibility criteria, 160 (76%) agreed to participate, and 138 (66%) completed the clinical examinations and the cardiac-and pulmonary function tests. For the present study, subjects who had received craniospinal radiotherapy (n=20) and/or allo-HSCT (n=3) were excluded (in total n=22). The medical tests included electrocardiography, echocardiography, pulmonary function tests, CPET and fasting blood tests. Three subjects could not perform CPET due to a physical incapability and two declined to do exercise. A total of 116 completed cardiac- and pulmonary function tests and 111 completed CPET. Different ALL treatment protocols had been used during the treatment period. We calculated cumulative intravenous chemotherapy doses adjusted for body surface area, individually in all survivors. The chemotherapeutic agents used in the protocols included vincristine, methotrexate, cyclophosphamide and anthracycline. Anthracycline doses were converted to doxorubicin isotoxic doses [19]. Intraspinal or oral administration of methotrexate was not included.

3.2. Survivors of allo-HSCT (Paper II and III)

The subjects included in this cross-sectional study comprised a national cohort of patients who had been treated with allo-HSCT in childhood, adolescence or young adulthood at Oslo University Hospital. Eligible survivors were identified through the hospital’s HSCT registry. Criteria for inclusion were age at allo-HSCT <30 years, age at survey >16 years and > 5 years of follow-up. All diagnoses prior to allo-HSCT were included, except mucopolysaccharidosis type I (Hurler syndrome). Eligible survivors (n=159) were invited to the health survey. In two cases the medical charts were missing, 38 subjects did not respond and 15 declined participation. A total of 104 survivors attended the present study, i.e. an attendance rate of 66%. Eight subjects were excluded from CPET due to cardiovascular contraindications (n=5) and physical disability (n=3). We also excluded patients who had received TBI (n=6) from the analysis in paper II. The myeloablative conditioning regimen prior to transplantation had consisted of cyclophosphamide and busulfan or cyclophosphamide alone for all but two of the survivors (who received none).

3.2.1. Healthy controls (paper III)

We recruited a control group of 105 healthy, never-smoking subjects among hospital and university employees. The controls had no history of malignancy or cardiac- or pulmonary disease, and they were age- and gender matched to the allo-HSCT survivors.
4. Methods

4.1. Clinical investigation and blood tests

In both study cohorts, all survivors underwent clinical examination, blood sampling and answered questionnaires. Age, gender, body mass index (BMI, kg/m²), smoking habits, cardiovascular- or pulmonary disease and current medication were registered. Overweight was defined as BMI ≥25 kg/m² and obesity as BMI ≥30 kg/m², according to classification of World Health Organization [39]. Blood samples included hemoglobin (Hb), N-terminal pro-brain natriuretic peptide (pro-BNP), lipids and glycated hemoglobin (HbA1c).

4.2. Pulmonary function tests

Pulmonary function tests included dynamic spirometry, measurement of static lung volumes and single breath gas diffusing capacity for carbon monoxide (DLCO). Registered variables were forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), FEV₁/FVC ratio, total lung capacity (TLC), residual volume (RV), DLCO and DLCO divided by alveolar volume, VA. DLCO measurements were also corrected for Hb-levels, obtained at the same day as pulmonary function testing. All tests were carried out by well-trained and experienced physiologists dedicated to the studies and under the guidance of a senior pulmonologist, and performed according to ATS/ERS guidelines [40] [41] [42]. Pulmonary function variables were reported in absolute values and as a percentage of predicted normal values. In paper I, all reference values were taken from the European Community for Steel and Coal (ECSC) [43]. In paper II, the predicted values for spirometric variables were taken from the Global Lung Initiative (GLI) [44] while the predicted values for static lung volumes and DLCO were taken from the ECSC [43]. In paper III, the predicted values for spirometry and DLCO were taken from GLI [44, 45], while for static lung volumes from the ECSC [43]. Restrictive impairment and impairment in DLCO were defined as lower than 80% of predicted, which correspond to the lower 5th percentiles in the reference material and according to ERS recommendations [46]. In paper I, obstructive impairment was defined as FEV₁/FVC <0.7 according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [47]. In paper II and III, obstructive impairment was defined according to National Institute of Health criteria for BOS [24] , which differ from GOLD criteria. In cohort 1, all pulmonary function tests were performed with Vmax Pulmonary Function Unit (VIASYS Respiratory Care Inc, Yorba Linda, CA). In cohort 2, the Jaeger Master Screen Body (Würzburg, Germany) was used for all tests. Table 2 gives an overview of equipment, reference values and cut-off values in both cohorts.
Table 2 Overview of equipment, reference values and cut-off values

<table>
<thead>
<tr>
<th>Study</th>
<th>Paper</th>
<th>Cohort</th>
<th>Patient group</th>
<th>Equipment</th>
<th>Reference values</th>
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<tr>
<td>Equipment</td>
<td>Pulmonary function tests</td>
<td>Vmax</td>
<td>Jaeger</td>
<td>Jaeger</td>
<td>Reference values</td>
<td>Cut-off values</td>
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<td>Cardiopulmonary exercise test</td>
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<td>Treadmill</td>
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<tr>
<td>Reference values</td>
<td>Spirometry</td>
<td>ERS 1993</td>
<td>GLI 2012</td>
<td>GLI 2012</td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC &lt; 0.7; FEV&lt;sub&gt;1&lt;/sub&gt;/FVC &lt; 0.7; FEV&lt;sub&gt;1&lt;/sub&gt;/FVC &lt; 0.7; FEV&lt;sub&gt;1&lt;/sub&gt;/FVC &lt; 0.7;</td>
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<td></td>
<td>Gas diffusing capacity</td>
<td>ERS 1993</td>
<td>ERS 1993</td>
<td>GLI 2017</td>
<td>Criteria obstructive impairment (BOS*)</td>
<td>GOLD</td>
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<td></td>
<td>Static lung volumes</td>
<td>ERS 1993</td>
<td>ERS 1993</td>
<td>ERS 1993</td>
<td>Restrictive impairment</td>
<td>TLC &lt; 80% predicted</td>
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<td></td>
<td>Cardiopulmonary exercise test</td>
<td>Jones 1982</td>
<td>Edvardsen 2013</td>
<td>-</td>
<td>DLCO impairment</td>
<td>&lt;80% predicted</td>
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4.3. Echocardiography

Transthoracic echocardiography was performed according to international guidelines [37], using Vivid 7 or E9 scanners and dedicated software from GE-Vingmed Ultrasound, Horten, Norway). All recordings were carried out by experienced sonographers and reviewed by a senior cardiologist. Left ventricular ejection fraction (LVEF) was assessed by the rule of Simpson’s biplane. In cohort 1, left ventricular systolic dysfunction was defined as a LVEF < 50% [36]. In cohort 2, left ventricular systolic dysfunction was defined as LVEF < 52% in men, < 54% in women [37].

4.4. Cardiopulmonary exercise test (CPET)

All CPETs were performed under guidance of a physician and an exercise physiologist. Metabolic gas exchange and variables of ventilation were continuously monitored during CPET, in addition to a 12-lead electrocardiogram and regularly measurements of blood pressure. The well-being of the test person was continuously observed, and perceived exertion was rated regularly, using the Borg scale. 20. Percutaneous oxygen saturation (SpO<sub>2</sub>) was measured by finger pulse oximetry. Prior to each test, the gas exchange units were calibrated. VO<sub>2</sub>peak was the primary outcome, and defined as the highest VO<sub>2</sub> measured over 30s, other recorded variables were RER and peak heart rate. In cohort 1 (bicycle test) low cardiorespiratory fitness was defined as VO<sub>2</sub>peak < 80% of predicted and in cohort 2 (treadmill test) VO<sub>2</sub>peak < 85% of predicted [35, 48].
4.4.1. Cohort I

CPET was performed using the Sensormedics Vmax unit (VIASYS Respiratory Care Inc., Yorba Linda, CA) with an Ergoline 800 bicycle (Bitz, Germany). The exercise test consisted of three stages: 1. Two minutes of warm-up with low work load. 2. An incremental exercise stage until exhaustion, ideally 8 to 12 minutes. 3. Recovery stage, lasting 2 minutes. The test was discontinued at exhaustion, with respiratory exchange ratio (RER) >1.10. Each day, calibration was conducted for the gas exchange units. Data recorded were primarily VO₂ peak, but also max oxygen pulse, maximum minute ventilation, maximum workload, RER, SpO₂ and Borg scale were gathered. The reference values for bicycle were those by Jones et al [49].

4.4.2. Cohort II

CPET was performed using a treadmill from Technogym Runrace (Forli, Italy). A modified Balke protocol was used, the same protocol that had been used for testing the reference population [50]. The exercise test consisted of three stages: 1. A warm-up stage with low work load, ending at 3 km/h and 2% inclination. 2. The incremental exercise with 2%-points increasing inclination every 60 sec until exhaustion. 3. Recovery stage. The measured values were compared to age- and gender adjusted reference values for CPET applying treadmill [50]. Subjects with impairment in VO₂ peak were divided into five categories: 1. Ventilatory limitation, defined as breathing reserve ≤15% or 11L/min. 2. Gas exchange limitation, defined as VE/Vco₂ slope ≥34. 3. Cardiac limitation, defined as oxygen pulse <80% of predicted, heart rate <90% of predicted and heart rate reserve <15 beats/min, in absence of pulmonary limitations. 4. Deconditioning, defined as low VO₂ peak, without other limitations. 5. Several organ-specific limitations.

4.5. High Resolution Computed Tomography (HRCT) of the lungs

HRCT was performed with a LightSpeed 16 Scanner (GE Healthcare, Milwaukee, Wisconsin, USA). The examination was obtained during deep inspiration while holding breath in a supine position. The current settings of the tube were adjusted according to the weight of each patient, using low dose references, with a noise index of 40 and no contrast material intravenously. Supplementary expiratory scans were obtained in all subjects with 1.25 mm section thickness at 10 mm intervals. The image reviewing system was Picture Archiving and Communication System, PACS. The images were assessed in random order and in consensus by two expert chest radiologists and one pulmonologist (OHM) with special training in interpreting HRCT. The Nomenclature Committee of the Fleischner Society was used to evaluate interstitial changes [51]. Reticular pattern and/or ground glass opacities were defined as interstitial lung disease. Other differential diagnoses to ground glass opacities
(pulmonary infection, malignant disease and cardiac oedema) were also evaluated and could be ruled out, as a quality check. The presence of bronchiectasis, air trapping, mosaic pattern, centrilobular micronodules was defined as airways disease. We categorized the HRCT findings into three major groups:

- Airways disease
- Apical irregular subpleural thickening
- Other signs of interstitial lung disease

Figure 4 The anatomic pulmonary zones. The figure is based on free-to-use images by Servier Medical art (Smart Servier Free Medical Images, available from smart.servier.com).

Four anatomic zones were defined and used to position the distribution of pathology, according to figure 4. For each anatomic zone, the extent of pathology was evaluated. The extent of air trapping in each zone was given a score based on the percentage of involvement of lung parenchyma. For each subject, an overall score of involvement was summarized from the four zones. Since some degree of air trapping is considered normal [52], we defined sub-segmental air trapping with <5% involvement of the lung parenchyma as normal.

4.6. Statistical analysis

Standard, established statistical tests were used for all analyses. Data are presented as mean, median or as a number with a percentage. To quantify the uncertainty of the effect estimate, standard deviation, range or 95% confidence interval were used. We used student’s t-test or Mann Whitney U test, as appropriate, to compare continuous data between groups. For categorical data, Chi-square test or Fisher’s exact test were used. A two-sided p-value < 0.05 was considered significant. Multivariate (linear or logistic) regression analyses were applied for estimation of associations. The statistical analyses were done with SPSS software, version 23-26.
**Paper I and II**

Pearson’s correlation coefficient was applied as a measure of the relationship between left ventricular ejection fraction, DL_{CO} and cumulative doses of anthracyclines, respectively in paper I. In paper II, Pearson’s correlation coefficient was used to detect any relationship between VO_{2peak} and respectively LVEF and DL_{CO}. Univariate and multiple linear regression analysis were used to detect associations between dependent continuous variables and appropriate covariates. The independent variables entered in the regression models, were hypothesized a priori for biological or clinical reasons, or found to be significant by univariate analysis at a 20% level. In paper I, the multiple linear regression analyses were used to assess the association between respectively VO_{2peak} and DL_{CO} as dependent variables and selected variables from the univariate analysis. In paper II, we used univariate (or unadjusted) linear regression analyses to assess the difference in VO_{2peak} among the long-term survivors. Factors associated with VO_{2peak} were included in the multiple (or adjusted) linear regression analysis to identify independent factors associated with VO_{2peak}. Only one variable from the pulmonary function tests were included in the final regression model in order to avoid problems with collinearity. The 95% CI was applied to express the uncertainty of the effect estimates from the univariate and multiple regression models.

**Paper III**

To investigate any relationship between pulmonary function variables and air trapping in HRCT, Pearson’s correlation coefficient was applied. We used a logistic regression model to estimate the odds ratio (OR) between ‘pathological findings on HRCT’ and ‘BOS’ as dependent variables, respectively and relevant exposure variables, controlled for possible confounders. The independent variables entered in the regression models were hypothesized a priori or found significant by univariate analysis at a 20% level. The uncertainty of the OR estimate was expressed as 95% CI.
5. Summary of results

5.1. Paper I

The main findings of this a cross-sectional study of 116 adult survivors of childhood ALL were that although mean pulmonary function and cardiorespiratory fitness were within the lower predicted range for the survivors as a group, 22% had impaired gas diffusing capacity (DLCO) and 42% had impaired cardiorespiratory fitness (VO2peak). The survivors were median (range) 5 (0.3-16) years at the time of diagnosis and 29 (19-47) years at follow-up. Median (range) follow-up was 23 (7-40) years. The chemotherapy regimens comprised vincristine in all patients, methotrexate in 95%, anthracyclines in 77% and cyclophosphamide 33%. Individual cumulative doses of intravenous cytostatic agents were calculated in all patients. We found that females had lower DLCO than males [mean (SD) 84 (13) versus 97 (14) % predicted, p<0.001], and DLCO impairment was found in 34% females compared to 7% males p=0.001. In a multiple linear regression model, female gender, BMI and smoking were found to be risk factors for reduced DLCO % predicted. Left ventricular ejection fraction (LVEF%) was normal (def. >50%) inn all but three subjects. No significant correlations were found between LVEF% and cumulative doses of anthracyclines, but patients who had received anthracyclines had lower LVEF% than those who had not been exposed (mean [SD] EF% 56.2 [4.3] vs 59.2 [5.2], p = 0.01). Risk factors for impaired cardiorespiratory fitness were BMI and smoking. Since the subjects were young (median age 29 years), longitudinal data are needed to determine if the observed impairments will progress or remain stable throughout their adulthood. In order to preserve cardiorespiratory fitness, we suggest that ALL survivors should be offered counselling on modifiable life-style factors with focus on inactivity, overweight and smoking.

5.2. Paper II

In a nationwide cross-sectional study of 90 long-term survivors of allo-HSCT (aged median (range) 35 (17-54) years, 56% females), we found that only 30% met the WHO-criteria for physical activity and 42% were overweight or obese. Malignancy had been the underlying diagnosis prior to allo-HSCT in 73% of the survivors, of whom 46% had received chemotherapy intravenously. Reduced gas diffusing capacity (DLCO) was found in 44% of the survivors, and those who had received chemotherapy prior to allo-HSCT had lower DLCO % predicted than those who had not received such treatment [mean (95% CI) 79 (75–84) vs 87 (83–92), p = 0.02]. Left ventricular systolic dysfunction was found in 31% of the survivors and was also associated with chemotherapy prior to allo-HSCT (p = 0.02). One third of the survivors had developed chronic GVHD of whom 40% were diagnosed with bronchiolitis obliterans syndrome (BOS).

