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DIFFUSE LARGE B-CELL LYMPHOMA – POPULATION-BASED STUDIES OF RELAPSE AND CARDIAC COMPLICATIONS

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DIFFUSE LARGE B-CELL LYMPHOMA – POPULATION-BASED STUDIES OF RELAPSE AND CARDIAC COMPLICATIONS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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“Don't be afraid of hard work. Nothing worthwhile comes easily.”

Gertrude B. Elion

POPULAR SCIENCE SUMMARY OF THE THESIS

Lymphoma is a type of blood cancer that occurs in white blood cells called lymphocytes. Depending on in which stage in the normal maturation of lymphocytes that the disease starts, different types of lymphoma develop.

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma type that affects almost 600 persons each year in Sweden. This is a fast-growing disease that, if left untreated, leads to death within weeks to months. It is however a highly treatable disease. With a combination of chemotherapy together with antibodies directed towards the CD20 protein present on the surface of the lymphoma cells, a majority of the patients will be cured. But some of the patients will not be cured or their disease will come back (relapse) and this thesis focuses on these patients. We wanted to both find out how common it is with relapse and also investigate how the relapse patients did.

It has been described in previous studies that the chemotherapy used to treat DLBCL can be toxic to the heart. Since many patients who fall ill in lymphoma are older and also have other diseases we wanted to study the risk of having a heart attack (acute myocardial infarction) following a DLBCL diagnosis.

All the studies in this thesis are observational studies where we have used data from Swedish health registers in combination with collected data from medical charts to answer the research questions. It is uncommon in research to have access to unselected data from the whole population, but thanks to the Swedish system with personal identity numbers and the high-quality Swedish health care registers we had access to such “real-world”-data and all of the studies in this thesis are population-based.

In the first study we found that not responding to primary treatment (refractoriness) or having a relapse of DLBCL is less common than previously described. After 5 years, only 23% of curatively treated patients had a relapse. We also found that a large proportion of older patients did not even start primary treatment with curative intent, probably because they were considered too sick or frail to tolerate chemotherapy. These patients are thus in need of new, more tolerable treatment alternatives. If the DLBCL relapse occurs within the central nervous system (CNS) it is known to be even more difficult to treat and with this study we could show that 3% of the curatively treated patients had a relapse in the CNS within two years.

In the second study we focused on the DLBCL patients who had a relapse and aimed at investigating how they did. We found that most patients relapsed early (within a year from primary diagnosis). Many patients either did not respond to the next round of chemotherapy or did not tolerate the treatment leading to short survival. Half of the patients survived for less than seven months (6.6) after having their relapse. In this study we also found that 35% of the relapse patients would be candidates for a new type of immunotherapy called chimeric antigen receptor (CAR) T-cell therapy and their survival with standard treatment was also very poor.

The third study focused on the subgroup of DLBCL relapse patients who had relapse in the CNS. Half of these patients survived for less than 3 months following their relapse and even those who received intensive treatment containing a chemotherapeutic drug called methotrexate had poor survival (half of them survived for less than 6 months). We estimated that one third of the CNS relapse patients fulfilled all the requirements for treatment with CAR T-cell therapy according to the criteria used in the clinical trial TRANSFORM.

The first three studies focused on DLBCL patients who relapsed or were refractory to treatment, but most DLBCL patients are actually cured by the standard primary treatment with chemotherapy and antibody. It is well known that some of the drugs that are used in lymphoma treatment can be bad for the heart. Also having lymphoma per se increases the risk of forming blood clots. In the fourth study we investigated the risk of having a heart attack among patients diagnosed with DLBCL who received primary treatment with curative intent. We found that the DLBCL patients had an increased risk of heart attack compared to people from the general population. The risk was highest shortly after the lymphoma diagnosis. After two years, DLBCL patients had the same risk as people in general. Older DLBCL patients who also had hypertension or diabetes had a higher risk. This study tells us that doctors who treat DLBCL patients should be more careful in monitoring risk factors for heart attack, such as high blood pressure, among these patients during and after their lymphoma treatment.

We conclude that in these large studies including all DLBCL patients in Sweden, having primary refractory disease or relapse was less common than what was previously thought. These patients did poorly with standard treatment and are thus highly in need of better and more tolerable treatment. We also conclude that curatively treated DLBCL patients had an increased risk of having a heart attack during and shortly after their treatment, but no increased long-term risk.

ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is an aggressive life-threatening disease, but it is often possible to cure with immunochemotherapy. Not responding to primary treatment or having a relapse is associated with poor prognosis. However, it has not been well described how large the proportion of patients with relapsed/refractory disease is. There is also a lack of knowledge regarding the outcome for these patients in a population-based setting. Standard primary treatment for DLBCL includes anthracyclines, which has been associated with an increased risk of heart failure in several studies. Most patients with DLBCL are also older (>70 years) and many have comorbidities that are associated with cardiovascular disease, but their rate of cardiovascular events such as acute myocardial infarction (AMI) is not well studied. We performed nation-wide studies to assess the cumulative incidence of relapsed/refractory disease, the outcome for the relapsed/refractory patients and patients with central nervous system (CNS) relapse in particular and also studied the AMI rate among DLBCL patients compared to the general population.

In study I we identified all newly diagnosed DLBCL patients (during 2007-2014, n=4243) in the Swedish Lymphoma Register, complemented with information on relapsed/refractory disease through a medical record review. Patients were followed from the time of DLBCL diagnosis until relapse or death of any cause. Their median age was 71 years (range 18-105) and 84% (n=3550) received primary treatment with curative intent resulting in 5-year overall survival (OS) of 65% (95% confidence interval (CI): 64-67). Fourteen percent of the DLBCL patients (median age: 84) did not receive curative intent treatment and their median OS was 2.9 months. The cumulative incidence of relapsed/refractory disease at any site among curatively treated patients, when assessed in the presence of the competing risk of death, was 23% (95% CI: 22-25) after 5 years. The cumulative incidence of CNS relapse at two years was 3% (95% CI: 2-4) overall and 8% (95% CI: 6-11) among high-risk patients with CNS IPI 4-6.

In study II the relapsed/refractory DLBCL patients identified in study I (n=736) were followed from the time of relapse/refractoriness until death of any cause. For the whole group the median OS was 6.6 months (95% CI: 5.8-7.9) and for patients below and above 70 years it was 9.6 and 4.9 months respectively. Having relapse within 12 months from diagnosis was associated with worse outcome. Among patients who were 70 years or younger at the time of relapse/refractoriness, two thirds (63%) received standard second-line intensive therapy and one third (35%) were consolidated with autologous stem cell transplantation (ASCT). For patients with early relapse (≤12 months) who received ASCT, 2-year OS was 40% (95% CI: 26-53) from the time of transplant, whereas for patients with late relapse (>12 months) 2-year OS was 66% (95% CI: 54-76). When applying inclusion/exclusion criteria commonly used in several clinical trials studying chimeric antigen receptor (CAR) T-cell therapy, in total 35% of patients ≤76 years fitted trial criteria and their survival was not much longer than those not eligible for CAR T (median progression-free survival (PFS): 4.8 months (95% CI: 2.9– 6.3) vs 3.1 months (95% CI: 2.6– 3.8)).

Study III included patients with CNS relapse identified in study II (n=145) who were followed from the time of CNS relapse until death of any cause with the aim to assess their survival and eligibility to CAR T-cell therapy. A majority had their CNS relapse at the time of their first relapse (81%, n=118) and most had isolated CNS-involvement (68%). Two year-OS for patients with CNS involvement at first relapse was 12% (95% CI: 7-18) and median OS was 3 months (95% CI: 3-4). Patients who could receive a second-line regimen containing high-dose methotrexate had 2-year OS of 18% (95% CI: 8-32). One third of the CNS relapse patients fitted the CAR T trial criteria and their median OS was 5 months (95% CI: 3-6).

In study IV the curatively treated DLBCL patients that were identified in study I (n=3548) and comparators from the general population matched by age and sex (n=35 474) were followed from the time of the DLBCL diagnosis to assess the rate of AMI. The cohort was linked to the National Patient Register and the Swedish Cause of Death Register to identify cases of AMI and to SWEDEHEART (Swedish web-system for enhancement and development of evidence-based care in heart disease evaluated according to recommended therapies) to obtain detailed information regarding the AMIs and their treatment. The DLBCL patients had a 33% excess rate of AMI compared to the general population (hazard ratio (HR) 1.33, 95% CI: 1.14-1.55) over the whole study period. The highest rate was observed during the first year following DLBCL diagnosis, but after two years there was no evident difference between DLBCL patients and comparators. Among older DLBCL patients, with mild or moderate comorbidity such as hypertension or diabetes, the excess rate of AMI was 61% (HR 1.61, 95% CI: 1.10-2.35). There was no significant difference in AMI characteristics, clinical management of the AMI or 30-day survival among DLBCL patients and comparators.

In summary we found that relapsed/refractory DLBCL was less common than previously described affecting approximately one in four curatively treated patients. However, the patients who experienced it had very poor prognosis, especially those with early relapse and those with relapse involving the CNS. DLBCL patients had an increased rate of AMI that motivates monitoring of other cardiovascular risk factors during and shortly after the DLBCL treatment.

LIST OF SCIENTIFIC PAPERS

I. Incidence of relapsed/refractory diffuse large B-cell lymphoma (DLBCL) including CNS relapse in a population-based cohort of 4243 patients in Sweden.
Harrysson S, Eloranta S, Ekberg S, Enblad G, Jerkeman M, Wahlin BE, Andersson P-O, Smedby KE.
Blood Cancer Journal. 2021 Jan 7;11(1):9.

II. Outcomes of relapsed/refractory diffuse large B-cell lymphoma and influence of chimaeric antigen receptor T trial eligibility criteria in second line - A population-based study of 736 patients.
Harrysson S, Eloranta S, Ekberg S, Enblad G, El-Galaly TC, Sander B, Sonnevi K, Andersson P-O, Jerkeman M, Smedby KE.
Br J Haematol. 2022 Jul;198(2):267-277.

III. Outcomes for patients with secondary CNS involvement in relapsed/refractory diffuse large B-cell lymphoma and estimation of eligibility to CAR T-cell therapy.
Harrysson S, Eloranta S, Ekberg S, Enblad G, Andersson P-O, Sonnevi K, Ljungqvist M, Sander B, Jerkeman M, Smedby KE.
Manuscript

IV. Myocardial infarction in diffuse large B-cell lymphoma patients - a population-based matched cohort study.
Ekberg S*, **Harrysson S***, Jernberg T, Szummer K, Andersson PO, Jerkeman M, Smedby KE, Eloranta S.
Journal of Internal Medicine. 2021 Nov;290(5):1048-1060.
*Both authors contributed equally to the study.

CONTENTS

LIST OF ABBREVIATIONS

ABC	Activated B-cell
AMI	Acute myocardial infarction
ASCT	Autologous stem cell transplantation
BEAM	Carmustine, etoposide, cytarabine, melphalan
BR	Bendamustin, rituximab
CAR T	Chimeric antigen receptor T
CI	Confidence interval
CNS	Central nervous system
CR	Complete remission
CRS	Cytokine release syndrome
CSF	Cerebrospinal fluid
DA-EPOCH-R	Dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab
DLBCL	Diffuse large B-cell lymphoma
GCB	Germinal centre B-cell
HR	Hazard ratio
ICANS	Immune effector cell-associated neurotoxicity syndrome
ICD	International classification of diseases
IPI	International prognostic index
Non-GCB	Non-germinal centre B-cell
OR	Odds ratio
ORR	Overall response rate
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial remission
R-CHOEP	Rituximab, cyclophosphamide, doxorubicin, vincristine, etoposide and prednisone
R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone
RCT	Randomised controlled trial

R-DHAOx	Rituximab, dexamethasone, cytarabine and oxaliplatin
R-DHAP	Rituximab, dexamethasone, cytarabine and cisplatin
R-GDP	Rituximab, gemcitabine, dexamethasone, and cisplatin
R-GemOx	Rituximab, gemcitabine and oxaliplatin
R-ICE	Rituximab, ifosfamide, carboplatin and etoposide
RIKS-HIA	Swedish register of information and knowledge about Swedish heart intensive care admissions
R-IME	Rituximab, ifosfamide, mitoxantrone and etoposide
SCAAR	Swedish coronary angiography and angioplasty register
SD	Stable disease
SEPHIA	Secondary prevention after heart intensive care admission
SWEDEHEART	The Swedish web-system for enhancement and development of evidence-based care in heart disease evaluated according to recommended therapies

1 INTRODUCTION

In my clinical work as a haematologist working mainly with lymphoma patients, one of the biggest challenges I meet in the clinic are the patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL). It was back in 2015 that I started discussing with my main supervisor Karin Ekström Smedby that we should try to study this group of patients in particular. We wanted to know who they were, how many they were and how they did on a group level. We knew what it looked like for patients who were included in clinical trials, but not for our patients that we meet every day in the clinic who are more diverse in terms of age and comorbidities. We knew that information regarding relapse had been possible to record in the Swedish Lymphoma Register since 2010, but we also knew that it was incomplete and realised that a large data collection would be necessary to be able to answer the research questions that we had.

During the course of the work with my thesis there has been a breakthrough in treatment for relapsed/refractory DLBCL patients with the discovery of chimeric antigen receptor (CAR) T-cell therapy. This led to new questions regarding our relapsed/refractory DLBCL patients. Could they also be candidates for CAR T-cell therapy? Or were the patients who were treated in the first CAR T studies highly selected since they were referred to and treated at big cancer centres in the US?

It was natural when starting with research to focus on the most challenging group of patients, but luckily most of the DLBCL patients that we treat will be cured and hopefully live long healthy lives after their lymphoma treatment. This made it logical to also study the side effects of the lymphoma treatment. I learned during my residency that anthracyclines could cause heart failure (it has been well described in several studies), but the knowledge regarding other heart complications was scarce. Although I had met these patients myself several times in the clinic, we could not find many publications regarding the risk among DLBCL patients to have an acute myocardial infarction (AMI). With the excellent Swedish health registers, we had a great opportunity to do that.

2 LITERATURE REVIEW

2.1 INTRODUCTION

DLBCL is the most common lymphoma that affects nearly 600 individuals each year in Sweden. The clinical presentation is diverse, but can often be severe with a rapidly progressive disease. Despite its aggressiveness a majority of DLBCL patients are cured, but a proportion of the patients will either not respond to primary therapy or relapse with a considerable worsening of the prognosis. This is a group that needs to be studied further with the aims of better tailoring of primary treatment as well as finding more effective and tolerable treatments in the relapse situation.

2.2 BIOLOGY AND PATHOLOGY

Lymphoma is a malignant disease that evolves in the white blood cells called lymphocytes. Depending on where in the normal maturation of the lymphocyte that genetic alterations occur, different lymphoma types develop. There are close to 80 different lymphoma diagnoses defined in the World Health Organization (WHO) classification based on morphologic features, immunohistochemical expression, genetics and clinical presentation. An update of the WHO classification was published in 2022 in parallel with the International Consensus Classification of Mature Lymphoid Neoplasms (1-4). DLBCL is the most common lymphoma and the name describes the histological picture as seen by the hematopathologist in the microscope (Figure 1).

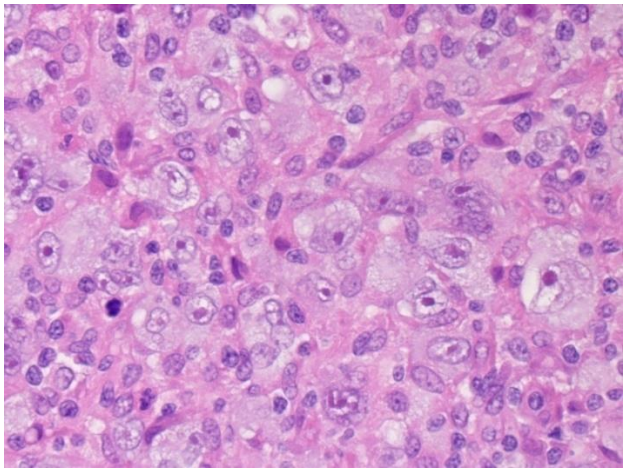


Figure 1. Histological image of diffuse large B-cell lymphoma. Copyright Birgitta Sander 2022.

The DLBCL cells are large, often multinucleated and grow in a diffuse pattern. There are different morphological variants, i.e., the anaplastic, the immunoblastic and the centroblastic variant (1). The DLBCL cells normally express the proteins CD20, CD19, CD22, CD79a and PAX5 when assessed by immunohistochemical staining or by flow cytometry. The DLBCL

cells can also express CD30 (10-20%, especially the anaplastic variant) or sometimes CD5 (5-10% of cases), where the latter is associated with worse outcome and specifically central nervous system (CNS) relapse (1, 5).

Gene expression profiling techniques were used to first identify the two different molecular subtypes germinal centre B-cell (GCB) and activated B-cell (ABC) DLBCL according to cell of origin. It was further shown that the ABC subtype has a worse prognosis when given the same treatment. The GCB subtype stems from lymphocytes who have reached the germinal centres and express CD10 and BCL6. The ABC subtype cells are similar to lymphocytes who have started their differentiation towards plasma cells expressing MUM1 and are characterised by activation of the NF-κB pathway. Approximately 10-15% cannot be included in any of these groups and are hence unclassified (6-8). More recent publications suggest that there are in fact several genetic signatures with prognostic significance and different potential targets (9-13).

In clinical work up, the subtyping is still often performed by immunohistochemical staining and by using algorithms, such as the Hans algorithm, in which staining for CD10, BCL6 and MUM1 is used for classification into GCB or non-GCB subtype (14) (Figure 2). So far, the molecular subtypes have limited value in clinical praxis and treatment choice.

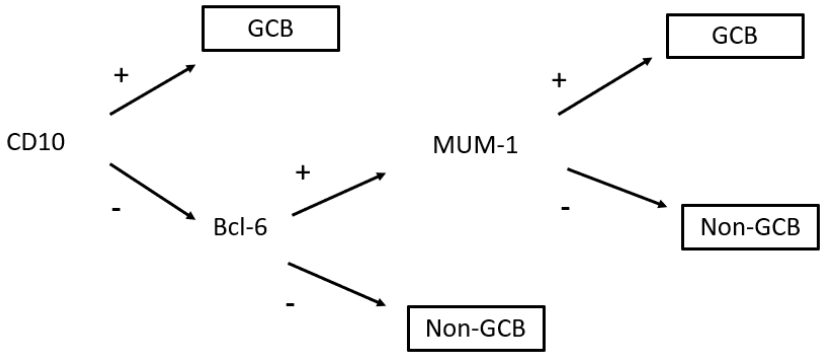


Figure 2. Hans algorithm used for characterisation of diffuse large B cell lymphoma (DLBCL) with immunohistochemical staining to divide cases into GCB (germinal centre B-cell) or non-GCB subtype (14).

There are other distinct subgroups of DLBCL as defined in the WHO classification, namely primary mediastinal B-cell lymphoma, primary CNS lymphoma and transformed low-grade lymphoma. These subtypes differ in both biology and clinical management and are not covered in this thesis. However, High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements that is a separate diagnosis in updated classifications is included as DLBCL in this thesis and so is testicular DLBCL.

2.3 EPIDEMIOLOGY AND RISK FACTORS

DLBCL is more common with increasing age and the median age at diagnosis in Swedish register data is 71 years (15). It has been described that the incidence increased from the middle of the 20th century for unknown reasons, but in the end of the 20th century the trend was broken (16). In the US, some of these cases could be explained by the HIV epidemic and some were due to improved registration, but not more than half of the cases (17).

The underlying causes for lymphoma development are to a great extent unknown. It is however known that several genetic alterations play a role in the disease evolution (18). Also the microenvironment is thought to play a role in the lymphomagenesis (19). Known risk factors for DLBCL are severe immunodeficiencies, including congenital immunodeficiency diseases and human immunodeficiency virus (HIV) infection. The rate among HIV patients has decreased markedly since the introduction of combination antiretroviral therapy (20, 21). Having an organ transplant or allogeneic stem cell transplantation, with the associated immunosuppressing medication, is also a known risk factor for what is then defined as a post-transplant lymphoproliferative disease (PTLD) that is often an Epstein-Barr virus (EBV) driven DLBCL (22).

Epidemiological studies have also pointed out the increased risk among patients with autoimmune or rheumatic disease to develop lymphoma, including DLBCL (23-25). The risk does not seem to be associated specifically to the treatment, but rather with more severe rheumatic disease with uncontrolled inflammation (26). However, further studies are warranted in this area. Other factors like for instance family history, exposure of pesticides, obesity and smoking have also been associated with an increased risk and it seems plausible to assume that the various factors contribute to the evolution of lymphoma in a multifactorial manner (27).

2.4 CLINICAL CHARACTERISTICS AND DIAGNOSIS

DLBCL is an aggressive disease that usually causes rapidly evolving symptoms of fever, night sweats and/or weight loss, referred to as B-symptoms. Common sites for DLBCL involvement are in enlarged lymph nodes and other lymphatic organs such as tonsils and spleen. It can however occur anywhere in the body and depending on where it is located, the disease can cause a wide variety of symptoms. Possible clinical presentations are for instance skin lesions, liver or kidney failure, ileus, gastrointestinal bleeding or vena cava syndrome. If the bone-marrow is involved, blood values can be affected, with anaemia, thrombocytopenia and sometimes neutropenia. Another common blood sample alteration is an elevated lactate dehydrogenase (LDH) level that comes from the rapid cell turnover of the lymphoma cells. This high cell turnover can also result in a tumour lysis syndrome, either spontaneously or more commonly when treatment is initiated. It presents with elevated uric acid, potassium and phosphate, a decrease in serum calcium and in worst case results in kidney failure.

Primary CNS lymphoma is a specific subgroup of DLBCL, representing DLBCL located in the central nervous system only. However, also a disseminated DLBCL can have CNS involvement at diagnosis and hence present with neurological symptoms.

The diagnosis of DLBCL is made from histopathological examination of a removed lymph node or core biopsy. A fine needle aspiration can raise the suspicion of lymphoma, but is generally not diagnostic (28). Clinical workup at baseline includes nowadays preferably a PET-scan for staging (28), or sometimes for practical reasons a computer tomography and a bone marrow biopsy. Lumbar puncture with diagnostic samples for cytology and flow cytometry is usually performed on patients with high risk for CNS relapse, with the addition of MRI of the brain in case of neurological symptoms or confirmed CNS involvement (29). This clinical workup is the basis for staging according to Ann Arbor with categories as follows: one lymph node region (stage I), two or more lymph node regions on the same side of the diaphragm (stage II), lymph node regions on both sides of the diaphragm (stage III) or disseminated disease with one or more extranodal sites (stage IV) (28, 30). It is also important to identify the number of extranodal sites as this has prognostic value (31). Blood workup include lactate dehydrogenase (LDH) that, if elevated, is of prognostic significance (31).

2.5 PRIMARY TREATMENT

2.5.1 Immunochemotherapy

Standard treatment has for many years been a combination of glucocorticoids and different chemotherapeutic agents: CHOP (containing 750 mg/m² cyclophosphamide, 50 mg/m² doxorubicin, 1.4 mg/m² (maximum dose 2 mg) vincristine and 50 mg/m² prednisone). Since the beginning of the 21st century, the addition of the monoclonal antibody targeting CD20, rituximab (R), has improved the outcome for DLBCL patients (32-35). The R-CHOP regimen is given every 14 or 21 days, with no evidence of difference in efficacy (36, 37). Usually patients receive 6 or sometimes up to 8 cycles of R-CHOP, but there is also evidence that 4 cycles of R-CHOP plus two doses of rituximab are non-inferior to 6 R-CHOP cycles among patients with low risk disease (38).

Randomised trials with more intensive treatment protocols have been conducted but have so far failed to show a clear outcome benefit (39, 40). However, there are reports of subgroups of patients who could benefit from treatment intensification, for instance a prospective phase II study included 53 patients with aggressive B cell lymphoma with MYC rearrangements who were treated with dose-adjusted-EPOCH-R and reported 2-year event-free survival (EFS) of 71% and overall survival (OS) of 77% (41). The Nordic Lymphoma Group trial CRY-04 included young DLBCL patients (<65 years), with age adjusted international prognostic index (aaIPI) 2-3, who received six courses of R-CHOEP followed by one course of high-dose cytarabine and one course of high-dose methotrexate (42). This phase II trial reported a 3-year failure free survival (FFS) of 65% and 3-year OS of 81%. The more recent CHIC-trial included similar high-risk patients who received an intensified treatment regimen

adding high-dose methotrexate, etoposide and cytarabine to R-CHOP and reported 5-year FFS of 74% and 5-year OS of 83%.(43). In the REMoDL-B trial, where long term follow-up was presented at ASH 2022, newly diagnosed DLBCL patients were included and from cycle 2 were randomised to either R-CHOP or R-CHOP with addition of bortezomib. The study showed improved 5-year progression-free survival (PFS) and OS with the addition of bortezomib among patients with ABC subtype and in a retrospectively defined molecular high-grade group (44, 45).