Cardiorespiratory fitness was impaired (def. VO2peak < 85% predicted) in 43% of the survivors. In a multiple linear regression analysis, low VO2peak was associated with low DLCO, low
LVEF, BOS, overweight and physical inactivity. Since half of the survivors had reduced cardiorespiratory fitness median 17 years after allo-HSCT, and since cardiopulmonary factors and deconditioning were equally common limitations to exercise, we encourage long-term monitoring of cardiac and pulmonary function in allo-HSCT survivors and recommend targeted advice on modifiable lifestyle factors in order to prevent deconditioning.

5.3. Paper III

In this study, we focused on pulmonary function and HRCT. We aimed to determine the occurrence and degree of late pulmonary sequelae after allo-HSCT. The same nationwide cohort (paper II) was expanded with 13 more survivors so that the study included 103 allo-HSCT survivors. We also recruited a group of 105 age- and gender matched healthy controls. We found that after median 17 years observation the survivors had significantly lower pulmonary function than the healthy controls and nearly half had pathological findings on HRCT. Although mean lung volumes and gas diffusing capacity were above 80% predicted for the entire study group, one third of the survivors had some kind of impairment (17% impaired gas diffusing capacity, 12% had developed BOS and 4% had restrictive impairment). The predominant pathological findings on HRCT were signs of airways disease, but also various patterns of interstitial lung disease were found. The survivors who had received chemotherapy prior to allo-HSCT had 2.4-fold and 4.5-fold increased risk for, respectively, pathological findings on HRCT and BOS. We recommend individually tailored lifelong monitoring of pulmonary function in allo-HSCT survivors. Pulmonary function testing is cost-effective, readily available, well-standardized and easy to perform. HRCT may provide valuable additional information, but is not suited for surveillance due to radiation exposure and should be reserved for clinical settings.
6. Methodological considerations

6.1. Study design

The studies were designed as cross-sectional cohort studies, as outlined by Hudson et al [53]. The design implies that an investigator samples a source population cross-sectionally and then retrospectively assesses the history of the subjects’ possible exposures and outcomes over a specified time. The cross-sectional cohort study is observational in nature and includes no intervention. The cross-sectional cohort study design is suitable for assessing the occurrence of a phenomenon – such as the burden of a disease at a specific time, i.e. detecting the prevalence - especially in situations with long interval between exposure and outcome. In our studies, we wanted the survivors to have at least 5 years disease-free survival, indicating that they were cured and had the likelihood of a normal lifespan. Generally, clinical cross-sectional follow-up studies tend to benefit from rather high attendance rates and the possibility to account for subjects lost to follow-up. In our two studies, the median observation periods were, respectively, 23 and 17 years, ranging between 7-40 and 6-26 years. In light of such very long follow-up, we think an attendance rate of 66% was quite acceptable (Coincidentally the attendance rate turned out to be 66% in both studies).

We were able to account for the number of subjects lost to follow-up due to death, and for those who declined to participate or did not respond to the invitation (non-responders). This information enabled us to find out if the non-responders differed significantly from the participants with respect to age, gender and diagnosis. In the study of ALL-survivors (paper I), the participants did not differ from the non-responders with respect to gender, age at diagnosis and years of observation, while in the study of allo-HSCT survivors (paper II and III) the non-responders were significantly younger than the participants and comprised more males. Regrettably, we did not have access to causes of death or occurrence of treatment-related morbidity, which entails a major limitation (survivorship bias).

A disadvantage of a cross-sectional design is lack of baseline, pre-treatment measurements. In our studies, baseline pulmonary function data might have been useful, but only to a limited extent. In the ALL-study (paper I) the patients were children, aged [median (range) 5 (0.3 -16)] years at diagnosis so that reliable pulmonary function tests might have been possible to obtain in some, but only in a subset. The subjects included in the allo-HSCT study (paper II and III) were somewhat older (median age 20 years), but one third were patients in the Paediatric ward at the time of allo-HSCT. Lack of baseline data for echocardiography and HRCT prevents us from concluding whether, respectively left ventricular dysfunction and pathological pulmonary findings, may have been present prior to treatment, or early after treatment, or have developed afterwards. Since cardiac and radiological pathology are more precisely defined than pulmonary function impairment, lack of
longitudinal data in the present study will influence interpretation of pulmonary function results more than results from echocardiography and HRCT.

6.2. Patient recruitment

The patients included in cohort 1 were long-term survivors of childhood ALL, identified by The Cancer Registry of Norway. Reporting cancer cases to the Registry is required by law, ensuring a high degree of completeness. Mandatory reporting, combined with the unique personal identification number used in Norway, make data from the Cancer Registry of Norway particularly well suited for research since the Registry provides basic demographic and clinical information for the entire population.

In cohort 2 (paper II and III), the patients were identified by the hospital’s HSCT registry which is nationwide due to Norway’s single centre policy for such treatment. The study cohort was limited to patients who had been less than 30 years at the time of allo-HSCT. This age-limit ensured that the large majority of the survivors would be in their 30s and the oldest around 55 years old at the time of follow-up. The reason for the age-limit was that we aimed to detect treatment-related late effects, and wanted to avoid concomitant effects of pulmonary and cardiovascular diseases (like COPD, hypertension and coronary artery disease) that are more frequently occurring after the age of 50. Further, distinguishing between treatment-associated risk factors and general risk factors (like smoking and blood lipids) would be more challenging in older survivors. Also, detecting treatment-related impairments and lifestyle risk factors in young adults with the prospect of a normal lifespan would be more beneficial than in older individuals since the effect of intervention would have a greater impact.

An age limit of 16 years at the time of the study (both cohorts) ensured that the participants would be old enough to perform complicated pulmonary function tests and CPET. Minimum 5 years observation (both cohorts) is commonly used as an indicator of cure after cancer treatment and it is also frequently suggested as a cut-off for detecting late effects of cancer therapy.

Due to the very long observation periods in our studies, mortality accounts for a substantial number of patients lost to follow-up. Mortality was 34% among ALL-patients and 44% among the patients treated with allo-HSCT. The mortality figures include relapse of hematological disease, which is most prevalent during the first years, and all other causes of mortality, which increases over time and is closely associated with adverse effects of treatment (Figure 3) [54]. Cardiovascular disease has been shown to be a leading cause of late morbidity and mortality after allo-HSCT [55] and survivors of allo-HSCT have a 4-fold higher risk of developing cardiovascular disease, compared to the general population [56]. The long-term survivors who participated in our studies represent a selected group.
that had been spared fatal complications. Such survivorship bias is an inevitable limitation in long-term follow-up studies.

Life-style risk factors for cardiovascular disease (overweight, smoking and physical inactivity) were quite prevalent among the survivors in our two cohorts. In cohort 1, 38% were overweight, of whom 17% were obese, and 19% had a history of smoking. While the occurrence of overweight was comparable to prevalence data reported from health surveys in Norway [57], the proportion of smokers was 4% points higher than the Norwegian population average at that time [58]. In cohort 2, the occurrence of overweight and smoking were comparable to the general population, but nearly two thirds of the survivors were physical inactive (i.e. they did not fulfil the WHO recommendations). In both cohorts, we found that cardiorespiratory fitness was impaired in more than 40% of the survivors. The impairment was significantly associated with BMI, inactivity and smoking. Since preserving cardiorespiratory fitness might be life-prolonging in cancer survivors [33, 59], we recommend targeted advice on modifiable lifestyle factors like smoking, diet and physical activity in all survivors of ALL and allo-HSCT.
6.3. The cardiopulmonary exercise test (CPET)

Exercise physiology and the interpretation of a CPET is complex and will be described in general terms. Figure 6 shows the physiological responses to exercise, from increased ventilation in the lungs to a higher level of O₂ at a cellular level in the muscles. The CPET is designed to assess physiological factors that may limit maximal exercise capacity: the cardiovascular-, respiratory-, musculoskeletal, autonomic nerve and hematological systems.

![Physiologic response to exercise](image)

**Figure 6** Physiologic response to exercise. Published with permission from Datta D, et. al. Cardiopulmonary exercise testing in the assessment of exertional dyspnea [60].

The most common method for CPET is maximal exercise test on a treadmill or a cycle ergometer, while monitoring breath gases. As earlier explained, VO₂peak is used instead of VO₂max to express the highest O₂ uptake during the CPET when a VO₂ plateau is absent. Different reference- or predicted values for VO₂max have been proposed (Table 12 [35, 50]). Additionally, different cut-off values for impairments have been suggested, the ATS/ACCP guideline suggest VO₂peak <85% of predicted value [35].
In addition to VO$_2$max, maximum minute ventilation (VE) is a central variable in the CPET, and may indicate ventilatory limitation. Other central variables are tidal volume and assessment of breathing reserve. In most healthy individuals, peak exercise ventilation approaches 70% of minute volume ventilation (MVV). Hence, a patient with low ventilatory reserve (<15%) has a ventilatory limitation. VE/VCO$_2$ is the ratio of minute ventilation to CO$_2$ output, and is termed the ventilatory equivalent for carbon dioxide. It is used as a non-invasive estimate of appropriateness of the ventilation. A high VE/VCO$_2$ (>34 at minimum, termed the respiratory compensation point) may reflect disease in several organs: An elevated VE/VCO$_2$ may be seen in ventilatory limited patients with COPD and in morbid obesity [35] but has also been found to be a sign of heart failure [61].

Maximal heart rate (HR$_{max}$) may be used as guidance in determining whether the test is done with sufficient effort. The ATS/ACCP guideline suggests HR$_{max}$ >90% of age-predicted value [35]. If HR$_{max}$ is below 90 %, the effort during the test may be inadequate or a lack of heart rate response is present. Heart rate reserve (HRR) is the difference between peak heart rate during CPET and maximal predicted value. Further cardiac function during the CPET can be evaluated using the product of VO$_2$/HR. The variable is a term for oxygen uptake per heartbeat, and increases during the incremental CPET, until reaching a plateau phase. VO$_2$/HR is also called oxygen pulse and can be compared to predicted values. A low value may reflect deconditioning or cardiovascular disease. The respiratory exchange ratio (RER), is a ratio of VCO$_2$/VO$_2$. The ratio reflects the tissue metabolic exchange of gases. Both lactic acidosis and hyperventilation must be considered when the RER is greater than 1.0.

Cardiorespiratory fitness was assessed in both cohorts, and VO$_2$peak was a main outcome in paper I (ALL-study) and paper II (allo-HSCT-study). In a systematic review that also gives practical recommendations for cardiorespiratory exercise testing in clinical oncology research, it is recommended that maximum tests should be done only in patients who are at low risk of an exercise-related adverse event [62]. For optimum patient safety, the clinical status of all patients should be assessed before exercise testing. It is further advised that the patients, prior to testing and for assessing potential contraindications to exercise, should be screened with respect to current treatments and physical-activity profile, resting BP and ECG, appropriate blood tests and echocardiography. In line with the recommendations, all survivors in our studies had a clinical examination, blood sampling, pulmonary function testing and echocardiography the day before CPET. In the ALL-cohort (paper I) five subjects did not perform CPET (three due to physical disability/wheelchair users and two declined). In the allo-HSCT cohort (paper II) five survivors had cardiovascular contraindications (severe, untreated hypertension, coronary fistula to the pulmonary artery, cardiomyopathy, aortic valve stenosis, and significant pulmonary hypertension) and three were not physically able to walk on a treadmill. All tests were performed in the presence of an experience exercise physiologist and a chest physician, and within the vicinity of nurses and doctors working in adjacent rooms. There were no CPET-related serious adverse events.
There are two different methods for conducting CPET: cycle ergometer and treadmill. Both modalities have advantages and disadvantages. For subjects who have problems with balance/coordination or peripheral neuropathy, it is preferable to sit on a stable, stationary bicycle. Due to less physical movements by the test subject and fewer disturbing artefacts, it is easier to obtain reliable recordings of exercise-response measures like BP, ECG and SaO₂ when the subject is seated. Further, it is recommended that individuals with overweight/obesity should preferably be tested on a cycle ergometer [35]. On a motor-driven treadmill, exercise intensity is progressively increased by a combination of speed and elevation. The main advantage of treadmill testing, lies in the fact that walking is a more natural and familiar activity than cycling for most individuals. Uphill treadmill testing places a higher demand to the cardiorespiratory system and is known to result in 5-10% higher VO₂peak, due to involvement of the larger muscle groups [35].

In cohort 1, the ALL-survivors were tested on a cycle ergometer. At that time, we did not have a treadmill in our lab. We had, however, extensive experience with CPET on a cycle ergometer. Since there were no Norwegian normative data available for cycle tests, we used the well-established reference values by Jones et al. that have been recommended by the ATS/ACCP [49]. The reference values are derived from a study of sedentary North Americans and dates back to the 1980s [49]. By the time we included participants for cohort 2 (paper II), our lab had been equipped with a treadmill in addition to the cycle ergometer. One reason for opting to test the survivors on a treadmill was that we also had access to rather new reference values derived from a Norwegian multicenter study involving 558 healthy adults within the same age range as the study population [50]. Since the gold standard is to choose normative data from individuals with similar characteristics to those of the participants who are being tested, we believe that the Norwegian reference values are the most representative for comparison with our cohort of allo-HSCT survivors.

### 6.4. Pulmonary function tests: reference values

Reference values (also known as predicted values) provide a context for comparing the pulmonary function values of an individual – or a study population - to measurements from a reference population. In general, a reference population should comprise healthy individuals with similar characteristics for the variables that affect pulmonary function; i.e. age, gender, height and ethnicity.

In 1993, the European Respiratory Society (ERS) published reference values for dynamic and static lung volumes and DLCO in adults of Caucasian descent [43]. In 2012, the Global Lung Function Initiative (GLI) published prediction equations for dynamic lung volumes for age 3 to 95 years [44]. In 2017 the GLI published prediction equations for DLCO, for age 5 to 85 years, endorsed by ERS and ATS [45]. GLI reference values for static lung volumes were published in 2021 [63], but were not available at the time when we wrote our papers. The pulmonary function tests in our two study cohorts
were obtained approximately 5 years apart. Several sets of reference values were used, as outlined in table 2.

**Spirometry (FVC, FEV₁)**
For cohort 1 (the ALL-study, paper I) all tests had been carried out before The Norwegian Respiratory Society recommended the use of GLI-2012 in 2016. When ERS endorsed GLI-2012, the Norwegian Respiratory Society appointed an expert group to prepare national recommendations for spirometric reference values. The group studied the fit of the GLI values to three sets of values from Norwegian population studies and compared to the ECSC-1993 values, and concluded that GLI-2012 fit the Norwegian data satisfactorily and recommended GLI-2012 for use in Norway [64]. In accordance with the Norwegian recommendations, we used the GLI-2012 for cohort 2 (the allo-HSCT study, paper II and III).

**Gas diffusing capacity (DLCO)**
For cohort 1 (the ALL-study, paper I) we used the 1993 ERS-recommended reference values. We submitted the paper before the GLI-2017 recommendations were published. For cohort 2, paper II, we also used the ERS-1993 reference values. All the analyses were carried out in 2019, and at that time our lab had not yet switched to GLI-2017. When we started analyzing data for paper III in 2020, we decided to use the GLI-2017, even though we had used different reference equations for the previous paper (that included 90 of the 104 subjects). The decision also implied that we saw it useful to compare with a group of age- and gender matched healthy controls.

**Static lung volumes (TLC, RV)**
We used the ERS-1993 recommended reference equations for the analyses in both cohorts (all three papers). There are no Norwegian reference values available, and the new GLI references had not yet been endorsed by ERS and published at the time when we wrote our articles.

**Reporting results**
The 2005 ERS/ATS guidelines recommend the 5th percentile to be used as the lower limit of normal [46]. Per definition, only 5 percent of healthy subjects will have a test below this limit. For pulmonary function, 80% of predicted value is commonly used as a cut-off value (% of predicted = observed value/predicted value x 100). It may be valid as a surrogate for the lower 5th percentile in young adults of average height – like the individuals included in our studies. However, for an index such as FEV₁ which is larger in taller subjects and also declines with age, the use of % predicted may lead to more elderly and shorter individuals being classified as abnormal than the original regression data would support [65]. In the analyses in our papers, results of pulmonary function tests have been used as continuous data. We used 80% of predicted as a cut-off (and not LLN) when it was considered useful to categorize (normal vs impairment). The main reason for choosing to use 80% predicted, was that the concept is still the most common way of expressing pulmonary function results. It is familiar and –
unlike lower limit of normal – it is easy to relate to for doctors with different specialties and other medical professionals. We would like our papers to be accessible not only to pulmonologist, but to oncologists, hematologists and general practitioners, as well as to those survivors who feel inclined to read papers in medical journals.

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) has recommended to use a post-bronchodilator fixed ratio of $\text{FEV}_1/\text{FVC} < 0.70$, as criterion for air flow limitation [66]. This fixed ratio is in contrast to the lower limit of normal recommended by ATS/ERS [67]. The GOLD stresses that the use of a fixed ratio to define airflow limitation, may result in more frequent diagnosis of COPD in older persons, and less frequent diagnosis in younger adults, compared to lower limit of normal [67].

6.5. Echocardiography

All survivors in both cohorts were examined with transthoracic echocardiography (paper I and II). The examinations took place in the same echocardiography-lab and the same types of scanners were used (Vivid 7 or E9 from GE-Vingmed Ultrasound, Horten, Norway). The recordings were performed by one single, highly experienced sonographer who did 91% of the recordings in cohort 1 (the ALL-survivors, paper I) [68] and 100% of the recordings in cohort 2 (allo-HSCT-survivors, paper II) [69]. All recordings were reviewed by senior cardiologists who were dedicated to the two respective studies.

For cohort 1 (paper I), control subjects were randomly selected from the North-Trøndelag Health Study (HUNT3) that was carried out in 2006-2008. The controls were matched 1:1 for gender, age, weight, body surface area and systolic blood pressure [68]. For cohort 2 (paper II) healthy volunteers responding to advertisements were recruited as controls [69]. With 104 survivors, it was estimated that 55 controls were needed to obtain a power >0.80. The controls were selected to match the survivors with respect to gender, age, height and BMI. The only exclusion criterion was established cardiovascular disease.

6.6. High Resolution CT (HRCT)

Inspiratory and expiratory high resolution CT scans were obtained from the allo-HSCT survivors in cohort 2 (Paper III). Methodologically, HRCT patterns can be evaluated either visually by a radiologist or by applying computer software technics. Computer software has limited clinical use today, except to estimate total lung volume and the degree of emphysema [70]. The method is not commonly used for evaluating interstitial changes. The visual evaluation of the radiologist is mainly done by pattern recognition, followed by determination of the pattern(s) extent. Visual evaluation is widely applied in evaluating interstitial changes. Each method has its strengths and weaknesses.
Traditionally, the radiologist’s analysis of HRCT after allo-HSCT was used to detect the different types of diseases that may occur after such treatment. Often, the implication of the HRCT findings would be interpreted in light of symptoms and pulmonary function tests, in order to evaluate the clinical significance and severity. The strength of this method is that it is well-established, and it is the result of collaboration between clinicians and radiologists. One methodological weakness is inter- and intra-observer variations in the visual evaluation of the HRCT findings [71], which may be reduced by standardized training of the interpreting radiologists.

A software-based analysis of HRCT has been suggested as an alternative to visual assessment by the radiologist. There are different ways to assess a software-based analysis, which is beyond the scope of this thesis to discuss. In short, software-based parameters from HRCT may be derived from density and volume measurements, relying on a threshold for normal values. For example, a small difference in density and volume measurement between expiration and inspiration may be indicative of air trapping [72, 73]. Software-based parameters derived from HRCT may be applied in different clinical settings. A review reported threshold values for emphysema, that has been validated clinically and pathologically [70]. Further, the authors highlight the heterogeneity between the studies and call for international consensus to reach a standardized protocol for how to conduct a HRCT [70]. Apart from a review, there is a paucity in documentation regarding software-based analysis of HRCT in pulmonary disease following allo-HSCT. One retrospective, international multicenter study has been undertaken in subjects with ‘moderately severe’ BOS after allo-HSCT. The authors found that geographical site had negligible effect on the HRCT results [74]. They concluded that such analysis is particularly accurate in diagnosis of early-stage disease BOS, despite its limitations [74].

Measured by HRCT, the term ‘lung attenuation’ reflects the combined influences of the volume of gas, blood (and extravascular fluid) and the density of lung tissue [52]. The lung attenuation is higher at the end of expiration than at full inhalation, due to lower volume (i.e. less area for the lung parenchyma). Air trapping is retention of air distal to an obstruction in the lung [51]. In HRCT at end-expiration, air trapping is seen as ‘less than normal increase in attenuation’ [51], i.e. low attenuation as if no expiration. Further, it may be difficult to differentiate air trapping from areas with decreased attenuation as a result of low perfusion [51]. Air trapping on HRCT, detected by the established visual method, would be done as described above. Software-based air trapping would be derived from density- and volume measurements, relying on a threshold value. Such threshold value may be validated in emphysema [70], but no threshold value is established in pulmonary disease following allo-HSCT. In addition, vascular disease may affect allo-HSCT survivors. Since lung attenuation reflects the volume of blood as well, any present vascular disorder will also affect the total lung attenuation. In our study of allo-HSCT survivors, we chose visual evaluation of HRCT, instead of a software-based evaluation, since the first is the only well-established method. We reported air trapping...
as a percentage of lung parenchyma involved. A software-based computer analysis may be promising for evaluating pulmonary disease in allo-HSCT survivors, but it is still not a fully validated method. Several studies have reported associations between air trapping and HRCT. This will be discussed in detail later, methodologically divided into visual- or software-based studies.

Pleuroparenchymal fibroelastosis (PPFE) is a pattern of interstitial disease that is recognized as a special type of late-onset noninfectious pulmonary complication following allo-HSCT [75]. In a recent review, PPFE post allo-HSCT was found to be mainly induced by cGVHD, since it had an association to BOS [75] and not to chemotherapy. In line with this perspective, we chose to report PPFE as an entity of its own, i.e. to report both PPFE and other interstitial diseases.

6.7. Statistics

Standard statistical methods and analyses were applied in all three studies. The tests were used to describe, to compare, to identify correlations and associations, and to test various hypotheses. A two-tailed p-value of <0.05 was regarded as statistically significant.

Random error may be caused by type I and type II errors, also known as alpha and beta errors. A type I error implies rejecting a null hypothesis that is true (false positive) and a type II error is rejecting a null hypothesis that is false (false negative). The value of alpha is determined in advance and usually set as 5%. The value of beta depends both on sample size and the size of the effect that is considered to be of interest. Usually, we refer to the power of the study; i.e. the power to detect an effect of a specific size. In our studies, small sample sizes give potential for type II error. Although our allo-HSCT cohort was nationwide, the sample numbered just above 100 survivors. Norway is a small country (5.7 mill at the time of the study) and allo-HSCT is a treatment offered to only a selected group of patients with severe hematological disorders. In general, small sample size and the risk of type II error will be the case in studies of rare diseases with rare outcomes. Our three studies were sub-studies within the context of two large projects. In both projects, it was assumed that approximately 70% of the eligible survivors would accept the invitation and attend the studies, based on experience from previous Norwegian studies of late-effects after cancer treatment. Our single-centre samples of around 100 young long-term survivors of, respectively, childhood ALL and allo-HSCT, represent larger samples from these two unique patient groups than what has previously been reported. In the majority of prior studies carried out in comparable survivor populations, fewer participants have been included. So, with our nation-wide cohorts, the likelihood of type I and II errors is reduced, compared to smaller sample-sized cohorts.
6.8. Internal validity

6.8.1. Selection-, survival and recall bias

The validity of our studies may be limited by selective non-response bias and survivorship bias. Due to very long-term follow-up and an attendance rate of 66%, selective non-response bias may influence the results. In cohort 1, we did not find any significant differences between study subjects and non-participants with respect to gender, age at diagnosis and years of observation while in cohort 2, the non-responders were younger and comprised more males than the study subjects. This may be interpreted as a lower risk of non-response bias in cohort 1 than in cohort 2. The potential impact of response bias has been addressed by Lie et al in a population-based Norwegian health survey among survivors of childhood, adolescence and young adulthood cancers [76]. They found low risk of non-response bias in their data, which may help strengthen the validity of our findings. In their survey, survivors of childhood cancer comprised one third and the time since diagnosis was (median, range) 16 (5-30) years, which both correspond well with diagnosis and follow-up in our two cohorts.

Mortality in our two cohorts was 34% for ALL and 44% for allo-HSCT. High mortality may cause survival bias, with a shift of results towards a more favourable outcome. Regrettably, we have no information on time of death or cause of death so we cannot distinguish between short-term and late occurring death, or between death because of relapse of hematological disease and other causes. However, one purpose for examining pulmonary function and cardiorespiratory fitness in the young cancer survivors in our studies was to provide a “post-treatment status” that may contribute to establish evidence-based recommendations and a strategy for further follow-up in their adulthood.

In cross-sectional studies with long-term follow-up, recall bias may affect the reliability of information obtained by questionnaires. In our studies, recall bias may in particular have affected information on smoking habits and especially data on pack-years must be interpreted with caution.

6.8.2. Causality, associations and confounding factors

Due to a cross-sectional design, we could not establish causal links between exposure and outcome, but we could identify significant associations. The risk of disease may be described as an association between an exposure and the probability of having developed disease, or, in our case, the probability of late-occurring adverse effects of chemotherapy. To determine the risk of disease, the study population needs be divided into exposed and unexposed. In our study cohorts, all subjects had been treated with chemotherapy, so the overall comparisons had to be with reference materials that include healthy subjects. In cohort 1, all subjects had been treated with vincristine and methotrexate, while additional anthracyclines had been given to 77% and cyclophosphamide 33% of the patients. We found that survivors who had been exposed to anthracyclines had lower left ventricular EF and lower % predicted VO2peak than those who had not been exposed. In cohort 2, the myeloablative regimens
comprised busulfan/cyclophosphamide or cyclophosphamide alone in all but two patients. Approximately 45% of the allo-HSCT survivors had also received chemotherapy intravenously for malignant diseases prior to allo-HSCT. That group had 2.4-fold and 4.5-fold increased risk for, respectively, pathological findings on HRCT and BOS, and also significantly lower DLCO % predicted and higher occurrence of left ventricular dysfunction than those who had not been exposed to intravenous chemotherapy prior to allo-HSCT.

The late effects of treatment for severe hematological diseases are difficult to isolate from each other due to potential and complex interactions between variables and the risk of confounding. A simple way of explaining the term confounding is ‘confusion of effects’. In order to be confounder, a variable must be associated with both the exposure and the outcome. In the setting of our studies, confounding denotes variables that compete with the exposures of interest in explaining the outcomes. Confounding variables may influence an association between an exposure and an outcome by either modifying or strengthening the observed association. We used multiple linear and logistic regression models to control for (or minimize) the impact of confounding variables. However, the possibility of unknown confounders must always be taken into account.

6.8.3. Healthy controls

For the first two studies (paper I and II) we did not recruit healthy controls. For the third study (paper III) we thought a group of age- and gender matched controls would be appropriate for comparison of pulmonary function. The main reason for adding a group of healthy controls was that we opted to use new reference values for DLCO in paper III. In 2017 the Global Lung Function Initiative (GLI) published new reference values for DLCO that were endorsed by the European Respiratory Society (ERS). In 2019, when we carried out the analyses for paper II, we did not have access to the “new” GLI material for DLCO in our lab and we therefore used the older ECSC reference values in that paper. However, when we analysed data for paper III in 2020/21, we decided to use the GLI-references. At the same time, our pulmonary function lab decided to upgrade to GLI. Since we had published results for DLCO % predicted based on the old ECSC-reference values in paper II and would use the GLI reference values in paper III, we thought it would be wise to see how a group of age- and gender matched healthy controls would fit the comparisons. Ideally, we would have liked to examine also the controls with HRCT, but found it unethical to expose healthy individuals to irradiation for research purpose only. For the echocardiographic study, 55 healthy volunteers were recruited. The sample size was estimated in order to obtain a power >0.80. The controls were matched for gender, age, height, and BMI [69].
6.8.4. Observation time

Both in cohort I and II, median follow-up of was very long (23 and 17 years respectively) and the range was wide (7-40 and 6-32 years respectively). The journal’s editor of paper II suggested that we should look into the possibility of analyzing data according to elapsed time (e.g. in categories of 5-year periods). Figure 5 shows the distribution (mean, SD) of survivors and years of follow-up for cohort 1 (ALL-study) and cohort 2 (allo-HSCT study). There are a few outliers in both cohorts, but the large majority of subjects cluster around the mean values. We therefore concluded that it would not be useful to divide into categories of 5-year periods since there would be very few subjects in some of the groups, resulting in lack of power to show significant differences.

![Figure 7 Histograms of number of survivors (Y axis) and follow-up time (X axis) in years with normal distribution curve.](image)

6.9. External validity

External validity refers to the extent to which the results can be generalized to a wider population. Ideally, we would like our findings to be universally generalizable to all long-term survivors of ALL and allo-HSCT, but in real life, some limitations to external validity will be the rule. Norway and the other Nordic countries (Sweden, Denmark, and Finland) have similar public healthcare systems. For all citizens, taxation funds the costs related to medical treatment, including follow-up examinations, drugs and supportive care. Thus, neither economic status nor access to private insurance will influence the ability to receive relevant medical care. These socio-economic factors, along with ethnicity, should be taken into consideration whenever generalizing results from studies like ours.
We think it is reasonable to assume that the results from the ALL-study (cohort 1) may be generalized to other ALL survivors within the same age- and time range in the Nordic countries, since they were treated in accordance with common Nordic protocols. Also, since the public healthcare systems are similar in the Nordic countries, similar strategies and measures for follow-up after treatment may be assumed. With due caution, the results may also be generalized to ALL survivors of different ethnicity, older age, other types of leukemia and after other multidrug chemotherapy regimens.

When it comes to generalizing results from the allo-HSCT studies, there are more limitations. Overall, the results may be generalized to allo-HSCT survivors of Caucasian ethnicity who were treated in childhood, adolescence and early adulthood (<30 years old) and conditioned with busulphan and cyclophosphamide. Only with caution may the results from our study be generalized to older patients, patients conditioned with total body irradiation or with underlying diagnoses other than hematological (i.e. lymphoma and solid tumors).

7. Discussion of main results

The overall hypothesis of the present research project was that survivors of severe hematological diseases in childhood, adolescence and young adulthood, treated with chemotherapy only or with allo-HSCT, may develop late-onset, non-infectious pulmonary complications that may affect pulmonary function and physical fitness.

7.1. Effects of chemotherapy for ALL on pulmonary function (Paper I)

The adult survivors of childhood ALL had pulmonary function in the lower predicted range median 23 years after treatment with chemotherapy, and approximately one fourth had impaired DLCO. Risk factors for impaired DLCO were female gender, smoking and BMI.

In a review, female gender was reported to be associated with higher risk of several adverse outcomes after cancer treatment, when compared to males [77]. An association between female gender and impairment in DLCO in survivors of lymphoma, leukemia and solid tumors was reported by Armenian et al in 2015, and more recently confirmed by Agrusa et al in a study of lymphoma survivors [12, 78]. Pathophysiologically, gas diffusing impairment may be linked to microvascular destruction, in addition to alveolar damage [79]. A possible explanation why females seem to be more susceptible to gas diffusing impairment than males may be related to female sex hormones in combination with smaller lung- and vascular volume [80]. We did not find any differences between the genders in smoking habits that could explain why females should have lower DLCO than males. However, we did not record pack-years, and also the study may not have been sufficiently powered to
detect an association. Although gender differences are most likely multifactorial and not fully understood, we should keep in mind that female ALL survivors probably have an increased risk of DL\textsubscript{CO} impairment compared to males.