Several other RCTs have tried to add or replace agents from the standard R-CHOP-regimen. In the GOYA-trial patients were randomised between standard R-CHOP and G-CHOP (obinutuzumab (G) instead of rituximab), but there was no difference in outcome (46). Neither did addition of ibrutinib to R-CHOP show a benefit compared to R-CHOP alone in the PHOENIX-trial (47). The ROBUST-trial tested the addition of lenalidomide to R-CHOP among ABC subtype DLBCL patients without evidence of improved outcomes (48). However, the RCT POLARIX published in 2022 reported slightly better 2-year PFS for R-CHP in combination with the antibody-drug conjugate polatuzumab vedotin than the standard arm with R-CHOP (76.7% (95% CI: 72.7-80.8) vs. 70.2% (95% CI: 65.8-474.6). There was no significant difference in OS between the groups (49).

Since the prognosis is dismal if DLBCL is left untreated, even patients with severe comorbidities are considered for treatment. An alternative for elderly or frail patients is dose-reduced R-CHOP; i.e. R-mini-CHOP (50). For patients where anthracyclines are contraindicated, either because of previous treatment with anthracyclines for another cancer or because of pre-existing cardiac disease, replacing the anthracyclines with etoposide is a commonly used alternative. This modified protocol has been studied in at least two recent retrospective studies with different interpretation (51, 52), reflecting the need of prospective trials for this patient group.

2.5.2 Radiotherapy

Consolidative radiotherapy has historically been recommended for patients with bulky disease and/or residual mass following completion of primary chemotherapy (53, 54). Most studies regarding radiotherapy in DLBCL are performed in patients treated without rituximab and its role in the rituximab era is thus somewhat unclear. The use of PET scan in the post treatment evaluation has resulted in more sensitive assessments with the ability to better separate remaining active tumour from residual scar tissue and many centres have changed practice accordingly. A retrospective study from British Columbia published in 2021 in Blood reported that DLBCL patients with bulky disease at diagnosis who were PET-negative at end-of-treatment evaluation and did not receive radiotherapy had similar outcomes to PET-negative patients without bulk. (55).

2.6 PROGNOSIS

A majority of the DLBCL patients will respond to standard primary treatment with R-CHOP and the prognosis is generally good. Clinical factors known to be associated with worse

outcome are summarised in the international prognostic index (IPI) (31). The risk factors included are age greater than 60 years, stage III or IV disease, elevated serum LDH, ECOG performance status of 2 or above and more than 1 extranodal site. Each factor yields one point and the sum of points strongly correlate with survival. In the original publication from 1993, the following figures were presented: low risk (0-1 points) had 5-year survival of 73%, low-intermediate risk (2 points) had 5-year survival of 51%, high-intermediate (3 points) had 5-year survival of 43% and high risk (4-5 points) had 5-year survival of 26% (31).

Perhaps more used in clinical practice (at least in Sweden) is the simplified aaIPI (age-adjusted IPI), developed for use within one age group above or below 60 and only including stage, LDH and ECOG performance status. In the original publication, it was divided into low risk (0 points) with 5-year survival of 83%, low-intermediate risk (1 point) with 5-year survival of 69%, high-intermediate risk (2 points) with 5-year survival of 46% and high risk (3 points) with 5-year survival of 32% among younger patients. Among patients >60 years the 5-year OS probabilities were 56, 44, 37 and 21% respectively (31).

The IPI has been reassessed in the rituximab era and is still considered predictive, but the authors of the study presenting the R-IPI suggested a revised risk-group classification divided into 3 risk groups; very good prognosis (0 points) with 94% 4-year overall survival (OS), good prognosis (1-2 points) with 79% 4-year OS and poor prognosis (3-5 points) with 55% 4-year OS (56). In 2014, the National Comprehensive Cancer Network published another revised version named NCCN-IPI. This includes the same factors as the IPI score but with finer categories for age and LDH and also certain sites of extranodal involvement (bone marrow, CNS, lung, liver and gastrointestinal tract). The risk groups are: low (0-1 points), low-intermediate (2-3 points), high-intermediate (4-5 points) and high (6-8 points) and the 5-year OS estimates range from 96% to 33% in the lowest and highest risk groups (57).

2.7 RELAPSED/REFRACTORY DISEASE

2.7.1 Introduction

DLBCL in general has good prognosis, but having primary refractory disease or relapse is associated with a much worse outcome (58-60).

It is often stated that 30-40% of the DLBCL patients will either not respond to primary chemoimmunotherapy or relapse during follow-up (61-64). However, there is a lack of large up-to-date population-based studies quantifying how big a proportion of patients that will actually experience primary refractory disease or relapse. With the new treatment options that have become available for relapsed/refractory DLBCL patients in recent years it has become even more important to have correct measures of the size of this patient group.

2.8 TREATMENT IN THE RELAPSE SETTING

2.8.1 Immunochemotherapy

For patients with relapsed/refractory disease there is no consensus regarding which relapse regimen is the best and consequently several regimens are in use. Commonly used are R-ICE (rituximab, ifosfamide, carboplatin and etoposide) and R-DHAP (rituximab, dexamethasone, high-dose cytarabine and cisplatin)). These two regimens were compared in the randomised controlled CORAL-trial with no significant difference in efficacy (65). R-DHAOx (66) replacing cisplatin with oxaliplatin is also widely used, as well as R-GDP (rituximab, gemcitabine, dexamethasone, and cisplatin) (62). If the patient is young and fit, there is evidence that it is beneficial to go for high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) (67). Even though this pivotal study is from before the rituximab era this management has stayed in clinical praxis. Commonly used today is the conditioning regimen BEAM (carmustine, etoposide, cytarabine, melphalan), but there is no randomised controlled trial (RCT) comparing conditioning regimens. A recent retrospective case-control study by the European Society for Blood and Marrow Transplantation (EBMT) however reported no difference in toxicity or outcome between BEAC (cyclophosphamide instead of melphalan) or BEAM (68). Maintenance therapy with rituximab following high-dose therapy with stem-cell support did not prove to have any effect on survival (69).

Patients who are not considered eligible for ASCT, have so far had limited treatment options. If aiming for remission, R-GemOx (rituximab, gemcitabine and oxaliplatin) or R-IME (rituximab, ifosfamide, methotrexate, etoposide) are chemotherapy combinations that are in use. New treatment options have recently become available or are under way and are covered in the section other immunotherapies below.

2.8.2 CAR T-cell therapy

The treatment arsenal in the relapsed/refractory setting has expanded recently with the development of the CAR T-cells. This is a type of immunotherapy where the patient's own T cells are harvested and a CAR T-cell product with a chimeric antigen receptor targeting CD19 (usually present on DLBCL cells) and a costimulatory domain (usually CD28 or 4-1BB) is produced. This process typically takes between 2-4 weeks. The CAR T-cell product is thereafter infused in the patient following a T-cell depleting conditioning, usually containing fludarabine and cyclophosphamide. CAR T-cells first proved to be efficacious in DLBCL in the ZUMA-1 trial (axi-cel) (70) and the JULIET trial (tisa-cel) (71). ZUMA-1 included DLBCL patients who had refractory disease (SD or PD as best response to the most recent chemotherapy line) or relapse within 12 months from ASCT and 54% achieved CR with CAR T. The JULIET trial included adult DLBCL patients who had received at least two previous lines of therapy and were ineligible for or had relapsed after ASCT (70, 71) and 40% achieved CR with CAR T. Further, randomised trials of CAR T-cell therapy compared to standard of care in second-line showed superior outcomes for CAR T in the ZUMA-7 (axi-cel) (72) and TRANSFORM (liso-cel) (73) trials. In ZUMA-7 a median PFS of 14.7 months

was observed in the CAR T arm compared to 3.7 months in the standard of care arm. In the TRANSFORM trial median PFS was14.8 months (CAR T) and 5.7 months (standard of care) respectively. The BELINDA trial on the other hand reported similar outcomes for patients treated with CAR T (tisa-cel) compared to standard of care with a median EFS of 3 months in both treatment arms (74).

The toxicities associated with CAR T-cell therapy are mainly cytokine release syndrome and neurotoxicity referred to as ICANS (immune effector cell-associated neurotoxicity syndrome) (75). The knowledge regarding management of these immunologic side-effects has improved and they are usually reversible with treatment containing the IL-6 inhibitor tocilizumab and steroids (76-78). Further, the IL-1 inhibitor anakinra has been described to have effect in patients with ICANS that were refractory to steroids and tocilizumab (79).

2.8.3 Other immunotherapies

In recent years several promising immunotherapeutic options have been developed. One example is the antibody targeted cytotoxic drug conjugate polatuzumab vedotin targeting the CD79b component of the B-cell receptor. It was studied in combination with bendamustin and rituximab (BR) in a randomised phase II trial including transplant ineligible relapsed/refractory DLBCL patients. Patients who received polatuzumab vedotin and BR had a median overall survival of 12.4 months (4.7 months in the BR arm) (80, 81).

Another antibody drug conjugate is loncastuximab tesirine, targeting CD19. It has been studied in a single-arm phase II trial among relapsed/refractory DLBCL patients who had received at least two prior lines of therapy and had an overall response rate (ORR) of 48% (82).

Tafasitamab is a monoclonal antibody directed towards CD19 that has been studied in a single-arm phase II trial in combination with lenalidomide. This treatment combination resulted in ORRs of 60% (43% complete remission (CR), 18% partial remission (PR)) among 80 relapsed/refractory DLBCL patients who had received 1-3 previous lines of therapy and were not candidates for ASCT (83).

Bispecific antibodies commonly targeting CD20 and CD3 have also been developed and several different constructs have shown promising results in phase I/II trials and are currently being further investigated (84), such as mosunetuzumab (91), glofitamab (85) and epcoritamab (86).

2.9 PROGNOSIS AFTER RELAPSE

Outcome for patients with relapse is generally poor. Studies, both before and during the rituximab era, have shown that time to relapse is an important prognostic factor for patients treated with standard second-line immunochemotherapy (65, 87). Both patients with primary refractory disease (sometimes defined as SD/PD as best response or relapse within 3 months from finishing R-CHOP treatment (59)) and patients with early relapse (within one year)

have been shown to have worse survival than patients with later relapse with standard regimens prior to the CAR T era (65). IPI and aaIPI have been assessed and showed prognostic value also in the relapse setting before the rituximab era (88, 89).

2.10 CNS RELAPSE

2.10.1 Introduction

CNS relapse or secondary CNS involvement is a rare event and it has been reported that rituximab diminishes the risk, but there are few population-based studies estimating the incidence (90, 91). When a CNS relapse occurs, it is often associated with very poor prognosis why it is important to try to prevent (92-95). CNS relapse most commonly occurs early after primary DLBCL diagnosis, sometimes even during the primary treatment (42, 96). Prophylactic treatment with intrathecal methotrexate has been widely used, but studies have failed to show any benefit from this approach (97, 98). In later years systemic high-dose methotrexate (and sometimes high-dose cytarabine) has been used as a prophylactic measure to try to minimise the risk of CNS relapse (99). However, this approach has also been questioned in more recent observational studies where the prophylactic effect was not evident (100-103).

The risk score CNS-IPI was developed as a tool to estimate the risk of CNS relapse and select patients for prophylactic treatment. It includes the same risk factors as in IPI but with the addition of kidney and/or adrenal gland involvement (104). The CNS IPI categorizes the patients into low-risk (0-1 points), intermediate-risk (2-3 points) and high risk (4-6 points) with probabilities of CNS relapse of 0.6-0.8%, 3.4-3.9% and 10.2-12.0% respectively. The sensitivity of CNS IPI has been questioned and better prediction tools and/or biological markers are desirable for better selection of which patients that would benefit most from prophylactic treatment (105).

2.10.2 Treatment of CNS relapse

CNS relapse is usually a complex situation where various factors including age, performance status, comorbidity, timing of relapse and which primary treatment the patient received, will influence the choice of second-line treatment. Fairly young and fit patients are probably best treated with a high-dose methotrexate-based regimen followed by high-dose chemotherapy and autologous stem cell transplantation (106-108). The conditioning regimen in these treatment protocols include carmustine and thiotepa. This approach has also been questioned because of its toxicity (109, 110). If a patient is not suited for high-dose methotrexate and has leptomeningeal involvement, intrathecal treatment with methotrexate, cytarabine and prednisone in combination can be an option. Patients with methotrexate-refractory disease; that is a CNS progression/relapse during or shortly after high-dose methotrexate treatment, should be considered for clinical trials and, if no such trial is available, whole brain irradiation is an option (29). Ibrutinib is another option that has shown promising results in primary CNS lymphoma as monotherapy (111, 112). There is at least one ongoing clinical

trial for DLBCL patients with CNS relapse with ibrutinib in combination with multiagent chemotherapy (NCT03964090, clinicaltrials.gov).

Because of concerns of neurotoxicity, later described as ICANS, patients with CNS involvement were not included in the first CAR T-cell studies. But experience from patients treated in clinical routine (113, 114) and also few patients included in clinical trials suggest that it is safe and that they can respond well to CAR T.

2.11 CARDIAC COMPLICATIONS

2.11.1 Introduction

With the improvement in outcomes for DLBCL patients in the last years the importance of side effects and late effects of the primary treatment has increased. Cardiovascular disease is the most common cause of death worldwide and it is more common in older age groups, when DLBCL is most often diagnosed. Anthracyclines that are an important part of DLBCL treatment are known to have cardiotoxic effects (115-121). The most well described cardiotoxicity following chemotherapy treatment for lymphoma is congestive heart failure (120-123).

2.11.2 Acute myocardial infarction

Cardiovascular disease includes several diseases involving the blood vessels, valves and myocardium of the heart. In study IV of this thesis we focus on AMI, which is a serious event with great risk of resulting morbidity and/or mortality. An AMI occurs when the perfusion of blood and oxygen to the myocardium is insufficient and the myocardial cells die as a consequence (124). The underlying cause is most often a thrombosis in a coronary vessel, triggered by the rupture of an atherosclerotic plaque that activate platelets and clotting factors (125). Other possible but more rare causes are coronary artery dissection, coronary artery embolism, hypotension and anaemia. Risk factors for AMI include high age, male sex, smoking, high alcohol consumption, physical inactivity, poor diet, hypertension, diabetes and hyperlipidaemia (126-131).

2.11.3 AMI and DLBCL

Several studies have investigated the incidence of congestive heart failure among DLBCL patients receiving CHOP or R-CHOP-like therapies (120-123). However, there are not that many studies assessing the risk of AMI following diagnosis and treatment of DLBCL. An increased risk of arterial thromboembolism following a cancer diagnosis, including lymphoma, was reported in a few studies (132-134). There is one previous study that report an increased rate of AMI in older DLBCL patients (>65 years) (135). In 2022 a population-based study from Denmark reported an increased risk of myocardial infarction among non-Hodgkin lymphoma patients who received radiotherapy involving the mediastinum (136).

2.12 POTENTIAL CARDIOTOXIC AGENTS

2.12.1 Anthracyclines

Anthracyclines, such as doxorubicin that is the backbone of R-CHOP are known to increase the risk of cardiac disease, especially congestive heart failure. Both acute effects and long-term side effects have been described. (96-101) The exact mechanism for this is not completely known. There is however evidence that the anthracyclines cause production of free radicals leading to oxidative stress that damages the myofibrils of the heart. It has also been described that doxorubicin decreases the presence of endogenous antioxidants resulting in even more oxidative stress (119, 137). Whether this also affects the coronary vessels is to my knowledge not described, but is not unlikely.

The cardiotoxic effect of anthracyclines has been described in various studies to be dose-dependent and cumulative doses above 450 mg/m^2 are not recommended (116, 117).

2.12.2 Other agents included in R-CHOP

Cyclophosphamide is an alkylator that has also been associated with an increased risk of heart failure in several studies (138-140). These studies however assess the effect when the drug is given at a much higher dose as in allogeneic stem cell transplantation conditioning regimens. Its potential role in the cardiotoxicity of the combination therapy R-CHOP is unclear.

Reports of cardiotoxic effects of vincristine or other vinca alkaloids are scarce. There is a case report of coronary spasm that was attributed to an injection of vincristine, but in a very sick patient treated in intensive care (141). Other publications claim that vincristine could diminish the cardiotoxic effect of doxorubicin, at least in a mouse model (142, 143).

R-CHOP contains a fairly high dose of glucocorticoids with 50 mg/m² prednisone. It is well known that corticosteroid treatment is associated with an increased risk of hypertension and secondary diabetes (144) that are both risk factors for cardiovascular disease. High steroid doses, at least when given during a prolonged time as in some lymphoma and acute lymphoblastic leukaemia protocols, also promotes weight gain which in turn can increase the risk for cardiovascular disease.

Rituximab is a monoclonal antibody targeting CD20 that is most often expressed on the surface of the DLBCL cells. Transfusion related symptoms such as fevers, chills, shortness of breath and hypotension are common, especially during the first treatment cycle. The reaction is caused by release of cytokines following lymphoma cell death and are associated with high tumour burden. There are published case reports of both myocardial infarction and cardiomyopathy associated with rituximab (145, 146) and it is probably wise to be vigilant with comorbid and frail patients. However, the cardiotoxic effects of CHOP do not seem to have increased to a large extent with the addition of rituximab (147).

2.12.3 Radiotherapy

Long-term cardiotoxic effects among patients who receive radiotherapy involving the heart are well described (148). Historically, it was more common to give larger fields that involved more organ tissue, for instance mantle fields in Hodgkin lymphoma. With improved knowledge regarding the side effects associated with radiotherapy, techniques have been developed to minimise the fields and especially the doses to sensitive organs, such as kidneys, lungs and heart. The proton therapy is a promising technique that will possibly even further reduce long term cardiotoxic effects among lymphoma patients who need consolidative radiotherapy (149).

3 RESEARCH AIMS

The overall aim of this thesis was to add to the knowledge regarding relapsed/refractory DLBCL including CNS relapse and to study cardiac complications related to DLBCL diagnosis and treatment. More specifically the aims of the different studies were to:

- provide population-based estimates of cumulative incidence of primary refractory disease and relapse at any site as well as for CNS relapse in DLBCL

- assess second-line line treatment intensity and eligibility and to present benchmark outcomes in different subgroups including patients that are eligible to CAR T-cell therapy using inclusion criteria from clinical trials

- assess outcomes for DLBCL patients with CNS relapse and estimate the proportion potentially eligible to CAR T-cell therapy

- assess the rate of AMI among DLBCL patients compared to the general population and to assess characteristics and outcome of the AMIs among DLBCL patients

4 MATERIALS AND METHODS

4.1 SETTING

Sweden has a long tradition of registering and keeping track of its citizens. Already in the late 17th century the churches started to register the members of their parish and information regarding their births and deaths. Since 1947 all citizens of Sweden receive a 10-digit personal identity number at birth (150, 151). Persons who immigrate to Sweden and intend to stay for at least one year will also receive a personal identity number. This is administered by the National Tax Board and registered in the Total Population Register held by Statistics Sweden. This register has since 1968 contained information regarding all births, deaths and emigration and immigration linked to the personal identity number. The personal identity number is also the basis for the good quality health registers that emerged in Sweden during the late 1900s and early 2000s and enable epidemiological research on population-based cohorts. The studies included in this thesis were possible to perform because of these presumptions.

4.2 DATA SOURCES

4.2.1 The Swedish Lymphoma Register

The Swedish Lymphoma Register is a national diagnosis-specific quality register steered by the Swedish Lymphoma Group, consisting of representatives of clinical haematologists and oncologists working with lymphoma patients in clinical routine and research all over Sweden. It is coordinated by the Regional Cancer Centres (RCC), which is an organisation that work on a national level to promote equal cancer care for patients in all parts of Sweden. Patients who are diagnosed with lymphoma in Sweden have been registered in this register since the year 2000. When compared to the mandatory National Cancer Register the coverage has been 95% during the last years (15). The register includes data on diagnosis, clinical characteristics and treatments regarding all lymphoma diagnoses (except chronic lymphocytic leukaemia that is registered in a separate register). Since 2010 data on relapse has also been collected, but the coverage has not been complete. For study I in this thesis the patients with a primary diagnosis of DLBCL in 2007-2014 were identified in the Swedish Lymphoma Register.

4.2.2 Data collection

Since the data regarding relapse was not complete in the Swedish Lymphoma Register, we decided to collect complementary information from medical charts. This was done in a two-step manner. First the patients with a primary diagnosis of DLBCL were identified in the Swedish Lymphoma Register. Lists were sent out to doctors and research nurses at the local hospitals who double checked information regarding response to primary treatment and relapse. We also hired research nurses who travelled all over Sweden to collect data at various hospitals. Data that was collected was first of all to validate the information regarding response to primary treatment and relapse that was already registered. If we found relapse data that was missing or incorrect, the register was updated with the correct information. In

the second phase, patients who did have primary refractory disease or relapse were, if still alive, asked for informed consent to collect additional medical record data. For patients who consented and those who were deceased we then collected detailed data regarding site of relapse, clinical characteristics, treatment and response both at primary diagnosis and first and later relapses. This data was used together with data from the Swedish Lymphoma Register in study I, II and III.

4.2.3 SWEDEHEART

The Swedish web-system for enhancement and development of evidence-based care in heart disease evaluated according to recommended therapies (SWEDEHEART) is a quality register of cardiovascular disease and interventions (152). It was founded in 2009, when the four registers; Swedish register of information and knowledge about Swedish heart intensive care admissions (RIKS-HIA), Secondary prevention after heart intensive care admission (SEPHIA), Swedish coronary angiography and angioplasty registry (SCAAR) and the Swedish register for heart surgery were merged into one. It contains data regarding symptoms, interventions, medications and complications from all hospital admissions due to acute myocardial infarction. In study IV of this thesis we used SWEDEHEART to obtain information regarding the characteristics of AMI events of DLBCL patients and controls from the general population. In 2018, the coverage was 96% for patients younger than 80 years who were admitted to a hospital for myocardial infarction (153).

4.2.4 Other health registers

The Total Population Register is regularly linked to the Swedish Lymphoma Register and vital status and dates of death are transferred. We used this information for the main outcomes in study I, II and III and to estimate survival after AMI in study IV.

The Swedish Cause of Death Register contains information regarding all deaths that occur in Sweden each year and is managed by the National Board of Health and Welfare. It contains date of death and cause of death (International Classification of Diseases (ICD) codes) as reported by clinical doctors and connected to the personal identity number. For study IV we used the cause of death information in this register to identify patients who died of AMI (the main outcome in study IV).

The National Cancer Register is the oldest health register in Sweden, founded in 1958. It is mandatory for all health care providers in Sweden (regulated in the law regarding health registers SFS 1998:543 and the regulation on the national cancer register 2001:709) to report newly diagnosed cancer cases to this register. This is performed mostly by the pathology/hematopathology departments but also by clinicians. The data is first sent to one of the six Regional Cancer Centres in Sweden for registration and quality check and then reported to the National Board of Health and Welfare. In study II and III we used this register to identify patients who had previous cancer diagnoses.

The National Prescribed Drug Register contains data on all prescribed drugs that are dispensed at pharmacies in Sweden. Data from this register was used in study IV to assess pre-existing comorbidities among DLBCL patients and their matched comparators.

The National Patient Register contains information regarding all in-patient care and all out-patient care to specialist clinics, but not primary care. It contains all main and secondary diagnoses (ICD codes) that the patients received during inpatient care or in connection with an outpatient visit. In study II we confirmed the dates of performed autologous stem cell transplantations using records in the National Patient Register. In study IV we used data from the National Patient Register to identify the main outcome AMI and pre-existing comorbidities.

4.3 STUDY DESIGN AND STUDY POPULATION

4.3.1 Hierarchy of study designs

There are various study designs in research and different designs are considered to have different scientific value. In medical research the RCT is considered the gold standard. The advantage of this study design is that you minimise confounding by randomising study participants to either exposure (for instance a new drug) or control group (for instance the standard of care treatment). Then you compare the two groups by measuring their outcome (for instance how long they lived). You also follow the groups prospectively which make it possible to study causal relationships. However, the most appropriate study design to choose depends on the research question that you want to answer. If you for instance want to look at a specific side effect of a new treatment that might be serious but very uncommon this could be difficult to study in an RCT because you would have to include a large number of study participants leading to higher costs. Observational post marketing studies instead offer a good complement to the RCT that showed the superior outcome of the new drug.

When it comes to research regarding DLBCL, one of the problems with RCTs is the risk of selection of patients that are included. This might have the consequence that the results are not generalisable to “real-world”-patients. Old and comorbid patients are rarely accepted for inclusion in RCTs. With median age for DLBCL patients being 70 years, they represent a large proportion of the patients. Since DLBCL is an aggressive disease there is also a risk that the patients with the most aggressive, fast-growing disease are not included in clinical trials. These patients might not be able to wait to start treatment for the days to weeks that are needed for the screening and inclusion process. This was high-lighted in a recent publication suggesting refined eligibility criteria for DLBCL patients in RCTs (154). The studies included in this thesis aimed at assessing relapse patterns, survival and complications for DLBCL patients “in the real world” and with this research question the observational cohort study design is the most suitable.

Table 1. Overview of the studies included in the thesis and their designs, data sources, study populations and main outcomes.