Nineteen percent of the ALL survivors reported to be daily smokers, which is 4%-points higher than the Norwegian population average at that time (Statistics Norway). When adjusted for age, the difference exceeds 5%-points (Statistics Norway). In addition to development of an obstructive impairment on long-term basis, smoking will cause a transient rise in the level of carbon monoxide (CO) in plasma. This effect is called “back-pressure”, and lowers the measured DL\textsubscript{CO} [79]. The study participants were instructed to refrain from smoking on the day of pulmonary function testing (prior to testing) in order to reduce the reversible effect on DL\textsubscript{CO} caused by CO back-pressure. Even so, in the multiple regression analysis we found that smoking was significantly associated with a reduction in DL\textsubscript{CO}. This may perhaps – in part - be explained by CO back-pressure. Since CO-Hb was not measured, we cannot be sure that all the smokers had followed the instructions and refrained from smoking prior to testing. The survivors had mean FEV\textsubscript{1} and FEV\textsubscript{1}/FVC well above lower normal limit, but 7 (6%) subjects were classified with mild obstructive impairment, none of them had asthma. We did not record pack-years, but since the survivors were in their early adulthood (median 29 years old), it is unlikely that the burden of smoking had been heavy enough to cause clinically manifest obstructive airways disease with elements of emphysema. We think it is more probable that reduced DL\textsubscript{CO} in ALL survivors may be an early sign of smoking-induced permanent lung injury, in spite of the survivors’ young age and relatively low burden of smoking. One may speculate that lungs exposed to multi-drug chemotherapy in childhood may be more sensitive and vulnerable to the detrimental effects of smoking than lungs with no such previous exposure. We may assume that the survivors had normal pulmonary function prior to chemotherapy, since they were children at the time of diagnosis (median 5 years old) and had presumably little or no history of smoking or other exposures that may affect lung function. At follow-up, five (4%) of the survivors reported doctor-diagnosed asthma, but otherwise there was no history of pulmonary disease. We do not know if the five subjects with asthma were diagnosed before or after they were treated for ALL, but 4% occurrence is in line with data from the Norwegian Institute of Public Health (NIPH) that reported a stable prevalence of asthma around 5% for the age group 0-44 years in the period 2008 to 2018 [81]. Furthermore, mild asthma is not likely to harm pulmonary function permanently, and in particular not affect DL\textsubscript{CO}.

Increasing BMI was weakly associated with increasing DL\textsubscript{CO} % predicted. Present data on the possible effects of obesity on DL\textsubscript{CO} is unclear, and the results from numerous studies are conflicting [82-84]. An association between obesity and both increased DL\textsubscript{CO} [79, 83] and decreased DL\textsubscript{CO} [82] has been reported. A recent review concluded that obesity generally has minor effect on DL\textsubscript{CO} [84]. In
our study, there were borderline significantly more females than males with overweight \( (p=0.047) \) and obesity \( (p=0.07) \).

No significant associations between impairment in \( DL_{CO} \) and cumulative doses of chemotherapy were found. This is in line with that reported by Armenian et al \[12\]. All the drugs that were used in the chemotherapy regimens for ALL have been linked to pulmonary toxicity. Probably due to concomitant treatment and clusters around the median doses, any significant relationship to individual drugs or cumulative doses were difficult to detect. Also, a dose-response relationship would not be possible to detect as there were small differences between the individual doses.

In a clinical context, a subclinical impairment in pulmonary function may become important in a future perspective. The young adult survivors of childhood cancer have the potential of a normal lifespan, but they also have an increased risk of both cardiovascular disease and secondary cancers \[85\]. If renewed treatment with potentially lung toxic agents should be indicated, it is not known how already vulnerable lungs may be affected. A subclinical pulmonary impairment may become clinically important also in combination with chronic cardiovascular disease. It is not known how pulmonary function will develop in the further adulthood of the survivors. It is therefore advisable for these young adults (in particular) to avoid harmful exposure such as smoking. To this end, tailored information and advice on smoking cessation should be provided in clinical oncology-settings.

### 7.2. Effects of chemotherapy for ALL on cardiorespiratory fitness (Paper I)

Forty-two percent of the ALL survivors had impairment in \( VO_2 \) peak, defined as <80% of predicted. For the survivors as a group, however, mean \( VO_2 \) peak was within normal range. Smoking and BMI were identified as risk factors for reduced \( VO_2 \) peak.

Impairment in \( VO_2 \) peak in survivors of childhood leukemia was reported in a review by van Brussel et al in 2005 \[86\]. Later, Tonorezos et al found that adult, long-term ALL survivors \( (n=115) \) had substantially lower \( VO_2 \) peak than matched healthy controls > 15 years after treatment \[87\]. In that study, two-thirds of the survivors were classified as having low cardiorespiratory fitness, which is a much higher proportion than that in our cohort \( (42\%) \). The explanation may possibly be differences in both test procedures and in definitions of low cardiorespiratory fitness. Tonorezos et al also found a relationship between cumulative dose of anthracyclines and impaired cardiorespiratory fitness in males, but not females. A similar relationship could not be found in our cohort. A more recent and larger study of 247 ALL survivors by Lemay et al reported mean \( VO_2 \) peak 86% of predicted \[88\], which is similar to 85% of predicted in our cohort. Two-third of the survivors in Lemay’s study were classified as physically inactive, and their cardiorespiratory fitness was reduced by 16% compared to the mean \( VO_2 \) peak % predicted \[88\]. The survivors in both Tonorezos’ and Lemay’s studies were comparable to the survivors in our study with respect to observation period and age at follow-up.
However, some differences between the studies may hamper the comparability. Like in our study, Lemay et al applied a cycle ergometer in the exercise test, while Tonorezos et al. used a treadmill. All three studies used different reference values and cut-off values for impairment in VO₂ peak. Tonorezos and Lemay included survivors treated with radiotherapy, while that was a criterion of exclusion in our study. However, and regardless of the differences between the studies: they all conclude that a substantial proportion of long-term ALL survivors had impaired VO₂ peak, and that reduced cardiorespiratory fitness was linked to lifestyle factors (overweight, physical inactivity and smoking).

We did not find an association between cumulative doses of chemotherapy (doxorubicin equivalent) and VO₂ peak. The explanation is most likely related to different treatment regimens consisting of several cytotoxic drugs. All subjects had received vincristine, 95% methotrexate, 77% anthracyclines (doxorubicin equivalent) and 33% cyclophosphamide. Additionally, there were clusters around the median doses, which represented a methodological challenge. An association between chemotherapy and VO₂ peak was recently reported by Caru et al in a study that included 216 ALL survivors, stratified with respect to prognostic risk group - standard or high-risk disease [89]. Most likely due to a large study population and large spread in treatment doses between standard and high-risk disease, the authors were able to detect an association between chemotherapy (doxorubicin) and VO₂ peak [89]. The high-risk group had received a higher dose of doxorubicin equivalents than standard risk survivors.

In our study, BMI and smoking were identified as risk factors for impairment in VO₂ peak % predicted. Regrettably, information about physical activity was not recorded. In general, smoking and overweight are associated with a sedentary lifestyle [90]. In the above-mentioned study by Lemay et al, it was found that two thirds of the ALL survivors were physical inactive according to national (Canadian) recommendations. One may assume that overweight and smoking were associated with physical inactivity also in our cohort, but we do not have data to prove it. The lack of information about physical activity was a limitation to our study. Therefore, in our subsequent study of VO₂ peak in allo-HSCT survivors, we made sure to include assessment of physical activity in the protocol (Paper II).

VO₂ peak, peak heart rate and SpO₂% were the main CPET variables recorded for analysis in our ALL study. This basic information may have been too scarce to find out whether the survivors were limited by pulmonary-, and/or cardiac factors or if they were just deconditioned. Therefore, in our subsequent allo-HSCT cohort, we expanded the number of variables from CPET in order to be able to categorize the survivors according to organ limiting factors (Paper II).
7.3. Long-term pulmonary sequelae in survivors of allo-HSCT (Paper III)

The term ‘pulmonary sequelae’ was applied to cover both impairments in pulmonary function and pathological findings on HRCT. Various types of sequelae were detected in the allo-HSCT survivors.

BOS is the best-described long-term pulmonary complication after allo-HSCT and may be defined as a manifestation of chronic GVHD in the lung. In the literature, BOS is reported to occur in 3-10% of allo-HSCT survivors [91, 92] and is usually diagnosed within the first two years after allo-HSCT [26, 92]. In our cohort of long-term survivors, the prevalence of BOS was 12%. Due to the cross-sectional design of the study, we do not know how early after allo-HSCT BOS occurred.

The NIH diagnostic criteria for BOS after allo-HSCT differ from those after lung transplantation [24, 93]. One of the NIH criteria is >10% decline in FEV₁ % predicted during a 2-year period. Due to the cross-sectional design of our study, this criterion could not be applied. In addition, the NIH criteria for BOS require the presence of a distinctive other-organ manifestation of cGVHD. If BOS is the only clinical manifestation, a biopsy is required to establish the diagnosis [24]. Since the allo-HSCT survivors did not undergo lung biopsy, we decided to classify airways obstruction as BOS if all other reasons for obstructive impairment could be ruled out. That was the case in all except two subjects. All the other survivors with BOS had other-organ manifestations of chronic GVHD and thereby fulfilled the NIH-criteria. The number of cases with BOS was 11 in Paper II and 12 in Paper III. The explanation is that 13 more subjects were included in paper III. The subjects who could not perform CPET due to medical contraindications or had received TBI were excluded in paper II, but could be included in Paper III.

Impairments in pulmonary function, with restrictive and/or obstructive pattern, in addition to reduced gas diffusing capacity, are known complications to allo-HSCT [94, 95]. However, similar findings have been reported after curative treatment of hematological malignancies without allo-HSCT [96, 97]. Also in our ALL cohort (paper I), different types of pulmonary impairments were found. Further, in late-onset, non-infectious pulmonary complications after allo-HSCT, chronic bronchitis may occur, in addition to BOS [95]. This means that airway obstruction may be an unspecific finding since it may originate from the treatment per se, without the involvement of cGVHD. That said, in our study, the survivors with obstructive impairment fulfilled the diagnostic criteria for BOS in all but two cases.

When compared to healthy age- and gender matched controls, the allo-HSCT survivors had lower lung volumes (TLC-, FVC-, FEV₁ % of predicted) and gas diffusing capacity (DLCO % of predicted). However, compared to reference values (see Table 2 for cut off values), the mean lung volumes (TLC-, FVC-, FEV₁ % of predicted) were within normal predicted range.

In the HRCT scans, almost half of the survivors had one or more pathological findings. The most frequent pathological pattern was airways disease that was found in 71%, while interstitial
changes were discovered in 35%, and apical subpleural interstitial thickening was detected in 24%. As earlier outlined, we chose to differentiate between the latter two patterns, even though they both represent interstitial pathology. Apical subpleural interstitial thickening may be indicative of pleuroparenchymal fibroelastosis (PPFE) that may be associated with BOS [75]. In our study of allo-HSCT survivors, however, no association between BOS and apical subpleural thickening was found. Only the collective finding termed ‘any pathological changes on HRCT’ was significantly associated with BOS in our cohort. The lack of associations between specific radiological changes and BOS may possibly be explained by the heterogeneity of pathological patterns, in addition to the overlap between various, different patterns. Such an assumption may be supported by a study by Meignin et al. who evaluated histological changes in lung parenchyma of 61 survivors of allo-HSCT who had developed LONIPCs [98]. They describe several different histological patterns and affected anatomical areas, including bronchiolar and alveolar/interstitial changes. They did not find any correlations between histopathology, radiology and pulmonary function tests [98].

The most frequent finding on HRCT was airways disease. The diagnostic pattern was based on one or several of the following findings: signs of air trapping, mosaic pattern, micronodules and bronchiectasis. Of these, air trapping was the single most frequent isolated finding on HRCT. Signs of increasing air trapping were associated with impaired pulmonary function (expressed as respectively RV % of predicted and FEV\textsubscript{1} % of predicted). Radiological changes and pulmonary function impairments have also previously been shown to be present in allo-HSCT survivors [74, 99-102]. Two of the studies reported air trapping visually evaluated [99, 102], while three studies demonstrated relationships between pulmonary function tests and computer software-based variables equivalent to air trapping [74, 100, 101]. Due to use of different techniques in HRCT analysis, as well as different definitions and methods of reporting results, comparisons between the studies are difficult to make. Our contribution to the literature will be that radiological changes and impairments in pulmonary function are evident also many years (median 17 years) after allo-HSCT, and that some of the radiological findings are significantly linked to pulmonary function.

We calculated air trapping as a percentage of lung parenchyma involved, in all images of the HRCT expiratory scan. Air trapping equal to or more than 5% of affected parenchyma was defined as pathological. Different ways to report the degree of air trapping visually evaluated have been suggested. Bergeron et al defined air trapping as a score of 1 to 4, scoring only three different HRCT levels [99]. In doing so, the detection level is likely to become lower than evaluating air trapping in every single image, as we did. We found correlation between the percent of air trapping as a continuous variable and pulmonary function in terms of FEV\textsubscript{1} and RV. Bergeron et al did not report any associations between pulmonary function and air trapping. A computer software analysis may be promising for evaluating pulmonary disease in allo-HSCT survivors, but the method is still at an experimental stage. There is no international consensus as to how air trapping should be analyzed and
reported after allo-HSCT, and the lack of consensus becomes evident when comparisons between studies are reported.

7.4. Long-term effects of allo-HSCT on cardiorespiratory fitness (Paper II)

In the multiple regression analysis, BOS and low DLCO were found to be independently associated with low VO2peak, and could thereby be identified as pulmonary limitations to cardiorespiratory fitness. The findings are in line with those reported in a Danish study of pediatric allo-HSCT survivors [103]. Although the studies are not directly comparable - the Danish one was performed in children and ours in young adults (mean age 14 vs 35 years) and the observation periods were 7 vs 17 years - both studies showed that airways obstruction and low DLCO were limiting factors to VO2peak.

In long-term survivors of allo-HSCT, cardiac limitations to cardiorespiratory fitness have been more widely reported in literature than pulmonary limitations. In 1992, Larsen et al described severely reduced cardiac reserve as a limiting factor to exercise performance in a small study of young allo-HSCT survivors [104]. In 2000, Hogarty et al reported impaired cardiovascular fitness in survivors with subclinical cardiac dysfunction, with dose of anthracycline as a predictor [105]. Armenian et al found that abnormal left ventricular global longitudinal strain was associated with reduced cardiorespiratory fitness in a small group of allo-HSCT survivors [106]. In our study, we found that low VO2peak was associated with low LVEF. Long-term effects of allo-HSCT on cardiac function is a separate and on-going PhD project within the same main project as our pulmonary project, and detailed echocardiographic results will be reported by co-researcher Massey et al. In brief: In the first study, Massey et al found that the allo-HSCT survivors had significantly reduced left ventricular systolic function compared to controls, and that left ventricular systolic dysfunction (found in 44%) was associated with anthracyclines, GVHD and hypertension [69].

We chose to categorize limitations in cardiorespiratory fitness as either organ-specific (pulmonary and cardiac) or deconditioning, based on variables derived from the exercise test. However, a cardiopulmonary exercise test is best suited to assess the global effects of cardiac and pulmonary limitations on an individual level, and interpretation of test results must always be done in light of the individual’s medical history and general physical fitness. For example, an abnormal VE/Vco2 slope may represent gas exchange limitations, but in the context of cardiac disease, it may also represent cardiac failure. The ATS/ACCP statement on cardiorespiratory exercise tests emphasizes that deconditioning may be difficult to distinguish from heart disease and that clinical history is “extremely helpful” in making the distinction [35]. Furthermore, an individual may have more than one organ-specific limitation, which adds to the difficulty of interpreting the test results.

We found that deconditioning was an important contributor to reduced cardiorespiratory fitness in allo-HSCT survivors. Among the 43% in our cohort with low VO2 peak, 56% did not have organ-
specific impairments and were categorized as deconditioned. Several variables from the cardiopulmonary exercise test may indicate deconditioning: low VO₂ peak, low anaerobe threshold and little or no heart rate reserve and submaximal VE [35]. Knowledge about deconditioning in allo-HSCT survivors is limited. In a study of 71 survivors one year after allo-HSCT, Dirou et al reported that 75% had abnormal VO₂ peak % of predicted and 38% were categorized as deconditioned [107]. The authors defined deconditioning as ventilatory threshold <40% predicted, while in our cohort deconditioning was defined as low VO₂peak in the absence of organ-specific limitations. The higher proportion classified as deconditioned in Dirou’s study than in ours (38% vs 24%) may partly be explained by different definitions of ‘deconditioning’ and partly by different observation periods (1 vs 17 years). One year after treatment, many patients will still be affected by inactivity related to long periods of hospital care, while after 17 years survival bias will influence the results.