	Study I	**Study II**	**Study III**	**Study IV**
Design	Cohort study	Cohort study	Cohort study	Cohort study with a matched comparison group
Data sources	-Swedish Lymphoma Register -Data collection	-Swedish Lymphoma Register -Data collection -National Patient Register -National Cancer Register	-Swedish Lymphoma Register -Data collection -National Cancer Register	-Swedish Lymphoma Register -Total Population Register -National Patient Register -National Cause of Death Register -SWEDEHEART -National prescribed drug register
Study population	All 4243 DLBCL patients in Sweden diagnosed in 2007-2014	736 relapsed or refractory DLBCL patients with a relapse in 2007-2018	145 CNS relapse patients with a relapse in 2007-2018	3548 curatively treated DLBCL patients diagnosed in 2007-2014 and 35 474 matched comparators from the general population
Main outcome	Relapse/refractoriness	Death	Death	Acute myocardial infarction

4.3.2 Cohort study

In study I, II and III our research questions were to assess survival for DLBCL patients in a population-based setting and we did that using the cohort study design. This is the most common design in epidemiological research. One way of defining a cohort is a group of individuals who share characteristics or a common event (155). All individuals in the cohort should be at risk for the outcome during the whole study period. In study I the common event that defined the cohort was having a primary diagnosis of DLBCL (in 2007-2014) and the patients were then consequently at risk of having primary refractory disease or relapse that was the main outcome. In study II the cohort consisted of all patients who were primary refractory or had a DLBCL relapse and the main outcome was all-cause death. In study III the event that was used to select the cohort was to be diagnosed with a CNS relapse and patients were followed until death of any cause. A cohort study can be both prospective and retrospective and all studies in this thesis were retrospective. An advantage of a retrospective observational cohort study is that it can be feasible if the data already exists. A disadvantage is that it is more difficult to study causal relationships.

4.3.3 Cohort study with external comparison group

In study IV, the research question was whether the DLBCL patients had an increased risk of AMI compared to the general population. Thus, study IV was designed as a retrospective cohort study with an external comparison group. There are two cohorts in the study. The curatively treated DLBCL patients who are followed from the time of lymphoma diagnosis and the external comparison group that was randomly selected from the general population with replacement and matched on sex and age. The comparison group was followed from the time of randomisation (which is the same as the time of DLBCL diagnosis for the corresponding DLBCL patient). The main outcome in this study was having an AMI.

4.3.4 Study population

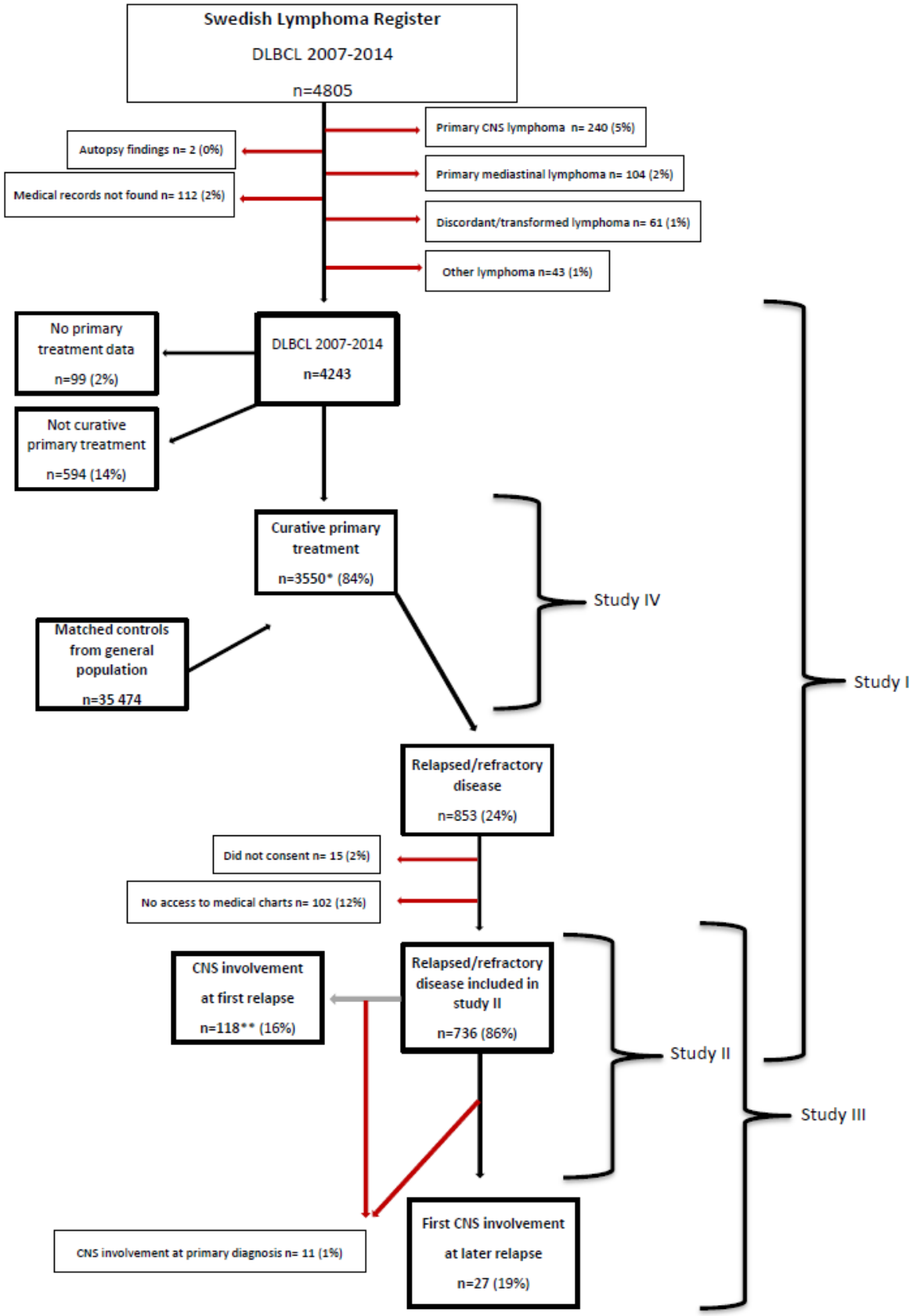
In study I the study population consisted of all patients diagnosed with DLBCL in Sweden from 2007 through 2014 and who were registered in the Swedish Lymphoma Register (n=4243). We excluded patients with primary CNS lymphoma (n=240), primary mediastinal lymphoma (n=104), transformed follicular lymphoma (n=61) and other lymphoma (n=43). Patients who were diagnosed at autopsy (n=2) or whose medical records were not found (n=112) were not included.

In study II the study population were the relapsed/refractory DLBCL patients who had received primary treatment with curative intent (n=3550) and either did not respond well to that (stable disease (SD)/progressive disease (PD)) or who responded (CR/PR) and had a later relapse (n=736). Living patients who did not consent to medical records review were excluded (n=15) as well as patients whose medical charts could not be accessed for the second phase of detailed data collection (n=102).

Study III included all DLBCL patients who were treated with curative intent and had a first involvement in the CNS during primary treatment or who had a first involvement in the CNS at first or later relapse (n=145). Patients who already had CNS involvement at primary diagnosis were excluded (n=11). For the main analyses we only included patients who had CNS involvement at first relapse (n=118).

In study IV the study population was all DLBCL patients with a primary diagnosis in 2007-2014 who received primary treatment with curative intent and to whom we could find a comparator (n=3548). Additionally, we randomly selected comparators from the general population (10 per DLBCL patient) who had the same sex and age as the DLBCL patient and who were lymphoma free at the time that the DLBCL patient got their diagnosis (n= 35 474). Each individual from the general population could be a comparator for more than one DLBCL patient.

Figure 3. Flowchart of the study populations in the different studies included in the thesis.



*For two DLBCL patients we could not find 10 matching comparators and thus only 3548 DLBCL patients were included in study IV.
**The 118 patients with CNS involvement at first relapse are included among the 736 relapsed and refractory patients in study II.

4.4 STATISTICAL CONSIDERATIONS

4.4.1 Survival analysis

All of the studies in this thesis include survival analysis. This is a term describing the statistical methods used in studies where you are investigating not only if an event occurred or not, but also the amount of time it took before the event of interest occurred. This time to the event is called survival time. In cancer patient survival studies the event of interest is often death, but the methods can be used for practically any event. Survival analysis was developed to be able to conduct studies where each study participant has an individual survival time and where you might not know the complete survival time for all individuals. It is common in research that you need to stop following individuals before everyone has had a chance to have the event and this is called censoring. Reasons for censoring can be that a study participant leaves the country before they had the event of interest (lost to follow up), or that the study participant did not have the event before the time that the study is closed (administrative censoring). These are examples of right censoring. This means that we do not know the exact survival time for the study participant, but we know that it is larger than the last observation time. In survival analysis, you also assume that the reason for study participants being censored is not related to their probability of having an event. This is referred to as non-informative censoring. Censoring is relevant in all studies included in this thesis, mainly due to administrative censoring.

4.4.1.1 Kaplan Meier estimator

One of the most commonly used methods in survival analysis is the Kaplan-Meier estimator (156). This is a non-parametric method that can be used to estimate the survival-function or the probability for a study participant to survive past a certain time point. It is a method that is very suitable to use in an RCT where you want to compare two groups who received different treatments. It can estimate the probability of responding to the treatment or the probability of not having relapsed or died from the disease under study in the two groups. If that is the setting, you can test for statistical differences between the groups using the log-rank test. If estimating for instance cancer-specific survival, the Kaplan-Meier estimator can provide an estimate of net survival. It is important to remember when interpreting the data from such a study that net survival does not provide estimates of the actual survival for the patients in the various groups as it does not consider the possible influence of competing risks (e.g. deaths due to other causes).

When interpreting the results of observational studies, it is also important to keep in mind that the Kaplan-Meier method gives you an unadjusted measure. For instance, it is not advisable to compare Kaplan-Meier curves for patients who received different treatments in an observational study, since the patients will have received treatments for different reasons that may also impact survival.

Overall survival (OS) is the most used outcome measure in cancer research. This means that you follow the study participants from the time when they start being at risk for the outcome

(for instance the time of randomisation or the time of being diagnosed with lymphoma) until death. This is the most robust and therefore recommended outcome in clinical trials of new cancer treatments. However, many clinical trials also include the outcome measure progression-free survival (PFS). This means that you follow the study participants from the time when they start being at risk for the outcome (e.g. randomisation) until they have progression of the studied disease or death. This measure is not as robust since the event progression can vary depending on various factors. For instance, it can be dependent on the clinicians varying judgement of the clinical situation or on how radiological or clinical check-ups were planned in the study. On the other hand, overall survival can be dependent on later treatments that the study participant might receive after the end of the study. In study I, II and III of this thesis we included both overall survival and progression-free survival analyses to be able to compare our population-based results to clinical trials.

4.4.1.2 Cox regression (Cox proportional hazards model)

Another commonly used method in survival analysis is Cox regression or Cox proportional hazards models (157). The Cox proportional hazards model compares the instantaneous outcome rate (hazard rate) between the exposed and unexposed during follow-up by estimating the hazard ratio (HR) (158). An advantage of this method is that you can add different covariates in a multivariable model to account for potential confounding. When using Cox regression, you assume proportional hazards. The proportional hazards assumption means that you assume that the hazards are proportional over time, or equivalently, that the association between the exposure and outcome is constant over time. If the proportional hazards assumption is not satisfied for some covariate one approach to relax the assumption is to stratify the model by the levels of that variable and assume proportionality within each stratum.

One tool that can be used to help deciding what covariates to adjust for in the multivariable models are directed acyclic graphs (DAGs). This is a way of visualising the different variables and their hypothesised causal relationship to one another. A very simple causal DAG, or causal model, can be drawn so that one arrow goes from the exposure to the outcome, and where potential confounders that are thought to have an effect on both the exposure and the outcome (confounders) are drawn with arrows pointing towards both the exposure and the outcome. In study II we used this method when discussing which clinical characteristics (exposures) that were associated with overall survival. To decide what confounders to adjust for we drew simple illustrations for each exposure with death being the outcome, see example below (Figure 4). There could of course be more confounders that we could not measure or account for.

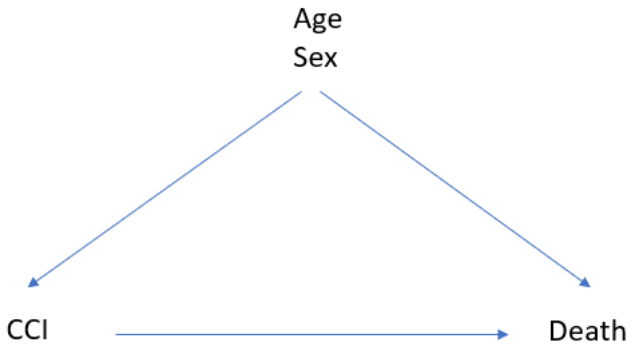


Figure 4. Illustration of the hypothesised causal relationship between Charlson comorbidity index (CCI) and death, where age and sex were considered to be confounders and were thus adjusted for in the multivariable model.

4.4.1.3 Competing risks (Aalen-Johansen method)

A competing event is an event that occurs that either makes it impossible for the event that you are studying to occur or that strongly modifies the probability of the event of interest to occur. In many cancer studies where cancer-specific death is the outcome, there will be competing events (e.g. other deaths) that will have to be considered if the study questions relates to estimating the absolute risk of dying from cancer. If one uses the traditional methods for survival analysis, such as the Kaplan-Meier method, the risk of cancer-specific death will be overestimated if it is not estimated in the presence of competing risks (159-162).

In study I, one of the outcomes of interest was the incidence of DLBCL relapse. In this analysis death without first having a relapse is a competing event, since if the study participant dies they are no longer at risk of having a relapse. In this example the Kaplan-Meier method would overestimate the incidence of relapse since it ignores the influence of death in the estimation process (where deaths are censored).

A similar example from study I is when we wanted to assess the incidence of CNS relapse. Using the Kaplan-Meier method, patients who die before having a CNS relapse will be censored. The risk of CNS-relapse will be over-estimated if the influence of competing risk (relapse at other sites and death due to any cause) are ignored in the estimation. Using the Aalen-Johansen estimator it is, however, possible to estimate the cumulative incidence in the presence of competing risks, sometimes called crude incidence or cumulative incidence (163). We used the Aalen-Johansen method to estimate the cumulative incidence of CNS relapse in different CNS IPI risk groups and found that the high-risk patients with CNS IPI of 4-6 had a cumulative incidence of CNS relapse of 8% (95% CI: 6-11). We also estimated the net risk of CNS relapse (1-Kaplan-Meier estimate) with the same outcome stratified by the same risk groups (Figure 5). In that analysis the high-risk patients had a probability of CNS relapse of 12% (95% CI: 8-15), which was similar to the estimate from the CNS IPI

publication (104). The cumulative incidence however, provide a more appropriate estimate of the real-world risk.

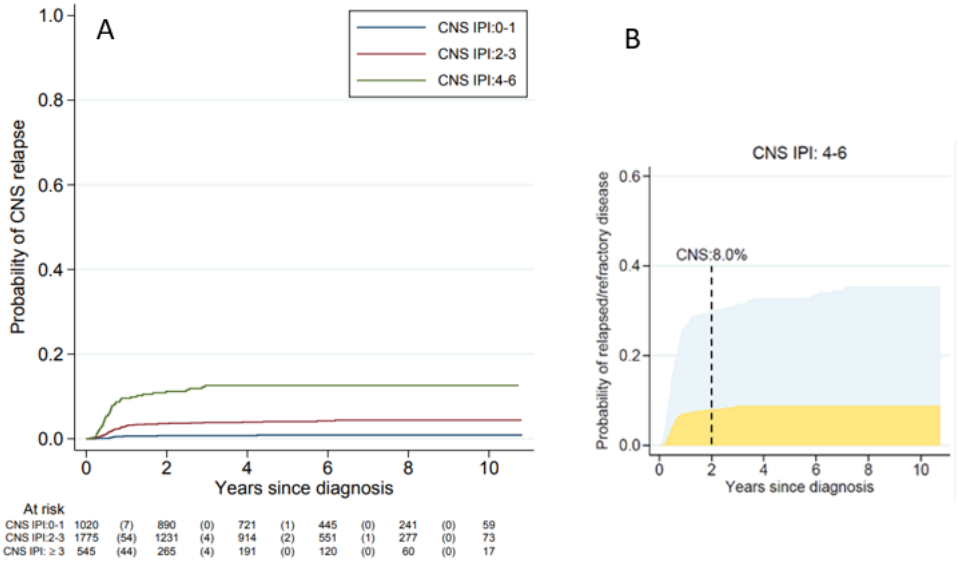


Figure 5. Cumulative incidence of central nervous system (CNS) relapse among curatively treated diffuse large B-cell lymphoma (DLBCL) patients estimated using the 1-Kaplan-Meier method (A) and the Aalen-Johansen method in the presence of the competing risk of relapse at other sites and death (B).

4.4.1.4 Flexible parametric survival models

As mentioned before, the Cox proportional hazards model is one of the most used methods in survival analysis, but one of its limitations is that it only provides an estimate of the hazard ratio. When the effect of interest is not proportional, the Cox model does not easily allow you to visualise how the effect changes with time. The flexible parametric survival model was first described by Royston and Parmar (164) and further developed by Lambert and Royston (165) and can do exactly this, as it also provides an explicit estimate of the baseline hazard rate function (in addition to the hazard ratio). We used a flexible parametric model in study IV to be able to describe visually how the risk of AMI varied over time for DLBCL patients compared to the general population.

4.4.2 Statistical methods in study I

Patients were followed from the time of primary DLBCL diagnosis until refractoriness (SD or PD as best response to primary treatment) or relapse, death of any cause or October 31 2017 (administrative censoring). This was the date when the data collection started. The cumulative incidence of relapsed/refractory disease was estimated in the presence of the competing risk of death using the Aalen-Johansen method. The same method was used when estimating the cumulative incidence of relapse specifically in the CNS and we then considered relapse at another site or death as competing events. To be able to compare with previous trials we also plotted 1-Kaplan-Meier curves with CNS relapse as the outcome.

We also estimated OS among non-curatively and curatively treated DLBCL patients using the Kaplan-Meier method. The definition that we chose for curative intent treatment was that the patient had received at least one course of a chemotherapy regimen that contained an anthracycline. Since some patients, due to cardiovascular comorbidity, might not receive anthracyclines, but instead etoposide in combination with cyclophosphamide, vincristine and prednisone, we also considered this as curative intent treatment. PFS was estimated among curatively treated patients and defined as time from primary DLBCL diagnosis to death of any cause or progression/relapse.

In this study we also investigated risk factors associated with having relapsed/refractory disease using Cox regression to estimate hazard ratios with 95% confidence intervals. Having relapsed/refractory disease was the event and patients were censored if they died. We tested the proportional hazards assumption by applying the Grambsch-Therneau test on the Schoenfeld residuals.

4.4.3 Statistical methods in study II

Patients were followed from the date of the diagnosed relapse/refractoriness until death of any cause or March 31 2019 (end of follow-up). OS was estimated using the Kaplan-Meier method. This analysis was performed among all relapsed/refractory DLBCL patients and stratified by age, time to relapse, secondary IPI and cell of origin. We chose 70 years as the age cut-off since the majority of patients above this age group will not be eligible for ASCT. We also performed analyses to study associations between different clinical disease characteristics and the probability of receiving intensive second-line therapy and to proceed to ASCT. When assessing the likelihood of receiving intensive second-line therapy or not we did not consider follow-up time in the analyses, but instead used logistic regression which estimates odds ratios with 95% confidence intervals. When studying the likelihood of obtaining an ASCT, the time from relapse/refractoriness until the ASCT, however, varied among the patients and we used Cox regression to estimate hazard ratios with 95% confidence intervals.

In this study we also aimed to estimate the proportion of relapsed/refractory patients who were 70 years or younger that went through ASCT. We used the Aalen-Johansen method estimating the cumulative incidence of ASCT in the presence of the competing risk of death among all patients (≤70 years) and stratified by age (</>60) and time to relapse (early (≤12 months) or late (>12 months)). Further we assessed overall survival for the patients who underwent ASCT by using the Kaplan-Meier method among all patients and stratified by time to relapse and age. In this analysis patients were followed from the time of transplant until death of any cause.

In study II we also estimated the proportion of relapsed/refractory patients who fulfilled the commonly used inclusion and exclusion criteria from the clinical CAR T trials (ZUMA-1, ZUMA-7 and TRANSFORM) by applying criteria by criteria and exclude the patients who did not fulfil each one of them. We also used the Kaplan-Meier method to assess OS and PFS

among the patients who did meet the CAR T trial criteria and all patients ≤76 years separately stratified by time to relapse and age.

4.4.4 Statistical methods in study III

All patients who had a primary diagnosis of DLBCL in 2007-2014, received primary treatment with curative intent and had a later CNS relapse/secondary CNS involvement were included. Patients who had CNS involvement at primary diagnosis were excluded. We described clinical characteristics for all CNS relapse patients, those with CNS involvement at first relapse and those who received methotrexate-based second-line treatment separately. We used the Kaplan-Meier method to estimate OS among all patients with CNS involvement at the time of their first relapse and at later relapse separately. Patients were followed from the time of their first CNS relapse until death of any cause or March 31 2019 (end of follow-up). We estimated PFS following the patients from time of CNS relapse until next progression/relapse at any site, death of any cause or end of follow-up. OS was also estimated stratified by second-line treatment intensity and response to second-line treatment. We used Cox regression for univariable and multivariable analyses estimating hazard ratios and 95% confidence intervals for associations between clinical characteristics and overall survival.

To estimate the proportion of CNS relapse patients that would potentially be candidates for CAR T, we applied inclusion and exclusion criteria from the TRANSFORM trial (73). In the TRANSFORM trial patients with relapse within 12 months from end of primary treatment were included. As we lacked date for end of primary treatment for all patients we chose a time cut-off of 18 months from primary diagnosis. We estimated this to be similar to 12 months from end of primary treatment in a majority of cases.

4.4.5 Statistical methods in study IV

Patients and their comparators were matched by sex and age at the time of the DLBCL diagnosis. We summarised demographic information and comorbidities as well as clinical presentation and characteristics of the AMI at the time of hospital admission (for the AMI) and used chi-square tests for comparison between the groups. Information regarding pre-existing comorbidities were collected from the National Patient Register, the National Prescribed Drug Register and the National Cancer register. We decided to categorise them into none, mild/moderate or severe. The categorisation was based on whether the comorbidity was likely to alter the DLBCL treatment or not. The comorbidity was considered mild/moderate if the patient only had atrial fibrillation, hypertension, diabetes or gastrointestinal bleeding. Patients who were previously diagnosed with stroke, angina pectoris, myocardial infarction, heart failure, peripheral arterial disease, renal failure, intracranial bleeding, dementia, chronic obstructive pulmonary disease or cancer were categorised as having severe comorbidity.

DLBCL patients and comparators were followed for rate of AMI from the time of DLBCL diagnosis (matching date for comparators) until death of any cause or 31 December 2017 (administrative censoring). In the main analysis, we used flexible parametric survival models

to model the incidence rate of AMI contrasted between DLBCL patients and comparators by using hazard ratios and 95% confidence intervals. Models were fitted separately among all patients and stratified by age and comorbidities.

Among the DLBCL patients, we also used Cox regression to assess associations between clinical characteristics of the DLBCL and pre-existing cardiovascular disease and related comorbidities and the risk of having an AMI. In multivariable analyses the models were adjusted for age at DLBCL diagnosis, sex, calendar year and pre-existing comorbidities.

We also estimated the cumulative incidence of AMI among the DLBCL patients non-parametrically with the Aalen-Johansen estimator stratified by age (≤/>70 years) and sex.

Finally, we estimated the 30-day OS for all study participants who were admitted to hospital for an AMI using both the Kaplan-Meier method and standardised survival measures applied to the flexible parametric survival models.

4.5 ETHICAL CONSIDERATIONS

In all research projects including human subjects there is a potential harm to the study participants' physical health and/or integrity. Since 1964 the declaration of Helsinki, adopted by the World Medical Association, have regulated medical research and important ethical concerns that should be applied by all medical researchers (166). In Sweden all researchers need to seek approval from the regional ethics committee before starting any research studies. For studies I, II and III in this thesis an application with the title "Observational studies of treatment and prognosis by tumour characteristics in malignant lymphoma" was approved by the regional ethics committee in Stockholm with reference number DNR 2015/2028-31/2. Amendments regarding study design with DNR 2018/1029-32 and additional linkage to health registers with DNR 2017/1335-32 were also approved. For study IV an application with the title: "Concomitant diseases in cancer and other prognostic factors" with the DNR 2007/1335-31/4 and amendments regarding linkage to the Swedish lymphoma register with DNR 2010/1624-32 and linkage to SWEDEHEART with DNR 2017/20-32 were approved by the regional ethics committee in Stockholm.