Physical inactivity and overweight are related lifestyle factors. Nearly two third of the allo-HSCT survivors in our study did not fulfil the WHO recommendations for physical activity and nearly half were overweight, of whom many had features of metabolic syndrome (hypertension and dyslipidemia). There is strong evidence that physical activity contributes to reduce the risk of the most common forms of cancer. In a review, the relative risk reduction was reported to be in the range 10-20% [108]. Thus, it is likely that life style changes may be even more important in cancer survivors (with a risk of secondary cancers) than in the general population, and counselling on modifiable risk factors should therefore be prioritized in clinical oncology settings.

When comparing the proportions of survivors with impairments in VO₂ peak in our two study cohorts, i.e. the ALL and the allo-HSCT cohorts (Paper I and II), we were somewhat surprised that they appeared to be similar (43% vs 42%). We assumed that the ALL survivors would have better preserved VO₂peak than the allo-HSCT survivors, due to a lighter burden of treatment. However, several differences between the studies may contribute to explain the seemingly similar results. One important difference is that CPET was carried out on a bicycle in the ALL study, while the allo-HSCT survivors were tested on a treadmill. It is well known that VO₂peak will be 5-10% higher on a treadmill than on a cycle ergometer [35]. Further, different reference values and different definitions of impairment in predicted VO₂peak were used. In the ALL-study, we used American reference values since Norwegian ones are not available for bicycle testing, and VO₂ peak <80% of predicted was used as cut-off. In the allo-HSCT study, we used the Norwegian reference values for treadmill testing and the ATS/ACCP recommended cut-off that is <85% of predicted. If the same test methods had been used in the both studies, possible differences between the two cohorts could have been detected.

7.5. Ethical considerations

The studies were approved by Regional Committee for Medical and Health Research Ethics for South Eastern Norway (reference 2.2007/522 and 2014/370) and the Data Protection Officer at Oslo
University Hospital (reference 2014/4331 and 2015/10025). Hence, the ethical aspects of the studies have been externally assessed and approved. All participants gave their written informed consent. The survivors who consented to participate were free at any time to declined participation in any parts of the investigations or withdraw their consent. I am not aware of any survivors who have withdrawn their consent, but some did not complete all investigations. In the ALL-cohort (paper I) two survivors did not want to perform the exercise test and in the allo-HSCT cohort (paper III) one survivor refrained from HRCT.

In long-term follow-up studies of survivors who have suffered severe or potentially fatal diseases, it is understandable that some of the survivors will feel morally obliged to participate as pay-back for the health care investments they have benefited from. Also, in studies of survivors of severe blood disorders, the treatment has usually been going on for a long periods of time (months, years), followed by at least 5 years with regular routine check-ups with respect to relapse. Many of the survivors will therefore have a long-lasting relationship with their doctors that in some cases may have stretched from childhood through adolescence and into early adulthood. Since such a close patient-doctor relationship will be asymmetrical in nature – i.e. the survivor owes a debt of gratitude – it may represent a conflict of interest. On the other hand, it may well be that the survivors’ motive for participating in the study stems from a genuine altruistic wish to ‘pay-back’ (to the hospital or to society). Other possible motives may be access to a free, high-quality health check offered by expert physicians within various specialties. Some survivors will be relieved to find out that they are healthy and that no serious pathology was observed while others will appreciate to get information about risk factors and/or impairments in order to take measures that may prevent further development.

For research purpose, we would ideally have wanted 100% attendance. From an ethical point of view, the actual attendance rate of 66% feels more comfortable. The fact that 34% of the ALL-survivors and 33% of the allo-HSCT survivors either declined to participate or did not respond to the invitation may indicate that participation was indeed voluntary and not morally coerced.
8. Conclusion

- Reduced pulmonary function and cardiorespiratory fitness are common long-term findings after curative treatment for severe blood disorders in childhood, adolescence and young adulthood.
- Impairments in pulmonary function is mostly subclinical, but may later become of clinical relevance in survivors who develop progressive impairment, or develop disorders that require treatment with other potentially lung-toxic agents.
- Cardiorespiratory fitness is limited by pulmonary and cardiac factors as well as by deconditioning. The latter is associated with inactivity, overweight and smoking.
- We recommend further follow-up, as appropriate, in survivors with both subclinical and clinically manifest pulmonary impairments, and targeted advice on modifiable lifestyle factors.

**Paper I:** Subclinical impairments in pulmonary function and cardiorespiratory fitness were common in long-term survivors of childhood ALL treated with chemotherapy only. Nearly one fourth of the survivors had reduced gas diffusing capacity, significantly associated with female gender, smoking and increased BMI. More than 40% had impaired cardiorespiratory fitness, associated with smoking and increased BMI. The findings highlight the need for advice on modifiable lifestyle factors such as smoking, overweight and inactivity.

**Paper II:** About half of the long-term survivors of allo-HSCT had reduced cardiorespiratory fitness. Factors significantly associated with impaired cardiorespiratory fitness were bronchiolitis obliterans syndrome, low gas diffusing capacity, low left ventricular ejection fraction, overweight and physical inactivity. Cardiopulmonary impairment(s) and deconditioning were equally common limitations to cardiorespiratory fitness. The findings not only indicate that long-term cardiopulmonary monitoring is warranted in survivors of allo-HSCT, but also the need for targeted advice on lifestyle factors.

**Paper III:** Late pulmonary sequelae were commonly found in long-term survivors of allo-HSCT. The survivors had significantly reduced pulmonary function compared to gender- and age matched healthy controls and nearly half had pathological findings on HRCT. Bronchiolitis obliterans syndrome (BOS), which is a clinical manifestation of chronic GVHD in the lung, was diagnosed in 12% of the survivors. Both pathological findings on HRCT and BOS were associated with chemotherapy prior to allo-HSCT. The cross-sectional design of the study prevents predicting whether the sequelae will remain stable or progress. Therefore, we recommend life-long monitoring of pulmonary function in allo-HSCT survivors with diagnosed sequelae. HRCT is not suited for surveillance, but may provide important diagnostic information and should be performed on liberal clinical indications.
9. Clinical implications and future perspectives

- All participants in the studies were individually informed about medical findings and potential clinical implications. In addition, a summarized written report was sent to their general practitioners and, if appropriate, to their local hospitals, with a plan for further follow-up.
- The impairments in pulmonary function detected in the young adult survivors were mainly of a subclinical nature. However, whether the impairments will remain stable or progress in their future adulthood is unknown. It is therefore strongly recommended that the survivors refrain from avoidable harmful exposure such as smoking. We recommend life-long monitoring of pulmonary function in survivors with both subclinical and clinically manifest impairments.
- A large proportion of the survivors were overweight/obese and physically inactive, and about half of them had reduced cardiorespiratory fitness. Since the survivors are at increased risk for cardiovascular disease, the importance of adhering to national guidelines for diet and physical activity should be encouraged on hospital discharge and at subsequent outpatient follow-up.
- All candidates for allo-HSCT now undergo pulmonary function testing as part of the routine workup prior to treatment, provided they are old enough to perform the procedures. Access to baseline data will enable future longitudinal follow-up studies.
- The National strategy plan for cancer, 2018-2022, from the Norwegian Ministry of Health and Care Services, highlights the need for increased knowledge on late effects of cancer treatment. Our studies have contributed to meet this request.
- Our pulmonary studies were carried out within the framework of two large, multidisciplinary research projects that involve several sub-studies and PhD projects. Results from these studies have been publicly shared among fellow researchers and clinicians at national and international conferences and meetings. Several articles have been published in international peer-reviewed journals, and several more will be published in the near future. In sum, the studies have made significant contributions to the present knowledge on late effects of treatment for severe hematological disorders. They also provide new information that will be useful with respect to evidence-based recommendations for clinical follow-up.
- The findings of our studies highlight the need for new studies designed to identify potential associations between physical activity, physical fitness and metabolic health outcomes. In 2018, a large project on Physical Activity in Childhood Cancer Survivors (PACCS) was initiated as collaboration between the University of Oslo, Oslo University Hospital, Norwegian School of Sports Sciences and hospitals in Finland, Denmark, Germany and Switzerland. The project will hopefully generate new empirical knowledge that will contribute towards the overall goal: to increase long-term survival after treatment for severe blood disorders and reduce the burden of late adverse effects.
10. Reference list


Determinants of cardiorespiratory fitness in very long-term survivors of allogeneic hematopoietic stem cell transplantation: a national cohort study

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Abstract

Purpose Survivors of allogeneic hematopoietic stem cell transplantation (allo-HSCT) are at risk for cardiopulmonary adverse events. Data on long-term effects on cardiorespiratory fitness are limited. To address the gap in knowledge, we aimed to determine peak oxygen uptake (VO2peak) and identify associations between cardiorespiratory fitness and clinical characteristics, self-reported physical activity, cardiac, and pulmonary function.

Methods In a nationwide, single-center cross-sectional study, 90 survivors [aged median (range) 35 (17–54) years, 56% females] were examined, 17 (6–26) years after allo-HSCT. Myeloablative conditioning comprised busulfan/cyclophosphamide or cyclophosphamide only. Methods included pulmonary function tests, echocardiography, and cardiopulmonary exercise test.

Results Chronic graft-versus-host disease (cGVHD) was found in 31% of the subjects, of whom 40% had bronchiolitis obliterans syndrome (BOS). Seventy-one percent of the survivors did not meet WHO recommendations for physical activity and 42% were overweight. Reduced gas diffusion (DLCO) and systolic ventricular dysfunction (LVEF) were found in 44% and 31%, respectively. For the group, mean (95% CI), VO2peak was 36.4 (34.7–38.0) mL/min/kg [89 (85–93)% of predicted]. VO2peak was low at 43%. Cardiopulmonary factors and deconditioning were equally common limitations for exercise. In a multiple linear regression model, low VO2peak was associated with low DLCO, low LVEF, BOS, overweight, and inactivity.

Conclusion Half of the survivors had reduced cardiorespiratory fitness median 17 years after allo-HSCT. Cardiopulmonary factors and deconditioning were equally common limitations to exercise. We encourage long-term cardiopulmonary monitoring of allo-HSCT survivors and targeted advice on modifiable lifestyle factors.

Keywords Allogeneic hematopoietic stem cell transplantation · Long-term follow-up · Cardiorespiratory fitness · Cardiac function · Pulmonary function

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Introduction

Survivors of allogeneic hematopoietic stem cell transplantation (allo-HSCT) are at risk for both cardiac and pulmonary adverse events, caused by the myeloablative conditioning prior to allo-HSCT and the immunological response induced by the hematopoietic stem cell graft after allo-HSCT [1, 2]. In addition, most patients with hematologic malignancies have received chemotherapy with or without radiation before they became candidates for allo-HSCT.

Late-onset non-infectious pulmonary complications (LONIPCs) occur in up to one-fifth after allo-HSCT and comprise leading causes of morbidity and mortality [3]. The most frequent LONIPC is bronchiolitis obliterans syndrome (BOS), which is linked to chronic graft-versus-host disease (cGVHD) [4]. Cardiovascular disease is another cause of morbidity and mortality in long-term allo-HSCT survivors [5]. Risk factors for cardiovascular disease have been reported to be >4-fold higher in allo-HSCT survivors than in the general population [6], with a clear association between multiple risk factors and subsequent development of cardiovascular disease [2].

Measurement of peak oxygen uptake (VO₂peak) through cardiopulmonary exercise test (CPET) represents the gold standard assessment of cardiorespiratory fitness and is useful to assess the global effect of cardiac and/or pulmonary impairments [7]. CPET also provides information about the risk of cardiovascular disease, all-cause mortality, and cancer mortality [8, 9].

Cardiorespiratory fitness in long-term survivors of allo-HSCT has been scarcely investigated [10–14]. Furthermore, most previous studies have been restricted to children [12, 14] or been limited by small and/or inhomogeneous patient materials [11, 13], and follow-up has not exceeded 10 years [10–14]. In Norway, all allo-HSCTs have been performed at Oslo University Hospital. The patients were recruited from the entire country, referred and selected according to uniform criteria, and subjected to standardized treatment procedures. Today, this single-center policy has provided us with a well-defined national patient population available for follow-up.

In the present study, our primary aim was to determine cardiorespiratory fitness assessed by VO₂peak in a nationwide cohort of young very long-term allo-HSCT survivors, who had received myeloablative conditioning with chemotherapy only and not total body irradiation (TBI). Secondly, we aimed to identify associations between cardiorespiratory fitness and clinical characteristics, self-reported level of physical activity, and cardiac and pulmonary function.

Methods

Design and study population

The study was part of a large, nationwide cross-sectional study covering a broad range of late treatment-related effects, conducted at Oslo University Hospital [15, 16]. All subjects were survivors of allo-HSCT, performed in childhood or early adulthood. Inclusion criteria were age <30 years at allo-HSCT, >16 years at the time of the survey, and minimum 5-year follow-up. At the time of the survey, 157 subjects were alive, and 104 subjects participated (Fig. 1). Exclusion criteria in the present study were TBI conditioning (n = 6), cardiovascular contraindications (n = 5), and physical inability to perform CPET, using wheelchair (n = 3). Cardiovascular contraindications included severe, untreated hypertension, coronary fistula to the pulmonary artery, cardiomyopathy, aortic valve stenosis, and significant pulmonary hypertension. In total, 90 subjects aged [median (range) 35 (17–54) years, 56% females] were included. The Regional Committee for Medical and Health Research Ethics (2014/370) approved the study. Written informed consent was obtained from all participants.

Clinical assessment

At follow-up, medical records were reviewed for treatment data. All participants underwent a medical examination, blood sampling, and a standardized interview. Age, gender, body mass index (BMI, kg/m²), smoking habits, physician-diagnosed cardiovascular or pulmonary disease, and current medication were registered (Table 1). Overweight was defined as BMI ≥25 kg/m² and obesity BMI ≥30 kg/m², according to the World Health Organization (WHO) Classification [17]. The self-reported physical activity level was assessed using the WHO recommendations [18]. The survivors were categorized as meeting the recommendations if their activity included ≥150 min/week with moderate-intensity exercise or ≥75 min/week with high-intensity exercise. cGVHD was diagnosed according to the National Institute of Health (NIH) criteria [19]. Hypertension was defined as current treatment with antihypertensive agents.

Blood samples

Fasting blood samples were collected at 8:00 am and analyzed for hemoglobin, lipids, glycated hemoglobin (HbA1c), and N-terminal proB-type natriuretic peptide (proBNP). For Hb and proBNP, the hospital’s reference values for males and females were used (Hb 13.4–17.0 g/dL and 11.7–15.3, and proBNP < 10 pmol/L and < 20 pmol/L in the age group 18–48 years, respectively). Hypercholesterolemia was defined as low-density lipoprotein > 4.1 mmol/L or the use of lipid-
lowering agents. Diabetes mellitus was defined as HbA1c > 6.5% or the current use of antidiabetic medication.

**Pulmonary function**

Pulmonary function measurements (Jaeger Master Screen Body, Würzburg, Germany) were performed in accordance with the guidelines of ERS [20, 21]. Recorded variables were total lung capacity (TLC), residual volume (RV), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and gas diffusion capacity (DLCO). The predicted values for FVC and FEV1 were taken from the Global Lung Initiative [22] and static lung volumes and DLCO from the European Community for Steel and Coal [23]. Obstructive impairment, i.e., BOS, was diagnosed according to NIH criteria [19]. Restrictive impairment was defined as TLC < 80% of predicted value, and gas diffusing impairment as DLCO < 80% of predicted value, corresponding to the lower 5th percentile of the reference material and in line with ERS recommendations [24].

**Echocardiography**

Echocardiography was performed according to international recommendations [25], using E9 scanners from GE (Horten, Norway). All recordings were obtained by one single sonographer and reviewed by a senior cardiologist, both blinded to all clinical data. Left ventricular ejection fraction (LVEF) was assessed by calculating the modified Simson’s method [25]. Left ventricular systolic dysfunction was defined as LVEF < 52% for men and < 54% for women [25].

**Cardiopulmonary exercise test**

All CPETs were performed with the subjects walking on a treadmill (TechnoGym Runrace, Forli, Italy) using a modified Balke protocol [26], the same protocol used for the reference population [27]. In short, after a warm-up phase at 3 km/h and a 2% inclination, the inclination was increased by 2% every 60 s up to 20%. Then, the speed was increased by 0.5 km/h every 60 s until exhaustion. During the test, metabolic gas exchange and ventilatory variables were measured continuously breath-by-breath and averaged over 30-s intervals (Vyntus CPX, CareFusion, Hoechberg, Germany) through a two-way breathing mask (2700 series, Hans Rudolph, Shawnee, USA). Calibration of flow and gas concentration was performed before each test. Maximal heart rate (HRmax) was recorded with a 12-lead ECG (CustoMed, Ottobrunn, Germany). The test was discontinued at exhaustion, reporting Borg scale 18–20 [28], and respiratory exchange ratio (RER) > 1.10. VO2peak predicted was calculated from the equations of Edvardsen et al. [27]. Low cardiorespiratory fitness was defined as VO2peak < 85% of predicted, in accordance with ATS/ACCP guidelines [29]. The ventilatory limitation was defined as breathing reserve ≤ 15% or 11 L/min [30], and gas exchange limitation was defined as VE/ VCO2 slope ≥ 34 [31]. The cardiac limitation was defined as oxygen pulse < 80% of predicted, HR < 90% of age-predicted, and HR reserve < 15 beats/min in absences of pulmonary limitations [29]. Oxygen pulse was calculated dividing VO2peak (mL min⁻¹) by HRmax [29]. Deconditioning was defined as low VO2peak in absence of cardiac or pulmonary limitations.
Table 1 Characteristics of 90 long-term survivors of allogeneic HSCT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female, n (%)</td>
<td>40/50 (44/56%)</td>
</tr>
<tr>
<td>Age at transplantation, median (range)</td>
<td>20 (0.3–30)</td>
</tr>
<tr>
<td>Age at follow-up, median (range)</td>
<td>35 (17–54)</td>
</tr>
<tr>
<td>Years of observation, median (range)</td>
<td>17 (6–26)</td>
</tr>
<tr>
<td>BMI, kg/m², median (range)</td>
<td>23.8 (15.8–43.8)</td>
</tr>
<tr>
<td>Overweight (BMI 25.0–29.9 kg/m²), n (%)</td>
<td>25 (28%)</td>
</tr>
<tr>
<td>Obese (BMI ≥ 30 kg/m²), n (%)</td>
<td>13 (14%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Physician-diagnosed asthma pre-allo-HSCT, n (%)</td>
<td>9 (10%)</td>
</tr>
</tbody>
</table>

Smoking

- Never, n (%) 61 (68%)
- Former, n (%) 16 (18%)
- Daily, n (%) 13 (14%)
- Pack-years, median (range) 3 (1–24)

Underlying diagnosis

- Malignancy, n (%) 66 (73%)
  - Acute myeloid leukemia, n (%) 30 (33%)
  - Chronic myeloid leukemia, n (%) 20 (22%)
  - Acute lymphoblastic leukemia, n (%) 9 (10%)
  - Other malignant, n (%) 7 (8%)
- Severe aplastic anemia, n (%) 15 (17%)
- Other non-malignant, n (%) 9 (10%)

Conditioning

- Cyclophosphamide/busulfan or cyclophosphamide, n (%) 88 (98%)
- None, n (%) 2 (2%)

Donor

- Matched related donor, n (%) 56 (62%)
- Matched unrelated donor, n (%) 27 (30%)
- Haploidentical donor, n (%) 7 (8%)

Graft-vs-host disease

Diagnosed earlier

- Acute, grades 0–I, n (%) 66 (73%)
- Acute, grades II–IV, n (%) 24 (27%)
- Chronic, limited, and extensive, n (%) 35 (39%)

Diagnosed at follow-up

- Chronic, grades 1–3 (NIH criteria), n (%) 28 (31%)
- Bronchiolitis obliterans syndrome, n (%) 11 (11%)

Statistical analysis

Student t test or Mann-Whitney U test was used, as appropriate, to compare continuous data between groups, and the chi-square test or Fisher’s exact test to compare categorical variables. Univariate and multiple linear regression analyses were used to detect associations between outcome variables and determinants of interest. The independent variables entered the regression models were those hypothesized a priori for biological or clinical reasons or found to be significant at a 20% level by univariate analysis. Due to collinearity, only one variable from the pulmonary function tests and one age-related variable were included in the final model. Pulmonary function in patients with BOS was analyzed separately since the diagnostic criteria for BOS are based on such tests [19]. Since the chemotherapy regimen used in the treatment of malignant blood disorders may affect both cardiac and pulmonary function, we chose to analyze the relevant variables according to chemotherapy prior to allo-HSCT (yes vs no). A two-sided p value ≤ 0.05 was considered significant. Standard statistical analyses were performed with SPSS (IBM SPSS, v26).

Results

Clinical characteristics are outlined in Table 1. In total, 42% of the subjects were overweight, 14% obese. Subjects with overweight were more frequently treated for hypertension (42% vs 21%, p = 0.03) and hypercholesterolemia (29% vs 8%, p = 0.008) than those with normal weight. Hematological malignancies comprised the underlying diagnosis in 66 (73%) of the patients of whom 41 (46%) had received treatment with chemotherapy regimens prior to allo-HSCT. Patients diagnosed with chronic myeloid leukemia did not receive chemotherapy routinely, in contrast to the other malignant disorders. Myeloablative conditioning with busulfan/cyclophosphamide or cyclophosphamide had been applied in 98% of the subjects. Two patients with severe combined immunodeficiency did not receive a conditioning regimen. cGVHD (grades 1–3) was diagnosed in 28 (31%) of the survivors and was associated with chemotherapy prior to allo-HSCT (p = 0.005). Eleven of the 28 patients with cGVHD had developed BOS. The genders were comparable with respect to all clinical characteristics. The non-responders were younger than those who participated in the study [(median, range) 25 (18–53) vs 35 (17–54) years, p < 0.001] and comprised more males (70% vs 44%, p = 0.01) but were comparable with respect to malignant vs non-malignant disease prior to allo-HSCT. Pulmonary and cardiac functions according to chemotherapy prior to allo-HSCT are summarized in Table 2. For the entire study group, mean lung volumes and DLCO were above 80% predicted; however, 44% had impaired

Reference population

The reference population has been described previously [27]. In brief, in a multicenter study involving regional centers throughout Norway, 759 healthy adults successfully completed CPET on a treadmill. In total, 558 subjects (48% females) were within the same age range as the participants in the present study.
Blood analysis

*BOS patients were separated in a column for the pulmonary function values. HSCT, hematopoietic stem cell transplantation; BOS, bronchiolitis obliterans syndrome; TLC, total lung capacity; RV, residual volume; FVC, forced vital capacity; FEV1, forced expiratory volume-one second; DLCO, diffusing capacity for carbon monoxide; LVEF, left ventricular ejection fraction; proBNP, pro B-type natriuretic peptide; CRP, C-reactive protein; Hb, hemoglobin concentration; HbA1c, glycated hemoglobin. Data are presented as mean (95% confidence interval), number (%) or † median (range).

Table 2  Blood test, pulmonary, and cardiac function of 90 long-term survivors of allo-HSCT according to chemotherapy prior to HSCT (no/yes)

<table>
<thead>
<tr>
<th>Pulmonary function</th>
<th>All</th>
<th>No</th>
<th>Yes</th>
<th>p value</th>
<th>BOS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC % predicted</td>
<td>n = 90</td>
<td>n = 46 (51%)</td>
<td>n = 33 (37%)</td>
<td>n = 11 (12%)</td>
<td>0.04</td>
</tr>
<tr>
<td>RV % predicted</td>
<td>104 (101–106)</td>
<td>105 (101–108)</td>
<td>103 (98–107)</td>
<td>0.71</td>
<td>105 (98–112)</td>
</tr>
<tr>
<td>FVC % predicted</td>
<td>117 (112–123)</td>
<td>115 (108–122)</td>
<td>120 (111–129)</td>
<td>0.89</td>
<td>157 (145–170)</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>93 (90–96)</td>
<td>96 (92–100)</td>
<td>90 (86–95)</td>
<td>0.36</td>
<td>80 (73–87)</td>
</tr>
<tr>
<td>FEV1 % predicted</td>
<td>88 (84–92)</td>
<td>92 (87–97)</td>
<td>84 (78–90)</td>
<td>0.17</td>
<td>56 (46–66)</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.78 (0.75–0.80)</td>
<td>0.79 (0.76–0.83)</td>
<td>0.76 (0.73–0.79)</td>
<td>0.19</td>
<td>0.58 (0.50–0.66)</td>
</tr>
<tr>
<td>DLCO % predicted</td>
<td>83 (80–87)</td>
<td>87 (83–92)</td>
<td>79 (75–84)</td>
<td>0.02</td>
<td>85 (74–95)</td>
</tr>
</tbody>
</table>

Cardiac function

<table>
<thead>
<tr>
<th>LVEF (%)</th>
<th>n = 90</th>
<th>n = 46 (51%)</th>
<th>n = 33 (37%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV dysfunction</td>
<td>55 (54–56)</td>
<td>57 (55–58)</td>
<td>54 (51–56)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>28 (31%)</td>
<td>6 (25%)</td>
<td>22 (33%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (17%)</td>
<td>1 (5%)</td>
<td>14 (25%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood analysis</th>
<th>All</th>
<th>No</th>
<th>Yes</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>proBNP, pmol/L</td>
<td>5.5 (0.7–79.0)</td>
<td>3.9 (0.7–19.0)</td>
<td>9.0 (1.4–79.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.5 (0.6–14.0)</td>
<td>1.2 (0.6–14.0)</td>
<td>1.7 (0.6–12.0)</td>
<td>0.11</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>14.3 (14.1–14.6)</td>
<td>14.3 (14–14.7)</td>
<td>14.3 (13.8–14.7)</td>
<td>0.78</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.4 (4.2–6.7)</td>
<td>5.3 (5.2–5.4)</td>
<td>5.4 (5.3–5.6)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

DLco. Patients who had received chemotherapy prior to allo-HSCT had lower DLco % predicted than those who had not received such treatment [mean (95% CI) 79 (75–84) vs 87 (83–92), p = 0.02]. Patients with BOS had increased RV and reduced FVC % predicted compared with those without BOS [76 (66–86) vs 91 (87–95), p = 0.01]. Those with BOS also had reduced breathing reserve (p = 0.002) and HR reserve (p = 0.01). There was a positive correlation between VO2peak % predicted and, respectively, LVEF and DLco % predicted (p = 0.04 and 0.06).

Detailed data on cardiorespiratory responses during CPET are presented in Supplement 1. All subjects were able to obtain peak effort (Borg scale ≥ 18 and/or RER ≥ 1.10). Of the 39 (43%) subjects with low cardiorespiratory fitness, 22 (56%) were deconditioned, while 17 (44%) had either cardiac (n = 6), lung volume (n = 6), gas exchange (n = 2), or several limiting factors (n = 3). In a multiple linear regression model, low VO2peak % predicted was significantly associated with high BMI, not meeting WHO recommendations for physical activity, BOS, low DLco % predicted, and reduced LVEF (Table 3).

Discussion

The study demonstrated that median 17 years after allo-HSCT, only half of the survivors had normal cardiorespiratory fitness assessed by VO2peak. Among the survivors with low cardiorespiratory fitness, both cardiopulmonary factors and deconditioning were found to limit exercise and were equally
Low cardiorespiratory fitness was associated with reduced gas diffusion capacity, reduced systolic ventricular function, BOS, and overweight and inactivity. In sum, these findings encourage long-term monitoring of survivors after allo-HSCT and highlight the need for increased focus on modifiable lifestyle factors in oncology survivorship clinics.

Several previous studies have reported reduced cardiorespiratory fitness after allo-HSCT [10–14]. However, nearly all those studies were performed in children and adolescents [11–14]. Eames et al. estimated that as much as 68% of their cohort had $\text{VO}_2\text{peak} < 80\%$ predicted [12]. Larsen et al. [11] and Hogarty et al. [13] found impaired cardiorespiratory fitness with reduced $\text{VO}_2\text{peak}$ in 55% and 69% of the patients, respectively. Those studies all had small sample sizes (<40), comprised heterogeneous groups including patients with both blood disorders and lymphomas, TBI conditioning, and mean follow-up was <7 years. One larger study including young patients (mean age 14 years) with blood disorders only reported reduced $\text{VO}_2\text{peak}$ in 25% of 63 survivors with mean observation 7 years [14]. To our knowledge, only one previous long-term study has been performed in adults [10]. That study was confined to an older population (median age 67 years), but included only 20 subjects (10 allo-HSCT and 10 auto-HSCT), and found, on average, 22% lower $\text{VO}_2\text{peak}$ in the survivors as compared with predicted. None of the studies above is directly comparable to ours since we studied very long-term effects of allo-HSCT in young adults conditioned with chemotherapy only.

We found that mean $\text{VO}_2\text{peak}$ was 89% of predicted, adjusted for gender and age. This is considerably higher than in the studies above. Furthermore, none of our survivors had $\text{VO}_2\text{peak} < 16\text{ mL kg}^{-1}\text{ min}^{-1}$, a threshold reported to be associated with a 9-fold risk of death in allo-HSCT patients [32]. Given the other studies, all had much shorter observation periods than ours, there is a possibility that $\text{VO}_2\text{peak}$ may improve over time. Survivorship bias is another potential explanation, suggesting that very long-term allo-HSCT survivors eligible for CPET comprise a selected group who has been spared serious complications. Another factor is the mode of exercise testing. The survivors in our study were tested on a treadmill while in the other studies—except one [12]—the

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted (β)</th>
<th>95% CI</th>
<th>p value</th>
<th>Adjusted (β)</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>5</td>
<td>(−2.6, 12.6)</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>−1.1</td>
<td>(−1.8, −0.4)</td>
<td>0.002</td>
<td>−1.4</td>
<td>(−2.0, −0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at allo-HSCT</td>
<td>0.2</td>
<td>(−0.2, 0.6)</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic GVHD w/o BOS</td>
<td>−2.5</td>
<td>(−10.8, 5.8)</td>
<td>0.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOS</td>
<td>−14.6</td>
<td>(−25.8, −3.4)</td>
<td>0.01</td>
<td>−15.2</td>
<td>(−24.9, −5.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>Physical activity</td>
<td>8.8</td>
<td>(0.6, 17.0)</td>
<td>0.04</td>
<td>7.3</td>
<td>(0.2, 14.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>$\text{DL}_{CO}$ % of predicted</td>
<td>0.2</td>
<td>(−0.01, 0.5)</td>
<td>0.06</td>
<td>0.2</td>
<td>(0.04, 0.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.6</td>
<td>(−0.03, 1.3)</td>
<td>0.06</td>
<td>0.6</td>
<td>(0.07, 1.2)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1 Adjusted for all shown variables. Allo-HSCT, Allogeneic hematopoietic stem cell transplantation; BOS, bronchiolitis obliterans syndrome; BMI, body mass index; GVHD, graft-versus-host disease; $\text{DL}_{CO}$, diffusing capacity for carbon monoxide; physical activity according to the World Health Organization recommendation; LVEF, left ventricular ejection fraction.
Subjects were tested on a cycle ergometer. Walking on a treadmill is a more functional way of moving than cycling; it places a higher demand on the cardiopulmonary system and is known to elicit 10–20% higher VO2peak because larger muscle groups are involved [29]. Finally, in contrast to the other mentioned studies, the survivors in our study had not been treated with TBI that may cause both cardiac and pulmonary injury [33].