One of the foundations to all research involving human subjects is that study participants should be completely informed about the study before being given the opportunity to take part in the study or not. When it comes to the type of large register-based studies described in this thesis, much of the data was already collected and it would be very impractical to ask all participants for informed consent. For study I, II and III we asked relapse patients who were still alive for informed consent to participate in the study since it also included medical chart review and initially we also planned to examine stored tumour material. We decided for patients who had died that we did not seek consent, from for instance relatives or family, as we thought this would be unethical. This decision could have caused an internal validity problem if the patients who were alive did not wish to participate. However, the experience from previous similar studies conducted by our research group was that cancer survivors are very motivated to participate in studies like this. This also turned out to be the result as very

few patients did not provide informed consent for study participation (in total 15 patients, 1.8%).

In the studies described in this thesis no medical interventions were performed, why there was no direct harm to the study participants' physical health. However, the data used in these studies include sensitive personal information and there is thus a potential harm to the study participants' integrity. To minimise this risk the data was pseudonymised, replacing the personal number with a study number. The code key and the data itself were stored separately in a server at the Division of Clinical Epidemiology to which only researchers involved in the studies had access. We also made sure that results were only presented on a group level so that individual patients were not identifiable.

In summary I estimate the risk of harm to the study participants from participation in these studies to be minimal. Neither did the study participants themselves benefit from their participation, as we are working with retrospective data, but their participation and the results of the studies can add to the knowledge regarding DLBCL and possibly improve treatment, prognostication and follow-up for future patients.

5 RESULTS

5.1 MAIN RESULTS FROM STUDY I

We identified 4243 patients with incident DLBCL in 2007-2014 in the Swedish lymphoma register. Their median age was 71 years (range: 18-105). When following these patients from diagnosis and onwards we found that 14% (n=594, median age: 84 years) did not receive primary treatment with curative intent. Treatment intent was uncertain for 2% of the patients (n=99). Overall, 84% (n=3550, median age: 69 years) of the DLBCL patients did receive curative intent treatment. Among curatively treated patients the 5-year OS was 65% (95% CI: 64-67) and 5-year PFS was 60% (95% CI: 58-62). The main question of this study was what proportion of patients had primary refractory disease or relapse and we observed that 23% (95% CI: 22-25) of curatively treated DLBCL patients experienced this within five years, when analysed in the presence of the competing risk of death. There was no large difference between younger patients ≤70 years (5-year cumulative incidence: 22% (95% CI: 20-23) and older patients >70 years (5-year cumulative incidence: 25% (95% CI: 23-27)). Most patients experienced relapsed/refractory disease within 1 year (62%) (79% within 2 years) (Figure 6).

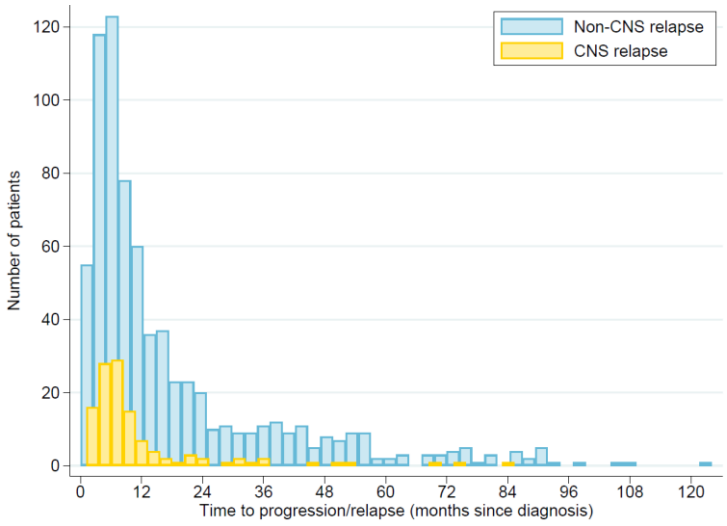


Figure 6. Histogram of time to progression or relapse among diffuse large B cell lymphoma (DLBCL) patients in Sweden (primary diagnosis 2007-2014) stratified by central nervous system (CNS) relapse and non-CNS relapse. Reproduced with permission from Springer Nature.

When assessing patients with IPI 0-1, 2, 3 and 4-5 separately the cumulative incidence of relapsed/refractory disease was 12%, 22%, 30% and 35% respectively (Figure 7). We also looked specifically at relapse in the CNS and saw that the 2-year cumulative incidence of CNS progression or relapse was 3% (95% CI: 2.5-3.6) among all curatively treated DLBCL patients. Patients who had CNS involvement at diagnosis were excluded from this analysis. Patients with a higher risk of CNS relapse (CNS IPI 4-6) had a 2-year cumulative incidence of CNS relapse of 8% (95% CI: 6-11).

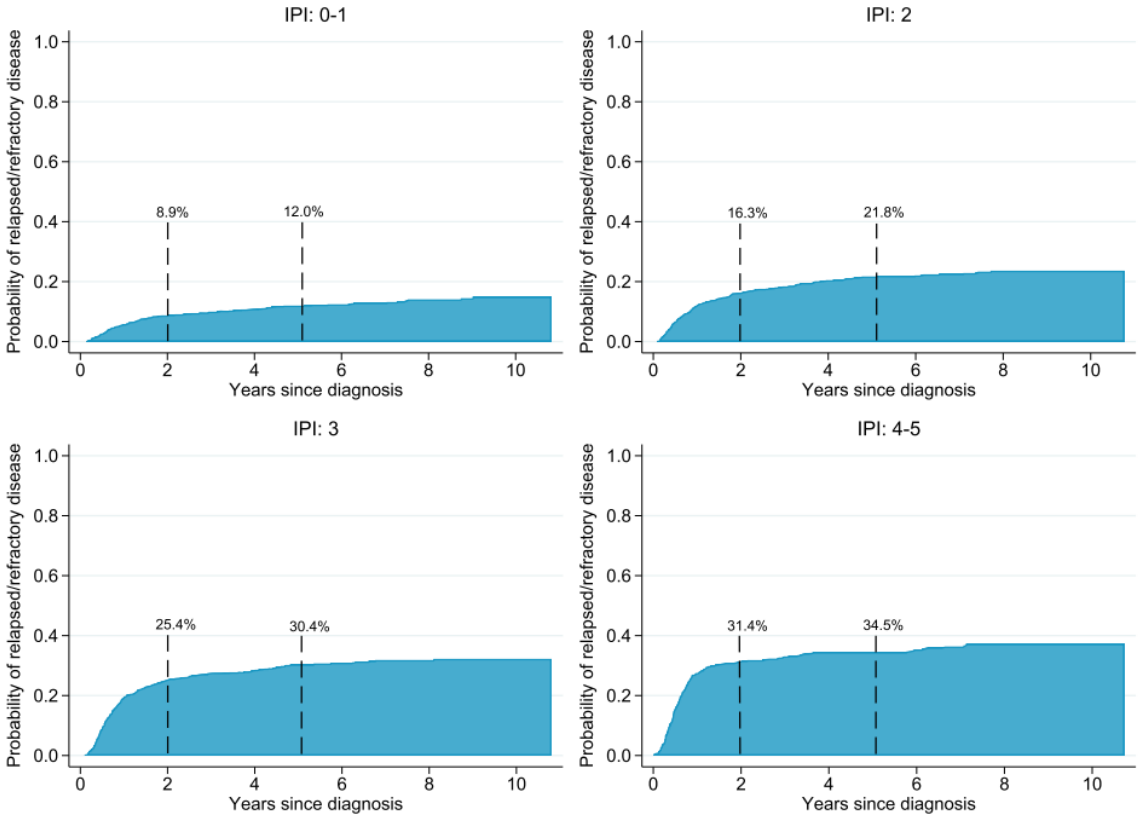
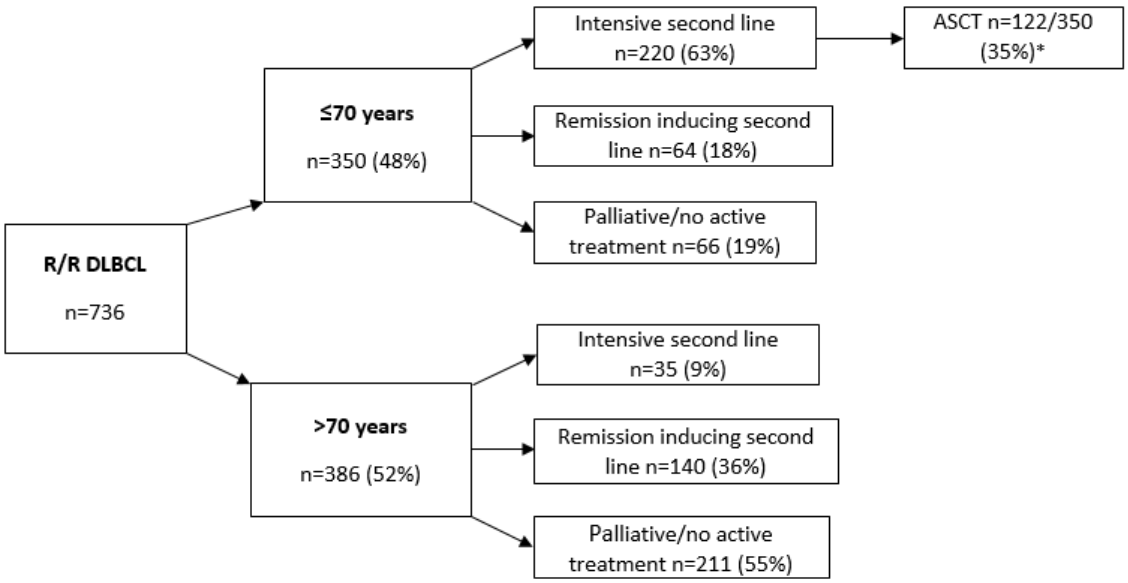


Figure 7. Cumulative incidence of relapsed/refractory disease among curatively treated diffuse large B cell lymphoma (DLBCL) patients stratified by international prognostic index (IPI). Cumulative incidence estimated in the presence of the competing risk of death, but deaths are not depicted in the figure. Reproduced with permission from Springer Nature.

5.2 MAIN RESULTS FROM STUDY II

In the second study we focused on the relapsed and refractory DLBCL patients and followed them from the time of relapse/refractoriness until death (OS) or until second relapse or death (PFS). We had identified in total 853 relapsed/refractory DLBCL patients but 117 patients were not included because of difficulties accessing their medical charts (n=102 (12%)) or because they did not consent (n=15 (2%)). The final cohort consisted of 736 DLBCL patients with relapsed or refractory disease and their median age at relapse was 71 years (range: 18–99).

We analysed the treatment patterns in the relapse setting and saw that 63% of patients who were 70 years or younger at the time of relapse/refractoriness received intensive second-line therapy (Figure 8). Among all patients who were 70 years or younger at relapse 35% (n=122) proceeded to consolidation with ASCT.



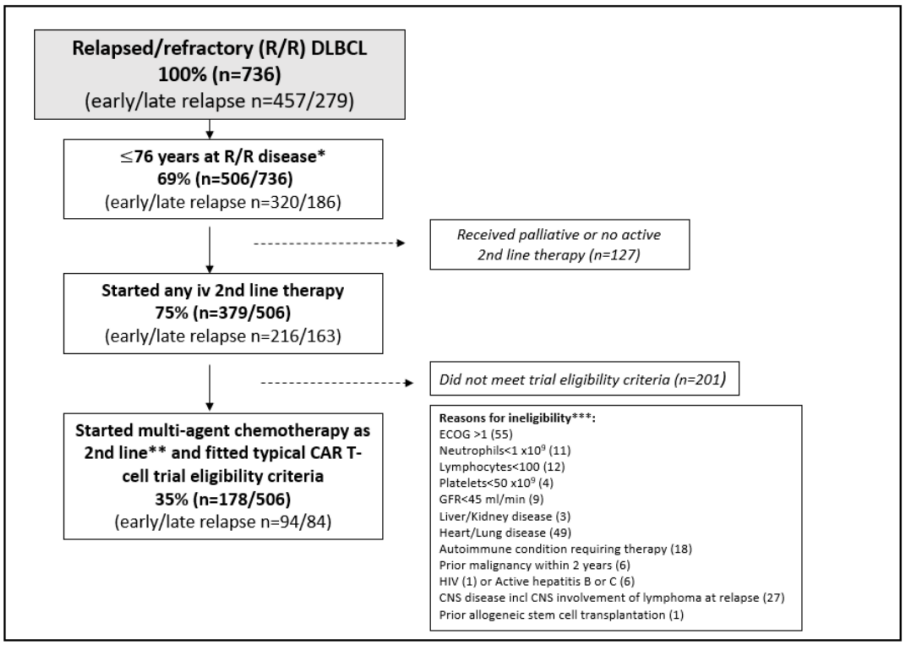
*114 patients were transplanted following second line and 8 patients received more lines before transplant. The percentage is not exactly the same as in figure 3 since that analysis account for competing risks during follow up. 5 patients >70 years also proceeded to ASCT.