About half of the survivors with low cardiopulmonary fitness were deconditioned. Deconditioning is closely linked to modifiable lifestyle factors such as overweight and inactivity. More than 70% of the survivors did not meet the WHO recommendations for physical activity, and 42% were overweight. Those with overweight had features of metabolic syndrome (hypertension, dyslipidemia). A report from the St. Jude Lifetime Cohort Study concluded that long-term adult survivors of childhood and adolescent malignancies were more likely to have metabolic syndrome with > 2-fold relative risk if they did not follow physical activity and diet guidelines set out by the World Cancer Research Fund and American Institute for Cancer [34]. The metabolic syndrome is associated with cardiovascular death and increased mortality by all causes, and even low levels of physical activity may reduce the risk [35].

Cardiovascular disease has been shown to be a leading cause of late morbidity and mortality after allo-HSCT [2] and survivors of allo-HSCT have a 4-fold higher risk of developing cardiovascular disease when compared with the general population [6]. A report from the Blood and Marrow Transplant Survivor Study-2 provided data on cause-specific late mortality after allo-HSCT [36]. In a large cohort (n = 764), the most common causes of death among those surviving > 10 years were secondary malignancies (36%), infection (22%), and cardiac disease (14%).

Late adverse effects of allo-HSCT on cardiac function have been reported in several studies [5, 6, 37, 38]. In a recent study, Vandekerckhove et al. found echocardiographic signs of systolic and diastolic dysfunction as well as reduced cardiopulmonary fitness in a cohort of 43 children (mean age 13.6 years, mean 6.6 years after allo-HSCT) compared with healthy controls [39]. In our study, none of the survivors had signs or symptoms of manifest left ventricular dysfunction at rest, and for the total study group, mean LVEF was normal. One-third of the subjects had systolic dysfunction, but only two subjects had LVEF < 40%. We found that LVEF was significantly associated with VO2peak % predicted, suggesting that subclinical left ventricular dysfunction may have contributed to low cardiopulmonary fitness.

Strengths and limitations

The main strengths of the present study are a homogeneous national cohort, very long-term follow-up, and comprehensive cardiopulmonary evaluation with methods that permit detecting key organ-specific impairments. A weakness is the lack of pre-treatment data, which prevents the analysis of longitudinal changes. Since age at diagnosis ranged from 0.3 to 30 years, it might have been possible to obtain reliable baseline CPET results from a subset of the patients. The survivors are young adults and longitudinal data are needed to determine if their cardiopulmonary fitness will improve, deteriorate, or remain stable throughout their adulthood. Furthermore, the cross-sectional design does not permit us to define causal relationships. Although we consider an attendance rate of 66% to be acceptable in a very long-term follow-up study, we cannot rule out that non-response bias has affected the external validity and generalizability of our results since the non-responders were somewhat younger than the participants and comprised more males. However, our non-response findings mirror those reported in a recent nationwide Norwegian study of young cancer survivors that found no evidence of non-response bias [40].

Conclusions and recommendations

Half of the survivors had reduced cardiopulmonary fitness median 17 years after allo-HSCT without TBI conditioning. Cardiopulmonary factors and deconditioning were equally common limitations to exercise. We recommend targeted advice on modifiable lifestyle factors and encourage allo-HSCT survivors to follow guidelines for physical activity and diet set out by national and international cancer federations. Life-long alertness for the prevention and detection of cardiopulmonary late effects is also recommended for this increasing population of vulnerable long-term allo-HSCT survivors.

Authors' contributions All authors contributed to the design of the study, to the interpretation of the results, and in revising the manuscript. O.H.M., R.I.M., and P.P.D. participated in data collection. O.H.M., M.B.L., and L.I.B.S. drafted the manuscript. The final version has been approved by all authors.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Regional Committee for Medical and Health Research Ethics (2014/370) and the Data Protection Officer at Oslo University Hospital.
Consent to participate  Written informed consent was obtained from all participants.

Code availability  Not applicable.

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Late-Onset, Noninfectious Pulmonary Complications following Allogeneic Hematopoietic Stem Cell Transplantation: A Nationwide Cohort Study of Long-Term Survivors

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Keywords
Allogeneic hematopoietic stem cell transplantation · Bronchiolitis obliterans syndrome · High-resolution CT · Pulmonary function · Long-term follow-up

Abstract
Background: Survivors of allogeneic hematopoietic stem cell transplantation (allo-HSCT) are at risk for pulmonary adverse events. Data on late-onset noninfectious pulmonary complications in long-term adult survivors of allo-HSCT are limited and incomplete. Objectives: This study aimed (1) to determine occurrence and degree of pulmonary sequelae in adult survivors of allo-HSCT and (2) to identify associations between pulmonary function, high-resolution CT (HRCT), and clinical characteristics. Method: In a nationwide, single-center cross-sectional study, 103 survivors (aged median [range] 35 [17–58] years, 53% females) were examined 17 (6–32) years after allo-HSCT and compared with healthy controls (n = 105). Methods included pulmonary function tests and HRCT. Results: Chronic graft-versus-host disease was diagnosed in 33% of survivors, including 12% with bronchiolitis obliterans syndrome (BOS). Mean lung volumes (TLC, FVC, and FEV\textsubscript{1}) and gas diffusing capacity were >80% of predicted for the survivors as a group, but significantly lower than in healthy controls. Pathological HRCT findings were detected in 48% of the survivors (71% airways disease, 35% interstitial lung disease, and 24% apical subpleural interstitial thickening). Air trapping (%) on HRCT correlated with % predicted FEV\textsubscript{1}, \( p < 0.001 \). In a multiple logistic regression model, both BOS and pathological findings on HRCT were associated with chemotherapy prior to allo-HSCT, \( p < 0.05 \). Conclusions: Long-term allo-HSCT survivors had significantly lower pulmonary function than age- and gender-matched healthy controls and nearly half had pathological findings on HRCT. Longitudinal data will determine if pulmonary sequelae will remain stable or progress. We recommend lifelong monitoring of pulmonary function in allo-HSCT survivors. HRCT provides additional information, but is not suited for surveillance.

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established, life-saving treatment for a diversity of malignant and nonmalignant disorders [1]. It is, however, associated with numerous adverse effects that may complicate long-term outcomes [2].

Pulmonary complications have been reported in one-third of allo-HSCT recipients, affecting morbidity and mortality [3]. Factors associated with pulmonary complications are graft-versus-host disease (GVHD), conditioning regimen, underlying lung disease, and factors related to treatment prior to allo-HSCT [3]. In brief, pulmonary complications may develop due to either infectious or noninfectious causes. Late-onset noninfectious pulmonary complications (LONIPCs) may be caused by chemotherapy used in the myelosuppressive conditioning regimen [4], total body irradiation [5, 6], or as an immunological response induced by the graft [7]. The latter is typically late-occurring in the form of chronic GVHD (cGVHD) [8]. The most frequent LONIPC is bronchiolitis obliterans, which is linked to cGVHD [9]. Bronchiolitis obliterans is diagnosed by its histological pattern of fibrogenic deposition in small airways. A lung biopsy is needed, which requires an invasive and potentially harmful procedure. Therefore, the clinically based diagnosis bronchiolitis obliterans syndrome (BOS) is usually preferred. Criteria for the diagnosis of cGVHD and BOS have been published by the National Institutes of Health (NIH) [10]. The diagnosis of BOS is primarily based on pulmonary function tests with supporting criteria based on high-resolution computed tomography (HRCT) and differs from the diagnosis of BOS following lung transplantation [11]. Also, interstitial lung diseases (ILDs) may occur in survivors of allo-HSCT and are often labeled LONIPCs as well [9]. The ILDs have different appearances and histological findings [12]. One is pleuroparenchymal fibroelastosis which is characterized by elastic fibrosis, involving lung tissue in the upper lobes [13]. Hence, the development of LONIPCs is complex, with a range of rare conditions from ILDs to BOS, and there may also be an overlap between these disorders in the same patient [3]. To our knowledge, HRCT and pulmonary function tests have been applied jointly in only a few clinical studies with focus on late complications in adult allo-HSCT survivors [14–16]. One was a large (n = 198), prospective study, median 6 years after allo-HSCT, reporting 44 survivors with LONIPCs, mainly in terms of BOS and ILD [14]. Other studies applying HRCT have been restricted to patients with previously diagnosed cGVHD/BOS [15–17]. Hence, little is known of LONIPCs in adult allo-HSCT survivors with observation periods exceeding 1 decade. The present study was carried out within the context of a large and comprehensive project investigating health conditions in young, very long-term survivors of allo-HSCT [18–21]. All survivors were examined with both HRCT and pulmonary function tests. We aimed to assess the occurrence and degree of late pulmonary sequelae in a nationwide cohort of survivors who had been treated with allo-HSCT in childhood, adolescence, or early adulthood median 17 years previously. We also aimed to identify associations between clinical characteristics, HRCT findings, and pulmonary function.

Materials and Methods

Design and Study Population

The study was conducted at Oslo University Hospital from August 2014 to February 2016, as a part of a single-center, nationwide cross-sectional study covering a broad range of late treatment-related effects after allo-HSCT [18–21]. The survivors were eligible for inclusion if they were younger than 30 years at transplantation, older than 16 years at examination, and had minimum 5 years of observation (n = 157) (Fig. 1). All participating survivors (n = 103) gave their written informed consent. The study was approved by the South-East Regional Committee for Medical and Health Research Ethics (2014/370).
Healthy Controls
For pulmonary function testing, 105 healthy, never-smoking subjects (age [median, range] 35 [20–59] years, 56% females) with no history of cancer or pulmonary disease were recruited among university and hospital employees through local advertisements. The controls did not undergo HRCT examination since it was considered unethical to expose healthy subjects to irradiation.

Clinical Assessment
Clinical data, including gender, age, BMI, smoking habits, medical history, physician-diagnosed lung disease, and current medication, were recorded. Routine blood tests included hemoglobin (Hb) levels.

Pulmonary Function
Measures of pulmonary function were performed according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines [22, 23] and included total lung capacity (TLC), vital capacity (VC), residual volume (RV), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and diffusing capacity of the lung for carbon monoxide (DLCO). DLCO values were also corrected for Hb levels, obtained on the same day as pulmonary function testing. Since Hb correction of DLCO did not significantly influence the results of the analyses, the variable DLCO/Hb was not reported in the Results section. The predicted values for spirometry and DLCO were taken from the Global Lung Initiative [24, 25] and static volumes from the European Community for Steel and Coal [26]. All tests were performed with the Jaeger Master Screen Body (Eric Jaeger, Wurzburg, Germany). BOS was diagnosed according to NIH criteria [10]: (1) new-onset airway obstruction with 10% decline over <2 years, and (3) absence of infection, and (4) one of 2 supportive features of air trapping, either on expiratory HRCT or by residual volume (RV) >120% of predicted. Due to the cross-sectional study design, we cannot tell if airway obstruction was new-onset or not, or if FEV1 had declined >10% in 2 years. Restrictive impairment and impaired gas diffusing capacity were defined as, respectively, TLC and DLCO <80% of predicted. We calculated the lower limit of normal in order to confirm that the cutoff 80% predicted fitted the lower 5th percentile of the reference values [27]. We used the term pulmonary sequelae for describing the combination of impairments in pulmonary function and pathological findings on HRCT.

Acquisition and Review of HRCT Images
CT examinations were performed with a LightSpeed 16 scanner (GE Healthcare, Milwaukee, WI, USA) and obtained in the supine position, during deep inspiration and breath-holding. Tube current settings were adjusted to each patient’s weight, but with low dose references (noise index: 40). No intravenous contrast material was used. In all subjects, supplementary expiratory scans were obtained with 1.25-mm section thickness at 10-mm intervals. Supplementary prone position scans were obtained when considered necessary to differentiate between fine reticular fibrosis and dependent atelectasis. The images were reviewed on a PACS (Picture Archiving and Communication System) screen in random order and in consensus by 2 experienced chest radiologists (T.M.A. and K.F.) and 1 pulmonologist (O.H.M.) with special training in interpretation of HRCTs. The reviewers were blinded to lung function and clinical data. The presence, extent, and distribution of interstitial findings were evaluated according to the HRCT criteria of ILD recommended by the Nomenclature Committee of the Fleischner Society [28]. HRCT-detected ILD was defined as reticular pattern and/or ground glass opacities. Airways disease was defined as bronchiectasis, and/or air trapping, and/or mosaic pattern, and/or centrilobular micronodules. In the setting of long-term survivors of allo-HSCT with the risk of LONIPCs, the HRCT findings were categorized into 3 major groups: airways disease, apical irregular subpleural interstitial thickening, and other signs of ILD. The distribution of pathology was reviewed in 4 zones: (a) above the aortic arch, (b) between the aortic arch and the level of the carina, (c) between the level of the carina and the level of the inferior pulmonary veins, and (d) below the inferior pulmonary veins. The extent of involvement of pathological findings was evaluated for each lung zone. The severity of bronchiectasis was scored either as bronchial wall thickening without distinct ectasis or bronchiectasis in localizations (a–d). The extent of air trapping in each zone was assigned a score based on the percentage of lung parenchyma involved, with an overall score

Late-Onset Pulmonary Complications after Allo-HSCT

Table 1. Characteristics of 103 long-term survivors of allo-HSCT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
<th>Male/female</th>
<th>Age at transplantation, median (range)</th>
<th>Age at follow-up, median (range)</th>
<th>Years of observation, median (range)</th>
<th>BMI, median (range), kg/m²</th>
<th>Smoking</th>
<th>Pack-years, median (range)</th>
<th>Physician-diagnosed asthma</th>
<th>Underlying diagnosis</th>
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<tbody>
<tr>
<td>Table 1. Characteristics of 103 long-term survivors of allo-HSCT</td>
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<tr>
<td>Male/female</td>
<td>48/55 (47/53)</td>
<td>20 (0.3–30)</td>
<td>35 (17–58)</td>
<td>17 (6–32)</td>
<td>24.1 (15.6–43.5)</td>
<td>Smoking</td>
<td>Pack-years, median (range)</td>
<td>Physician-diagnosed asthma</td>
<td>Underlying diagnosis</td>
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<tr>
<td>Benign hematological disorders</td>
<td>18 (18)</td>
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<tr>
<td>Immunodeficiencies and metabolic disorders</td>
<td>8 (8)</td>
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<td>Malignancy</td>
<td>77 (75)</td>
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<tr>
<td>Acute myeloid leukemia</td>
<td>32 (42)</td>
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<tr>
<td>Chronic myeloid leukemia</td>
<td>26 (34)</td>
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<tr>
<td>Acute lymphoblastic leukemia</td>
<td>12 (16)</td>
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<td>Others</td>
<td>7 (8)</td>
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<tr>
<td>Pre allo-HSCT treatment and conditioning</td>
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<tr>
<td>Intravenous chemotherapy for malignancies</td>
<td>46 (45)</td>
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<tr>
<td>Chemotherapy, busulfan/cyclophosphamide</td>
<td>94 (91)</td>
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<td>Chemotherapy + total body irradiation</td>
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<td>None</td>
<td>2 (2)</td>
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<td>Donor</td>
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<tr>
<td>Matched related donor</td>
<td>65 (63)</td>
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<td>Haploidentical donor</td>
<td>7 (10)</td>
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<tr>
<td>Matched unrelated donor</td>
<td>31 (30)</td>
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<tr>
<td>Gender mismatch</td>
<td>41 (40)</td>
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<tr>
<td>Graft-versus-host disease</td>
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<tr>
<td>Acute, grade II–IV</td>
<td>33 (32)</td>
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<tr>
<td>Chronic</td>
<td>34 (33)</td>
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</table>

Data are presented as median (range) or n (%). Allo-HSCT, allogeneic hematopoietic stem cell transplantation; pack-years, the number of packs with 20 cigarettes smoked/day, multiplied by years of consumption; BMI, body mass index.
of involvement for each patient derived by summing the scores of the 4 CT levels. Subsegmental air trapping comprising <5% of the lung parenchyma was considered normal [27].

**Statistical Analysis**

Student’s t test or Mann Whitney U test was used, as appropriate, to compare continuous data between groups. χ² test or Fisher’s exact test was used to compare categorical variables, controlling for extraneous effects. The independent variables which entered the regression models were those hypothesized a priori for biological or clinical reasons or found to be significant at a 20% level by univariate analysis. Pulmonary function in patients with BOS was analyzed separately since the diagnostic criteria for BOS are based on such tests [10]. A 2-sided p value <0.05 was considered significant. Standard statistical analyses were performed with SPSS software (IBM SPSS statistics, version 26).

**Results**

A total of 157 survivors fulfilled the inclusion criteria and 103 (66%) participated in the study (Fig. 1). The 54 nonparticipating survivors included more males than females (69% vs. 47%, p = 0.01), were (median [range] years) younger at the time of allo-HSCT 14 (0.8–30) versus 20 (0.3–30), p = 0.02, and had shorter observation time 12 (5–24) versus 17 (6–32), p < 0.001 than the included survivors. The nonresponders were comparable to the included survivors with respect to diagnosis prior to transplantation.

**Patient Characteristics**

Clinical characteristics are outlined in Table 1. Hematological malignancies comprised the underlying diagnosis in 77 (75%) survivors of whom 46 (60%) had received chemotherapy prior to allo-HSCT. Patients with chronic myeloid leukemia had not received chemotherapy routinely, in contrast to those with other malignancies. Myeloablative regimens with cyclophosphamide/busulfan or cyclophosphamide had been applied in 101 (98%) survivors. Only 7 subjects had been treated with total body irradiation. Chronic GVHD was diagnosed in 34 (33%) survivors. Survivors with cGVHD were comparable to those without cGVHD with respect to gender, age, observation time, BMI, and smoking habits. Among subjects with benign underlying disorders (n = 26), 4 (15%) had developed cGVHD compared to 30 (39%) among those with malignant disorders (n = 77) (p = 0.03). Chronic GVHD was associated with chemotherapy prior to allo-HSCT (p = 0.01) and history of acute GVHD (p = 0.001), but not with donor match. Mean (SD) Hb was 15.3 (1.0) for males and 13.6 (0.9) for females.

**Pulmonary Function**

Pulmonary function for the allo-HSCT survivors and the healthy controls is shown in Figure 2. Twelve survivors were diagnosed with BOS. They had increased RV and reduced FVC and FEV₁, hence confirming that they met the NIH criteria. The survivors without BOS had significantly lower TLC, FVC, FEV₁, and DLCO than the healthy controls, whereas RV was comparable. Among the allo-HSCT survivors, 4% had restrictive impairment and 17% had impaired gas diffusing capacity. No case of impairment was observed among the healthy controls. Twelve of the 13 survivors with asthma had been diagnosed after HSCT, and 4 of them had BOS. Ever-smokers

![Fig. 2. Pulmonary function in 103 long-term survivors of allo-HSCT and 105 healthy controls. Comparison between controls and allo-HSCT w/o BOS. Data presented as % predicted (mean [95% CI]). Allo-HSCT, allogeneic hematopoietic stem cell transplantation; BOS, bronchiolitis obliterans syndrome.](image-url)
Late-Onset Pulmonary Complications after Allo-HSCT

(daily + former) had comparable pulmonary function to never-smokers. There was no significant association between smoking status and BOS. Patients who had received chemotherapy prior to allo-HSCT had lower DLCO% predicted than those who had not received such treatment (mean [95% CI] % predicted 78 [73–82] versus 87 [82–91], \( p = 0.003 \)). Of the survivors with impaired DLCO, 2 had BOS.

**HRCT Findings**

HRCT findings are presented in Tables 2 and 3, Figure 3, and online supplementary Figure 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000520824). Fifty-four (52%) survivors had normal HRCT (online suppl. Fig. 1a). Among the remaining 49 survivors, 64 pathological HRCT findings were detected (signs of airways disease \( n = 35 \), apical irregular subpleural interstitial thickening \( n = 12 \), and other findings of ILD \( n = 17 \)). Ten subjects had concomitantly 2 types of pathology and 2 had all 3 types. A picture demonstrating typical CT findings is shown in Figure 3.

**Table 2.** Pulmonary function in 103 long-term survivors of allo-HSCT according to findings on HRCT

<table>
<thead>
<tr>
<th></th>
<th>Survivors w/ normal findings</th>
<th>Survivors w/ pathological findings</th>
<th>( p ) value</th>
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<tbody>
<tr>
<td></td>
<td>( n = 54 )</td>
<td>( n = 49 )</td>
<td>( n = 35 )</td>
</tr>
<tr>
<td>TLC</td>
<td>105 (102–109)</td>
<td>102 (98–106)</td>
<td>0.13</td>
</tr>
<tr>
<td>RV</td>
<td>112 (106–118)</td>
<td>121 (113–130)</td>
<td>0.08</td>
</tr>
<tr>
<td>FVC</td>
<td>96 (93–100)</td>
<td>90 (86–94)</td>
<td>0.06</td>
</tr>
<tr>
<td>FEV(_1)</td>
<td>94 (90–98)</td>
<td>82 (76–88)</td>
<td>0.001</td>
</tr>
<tr>
<td>FEV(_1)/FVC</td>
<td>0.81 (0.78–0.82)</td>
<td>0.75 (0.71–0.78)</td>
<td>0.002</td>
</tr>
<tr>
<td>DLCO</td>
<td>93 (88–98)</td>
<td>95 (91–100)</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Comparison is between the different findings and normal. One subject may have several findings. Data presented as % of predicted value (95% CI). Allo-HSCT, ALLOGENEIC hematopoietic stem cell transplantation; TLC, total lung capacity in % of predicted value; RV, residual volume; FVC, forced vital capacity; FEV\(_1\), forced expiratory volume in 1 s; DLCO, diffusing capacity for carbon monoxide.

Fig. 3. Axial (a), coronal (b), and sagittal (c) inspiratory HRCT images in a long-term survivor of allo-HSCT with BOS shows airways disease with bronchiectasis (arrows) and mosaic pattern, with heterogeneous lung attenuation comprising geographic areas of decreased attenuation (*) adjacent to the normal lung. In addition, there is irregular subpleural apical interstitial thickening (arrowheads). Allo-HSCT, allogeneic hematopoietic stem cell transplantation; BOS, bronchiolitis obliterans syndrome; HRCT, high-resolution computed tomography.
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(Except one) of the survivors who fulfilled the pulmonary function criteria for BOS had findings consistent with airways disease. The most frequent single finding on HRCT was air trapping, which was detected in 24 survivors, with mean 29% of the lung parenchyma involved, ranging from 5 to 77%. The degree of air trapping in % of lung volume correlated with RV% predicted ($r = 0.610, p = 0.002$) and inversely with FEV1% predicted ($r = -0.771, p < 0.001$) (online suppl. Fig. 1c). There was no association between pathological findings on HRCT and cGVHD without BOS ($p = 0.926$). Age at allo-HSCT was not a risk factor for pneumological abnormalities in our cohort ($p = 0.728$). In a multiple logistic regression model, controlling for gender, age at allo-HSCT, and observation time, pathological findings on HRCT and BOS were both associated with chemotherapy prior to allo-HSCT ($p < 0.041$ and $p < 0.040$) (Table 3).

Discussion

The main findings of this study were that long-term allo-HSCT survivors had significantly lower pulmonary function than age- and gender-matched healthy controls and nearly half of them had pathological findings on HRCT. Although mean lung volumes (TLC, FVC, and FEV1) and gas diffusing capacity were above 80% predicted for the entire study group, one-third of the survivors had some kind of impairment (17% impaired gas diffusing capacity, 12% had developed BOS, and 4% had restrictive impairment). Survivors who had received chemotherapy prior to allo-HSCT had 2.4-fold and 4.5-fold increased risk for, respectively, pathological findings on HRCT and BOS. The predominant pathological findings on HRCT were signs of airways disease, but also various patterns of ILD, including apical irregular subpleural interstitial thickening suggestive of pleuroparenchymal fibroelastosis, were found. The latter is a rare radiological pattern seen, in particular, after allo-HSCT and lung transplantation [13]. Our findings were indicative of pleuroparenchymal fibroelastosis because upper lobe pleural thickening with associated subpleural fibrosis was present and without involvement of lower lobes. However, detailed radiological features of pleuroparenchymal fibroelastosis are still largely unknown, and definite diagnosis requires histological confirmation [29].

The survivors included in the present study were young adults (median 35 years old), and approximately three-quarters were never-smokers. The former/current smokers had few pack-years (median 3 pack-years) which probably explains why their pulmonary function was comparable to the never-smokers. Four of the 12 survivors with physician-diagnosed asthma after HSCT also had BOS. It is possible that respiratory symptoms caused by BOS may have led to misclassification of asthma.

Pulmonary function was well preserved for the survivors who had not developed BOS. However, compared to the healthy controls, also the survivors without BOS had significantly reduced TLC, FVC, FEV1, and gas diffusing capacity. The latter was reduced, in particular, in subjects who had received intravenous high-dose courses of chemotherapy for malignant blood disorders prior to allo-HSCT. This finding is in line with reports from various other studies indicating that DLCO is the most sensitive test for detecting chemotherapy-induced lung injury [30–35]. In previous studies, we have found impaired gas diffusing capacity in long-term lymphoma survivors after high-dose therapy with autologous stem cell transplantation [33] and in very long-term adult survivors of childhood acute lymphoblastic leukemia [34]. Late adverse ef-

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Pathological findings on HRCT</th>
<th>BOS</th>
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<tbody>
<tr>
<td></td>
<td>odds ratio 95% CI  p value</td>
<td>odds ratio 95% CI  p value</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.7 0.7–3.9 0.205</td>
<td>2.3 0.6–9.2 0.222</td>
</tr>
<tr>
<td>Age at allo-HSCT, years</td>
<td>1.0 1.0–1.1 0.502</td>
<td>1.0 0.9–1.1 0.970</td>
</tr>
<tr>
<td>Observation time, years</td>
<td>1.0 0.9–1.1 0.565</td>
<td>0.9 0.8–1.0 0.149</td>
</tr>
<tr>
<td>Chemotherapy prior to allo-HSCT</td>
<td>2.4 1.0–5.4 0.041</td>
<td>4.5 1.1–18.6 0.040</td>
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Findings on HRCT include signs of airways disease, interstitial lung disease, and apical irregular subpleural, interstitial thickening. Allo-HSCT, allogeneic hematopoietic stem cell transplantation; BOS, bronchiolitis obliterans syndrome.

Table 3. Odds ratio for selected risk factors related to, respectively, pathological findings on HRCT and BOS in 103 long-term survivors of allo-HSCT

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fects of chemotherapy on gas diffusing capacity have also been shown in patients treated for lung cancer [32] and breast cancer [35]. Although the underlying mechanisms of cytotoxic lung injury are multifactorial and remain unclear, it is thought that microvascular damage may be a common denominator and an important feature [30, 31].

Our findings suggest that in combination with pulmonary function tests, HRCT is a useful tool for diagnosing BOS. In the present study, all but one subject with BOS also had pathological findings on HRCT consistent with airways disease. However, signs of airways disease (air trapping, small airway wall thickening, bronchiectasis, and mosaic attenuation) on HRCT are too common and too unspecific to be used alone to diagnose BOS. Air trapping was the single most frequent finding on HRCT in our study and occurred in nearly one-fourth of the survivors of whom only half fulfilled the pulmonary function criteria for BOS. To our knowledge, only one other large-sample study has used both HRCT and pulmonary function tests in order to identify long-term noninfectious lung complications in young adult survivors of allo-HSCT [14]. A few smaller studies have reported HRCT findings in survivors who had already been diagnosed with airway obstruction by previous pulmonary function testing [15–17]. It is, however, difficult to compare HRCT findings from different studies due to various study designs, definitions, and methods of reporting. Air trapping is defined as retention of air distal to airway obstruction, best visualized on expiratory HRCT scans. It is the most common finding on HRCT suggestive of BOS. We chose to report air trapping as a percentage of lung parenchyma, which is a well-established method [16, 28, 36, 37]. We found a strong correlation between % air trapping on HRCT and FEV1% predicted and RV% of predicted. This is in agreement with previous studies that have reported similar associations between airflow obstruction and air trapping in comparable allo-HSCT study populations [15–17]. The prevalence of BOS was 12% in our study, which is comparable to 11% reported by Bergeron et al. [14]. This is interesting, given the difference in follow-up between the 2 studies (median 17 vs. 6 years). Due to the cross-sectional design of our study, we cannot make assumptions regarding longitudinal changes. On the other hand, since our data are comparable with that of Bergeron et al. [14] with a median follow-up time of 6 years, we may assume that most of the cases with BOS do occur within the first few years after allo-HSCT. This is in line with reports from earlier studies [38, 39]. Although we do not know if the occurrence of pulmonary sequelae found in our study will increase or remain unchanged – or even regress – in the future, we think it is unlikely that the findings on HRCT and the impairments in pulmonary function will change in a clinically significant degree since they were – in general – of a mild nature. However, this assumption may be challenged by new biological research focusing the cellular processes of aging and their association with the premature development of age-related diseases seen in cancer survivors [40]. Since long-term comorbidities observed in cancer survivors seem to mimic the phenotypes of aging, they may be caused by some kind of interaction between therapeutic exposures and the underlying biology of aging [40].

One strength of the present study is the single-center, national patient cohort, uniformly treated according to standardized national protocols. Also, at follow-up, all medical tests were undertaken at the same site and carried out by a limited number of highly selected and experienced staff dedicated to the study, and the same type of equipment was used for, respectively, HRCT and pulmonary function testing. Another strength is an age- and gender-matched group of healthy controls. HRCT was applied in all survivors, not only those with diagnosed or suspected pulmonary complications, which has been the choice in other studies [16, 37]. From a research point of view, we obviously would have liked to have HRCT results also for the healthy controls, but since it was considered unethical to expose healthy subjects to irradiation for study purposes, the controls were used for comparing pulmonary function data only. Since we aimed to investigate late pulmonary sequelae in very long-term survivors, the long observation time (median 17 years) may be seen as a strength. However, very long observation time also implies survival bias. In our study, 45% of the allo-HSCT patients were deceased at the time of survey. Since national legislation prevents access to information on individual causes of death, we do not know to what extent pulmonary complications may have affected mortality. In a recent long-term study of mortality including almost 4,500 allo-HSCT survivors, Wong et al. [41] reported that if a patient survives the first 2 years after allo-HSCT, the 5-year survival rates the next 15 years exceed 85%. Furthermore, they found that cGVHD accounted for increasingly fewer deaths among long-term survivors and also a relatively stable mortality rate for pulmonary cause of death throughout the years [41]. Another weakness is the lack of pretreatment data that prevents analysis of longitudinal changes. The patients’ age at diagnosis ranged from 0.3 to 30 years. Although pulmonary function testing had not been routinely carried out prior to allo-HSCT and about one-third of the patients would have been too
young to obtain reliable tests, it might have been possible to track down baseline pulmonary function data from a subset of the patients. Pretreatment HRCT, on the other hand, was not accessible for any patient since it had not been part of the initial routine before allo-HSCT workup for any of the survivors. Furthermore, the cross-sectional design of the study does not allow us to study causal relationships, just describe associations. In a very long-term follow-up study, we think an attendance rate of 66% is satisfactory. However, we cannot rule out that non-response bias may have affected the external validity and generalizability of the results since the nonresponders comprised more males, were younger at the time of treatment, and had shorter observation time. In conclusion, after a median 17 years of observation, allo-HSCT survivors had significantly lower pulmonary function than age- and gender-matched healthy controls and nearly half of them had pathological findings on HRCT. The survivors were young adults (median age 35 years), and longitudinal data are needed to determine if their pulmonary sequelae will remain stable or progress throughout their adulthood. We therefore recommend lifelong monitoring of pulmonary function in allo-HSCT survivors. Pulmonary function testing is cost-effective, readily available, safe, and easy to perform. HRCT may provide valuable additional information and may be indicated for clinical reasons in selected patients. However, HRCT is still a cumbersome and expensive method, and it is also poorly suited for surveillance due to radiation exposure.

Statement of Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics (2014/370) and the Data Protection Officer at Oslo University Hospital. Written informed consent was obtained from all participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.B.L., T.M.A., L.B., and E.R. designed the study. O.H.M., P.P.D., and L.I.S. collected the data; O.H.M., L.I.S., T.M.A., J.K., and M.B.L. interpreted the results and drafted the manuscript. The final version was approved by all authors.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

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References

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Respiration

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