Figure 8. Treatment patterns for relapsed/refractory diffuse large B-cell lymphoma (R/R DLBCL) patients treated in Sweden in 2007-2018.

Survival for relapsed/refractory DLBCL patients was generally poor with a median OS in the whole cohort of 6.6 months (95% CI: 5.8-7.9). Among patients who were 70 years or younger the median OS was 9.6 months (95% CI: 8.0-11.8) and among patients who were 70 years or older it was 4.9 months (95% CI: 4.1-5.7). In multivariable Cox regression analyses, having an early relapse, advanced stage, poor performance status and high secondary IPI was associated with worse outcome. Patients who relapsed within 6 months had a 2-year OS of 19% (95% CI: 14–24) and patients who relapsed after more than 2 years had a 2-year OS of 55% (95% CI: 47–63).

To ensure that the study cohort was representative of the underlying cohort of relapsed/refractory patients before exclusions we also performed a survival analysis with all 853 relapsed/refractory patients. In this complementary analysis, 2-year OS among all 853 patients was 27% (95% confidence interval, CI, 24-30%) which was identical to the 2-year OS in the final study cohort of 736 patients (27% (95% CI 24-30%). Also, median age, sex and distribution over time from diagnosis to relapsed/refractory disease were similar in the two cohorts.

We also estimated the proportion of patients that could be candidates for CAR T as second-line by applying the inclusion and exclusion criteria from the clinical trials ZUMA-1, TRANSFORM and the recently published ZUMA-7 (71-73). We found that 35% of all patients below 76 years of age received two lines of therapy and could have been eligible for CAR T in our population-based material (Figure 9). We also assessed survival for the CAR T eligible patients compared to all patients in the same age group who received any iv second-line therapy and with early versus late relapse. We saw no large differences in survival between patients who were eligible for CAR T and those who were not.



* Upper age cut-off was 76 years in the ZUMA-1 trial, 75 years in the Transform trial, and there was no defined upper age cut off in the ZUMA-7 trial

** The ZUMA-7 and TRANSFORM trials required eligibility to intensive second-line chemotherapy and autologous stem-cell transplantation (ASCT), but since remission-inducing treatment is standard for patients >70 years, patients were not deemed ineligible based on this criterion for this study

*** Patients were included even if data regarding neutrophils, lymphocytes or thrombocytes were missing. We did not have information on liver, cardiac or lung function beyond comorbidity data, nor on measurable disease.

Figure 9. Flowchart of retrospective assessment of eligibility to CAR T-cell therapy among relapsed/refractory diffuse large B-cell lymphoma (R/R DLBCL) patients according to typical trial criteria (based on the ZUMA-7, TRANSFORM and ZUMA-1 trials). Since inclusion criteria differed by trial regarding time between primary diagnosis and relapsed/refractory disease, we present numbers independently of time to relapse, as well as by early and late relapsed/refractory disease (≤12 versus >12 months from primary diagnosis).

5.3 MAIN RESULTS FROM STUDY III

All DLBCL patients who received primary treatment with curative intent and had a later relapse in the CNS (secondary CNS involvement) were included in this study. The cohort consisted of 145 patients with CNS relapse (median age: 68 years (range: 18-89)) and most of them (81%, n=118) had CNS involvement at their first relapse. A majority of patients with CNS involvement at first relapse had isolated CNS relapse (68%, n=80). One third (32%, n=38) of the CNS relapse patients received high-dose-methotrexate based chemotherapy regimens as second-line treatment and their ORR was 42% (24% CR and 18% PR). Survival was generally poor with a median OS of 3 months (95% CI: 3-4) among all patients with CNS involvement at first relapse. Patients who had received methotrexate-based second-line therapy also had poor survival (median OS: 6 months (95% CI: 4-8)).

Since the first publications of CNS relapse patients treated with CAR T-cell therapy were published suggesting that it could be safe and efficient also in this group we also assessed the proportion of patients who would potentially be eligible for CAR T-cell therapy by using the inclusion and exclusion criteria from the recently published CAR T trial TRANSFORM (that allowed patients with CNS relapse). We found that of the 84 patients who were <=75 years at the time of CNS relapse, 85% (n=71/84) had received an intensive second-line regimen as a proxy for being ASCT-eligible and 61% (n=51/84) had their relapse within 18 months. In total 32% (27/84) of the CNS relapse patients were eligible for CAR T-cell therapy when retrospectively applying the TRANSFORM trial criteria (Figure 10). The CAR T eligible CNS relapse patients had an ORR to the second-line therapy that they received of 26% (15% CR, 11% PR). Their 2-year OS was 7% (95% CI: 1-21) and median OS was 5 months (95% CI: 3-6).

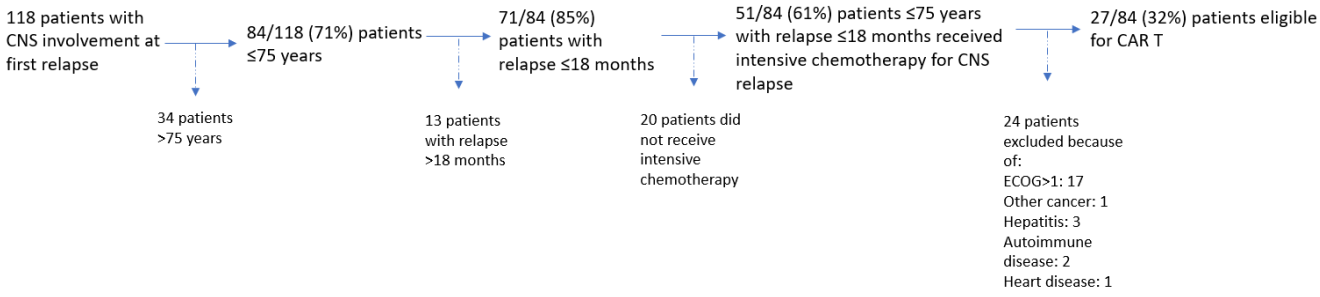


Figure 10. Flowchart of potential eligibility among diffuse large B-cell lymphoma (DLBCL) patients with central nervous system (CNS) involvement at first relapse to CAR T-cell therapy in second-line retrospectively applying TRANSFORM trial inclusion/exclusion criteria.

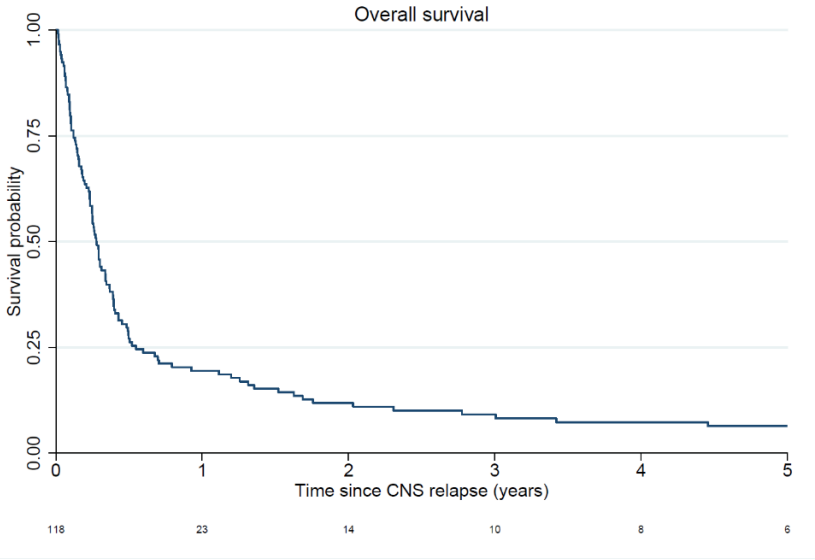


Figure 11. Overall survival (OS) among diffuse large B-cell lymphoma (DLBCL) patients with central nervous system (CNS) involvement at first relapse, n=118.

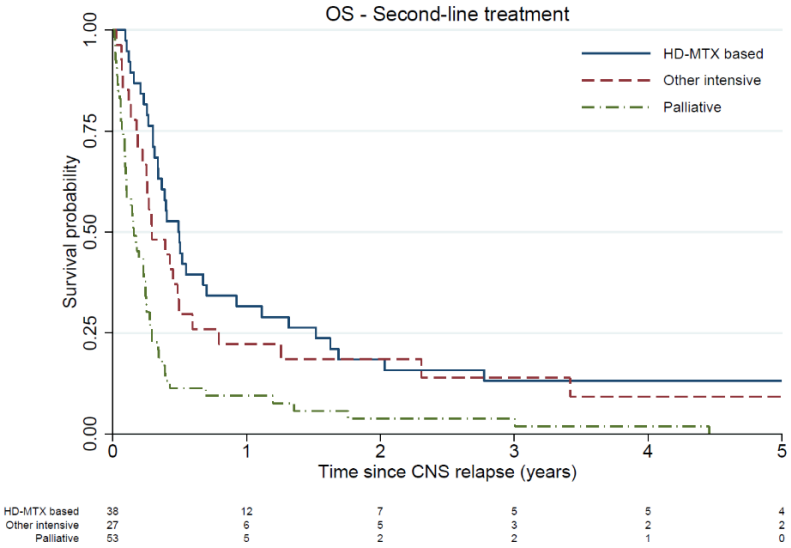
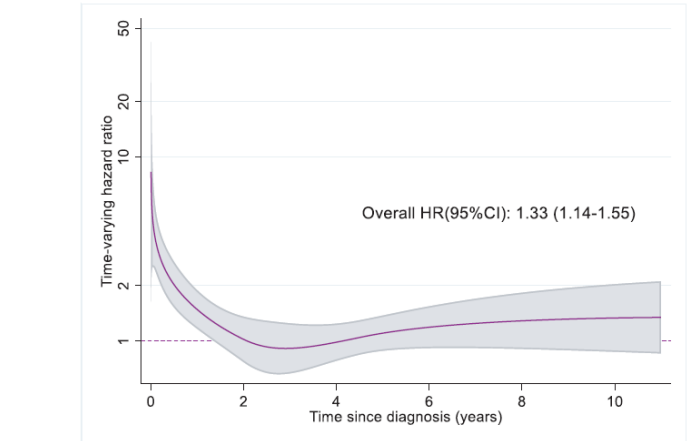


Figure 12. Overall survival (OS) among diffuse large B-cell lymphoma (DLBCL) patients with central nervous system (CNS) involvement at first relapse (n=118) stratified by second-line treatment intensity.

5.4 MAIN RESULTS FROM STUDY IV

The study included 3548 curatively treated DLBCL patients that were identified in study I and for whom we could identify 10 matched comparators from the total population register (n=35 474). Median age among the study participants was 69 years (range 18-99). Pre-existing cardiovascular comorbidities were equally common among DLBCL patients and comparators. Twenty-two percent of the DLBCL patients were categorised as having mild/moderate comorbidities and 34% as severe.

The DLBCL patients had a 33% excess rate of AMI (HR: 1.33, 95% CI: 1.14-1.55) compared to the matched comparators over the whole time period (Figure 12). The rate was highest during the first months following DLBCL diagnosis and after 2 years the DLBCL patients had the same risk as the general population on average. When stratified by age (>/≤70 years) and comorbidities (none, mild/moderate, severe) we saw that older DLBCL patients (>70 years) had an overall excess rate of AMI whereas younger DLBCL patients (≤70 years) did not. It was 61% (HR: 1.61, 95% CI: 1.10-2.35) among older DLBCL patients with mild/moderate comorbidities and 28% (HR: 1.28, 95% CI: 1.01-1.64) among older DLBCL patients with severe comorbidities. However, when estimating the hazard ratios over the first 6 months and first 2 years separately, younger DLBCL patients with severe comorbidities also had an excess rate up to 2 years following DLBCL diagnosis (HR 2.08 (95% CI: 1.18, 3.67).



At risk						
DLBCL	3548	2658	2197	1373	731	213
Comparators	35484	33587	28703	18748	10401	3105

Figure 12. Time-varying hazard ratio (HR) and overall HR with 95% confidence intervals (CI) illustrating the relative risk of acute myocardial infarction (AMI) among curatively treated diffuse large B-cell lymphoma (DLBCL) patients compared to matched comparators from the general population.

When assessing risk factors for AMI among the DLBCL patients we found as expected that higher age, male sex and pre-existing comorbidities were strongly associated with a higher rate of AMI. Clinical characteristics of the DLBCL diagnosis were not significantly associated to rates of AMI in multivariable Cox models.

The characteristics of the AMIs, such as infarction type, Killip class and ECG rhythm, that were registered in SWEDEHEART were similar among DLBCL patients and comparators. We also estimated survival among all DLBCL patients and comparators who were admitted to a hospital for AMI and found no significant difference in 30-day all-cause mortality.

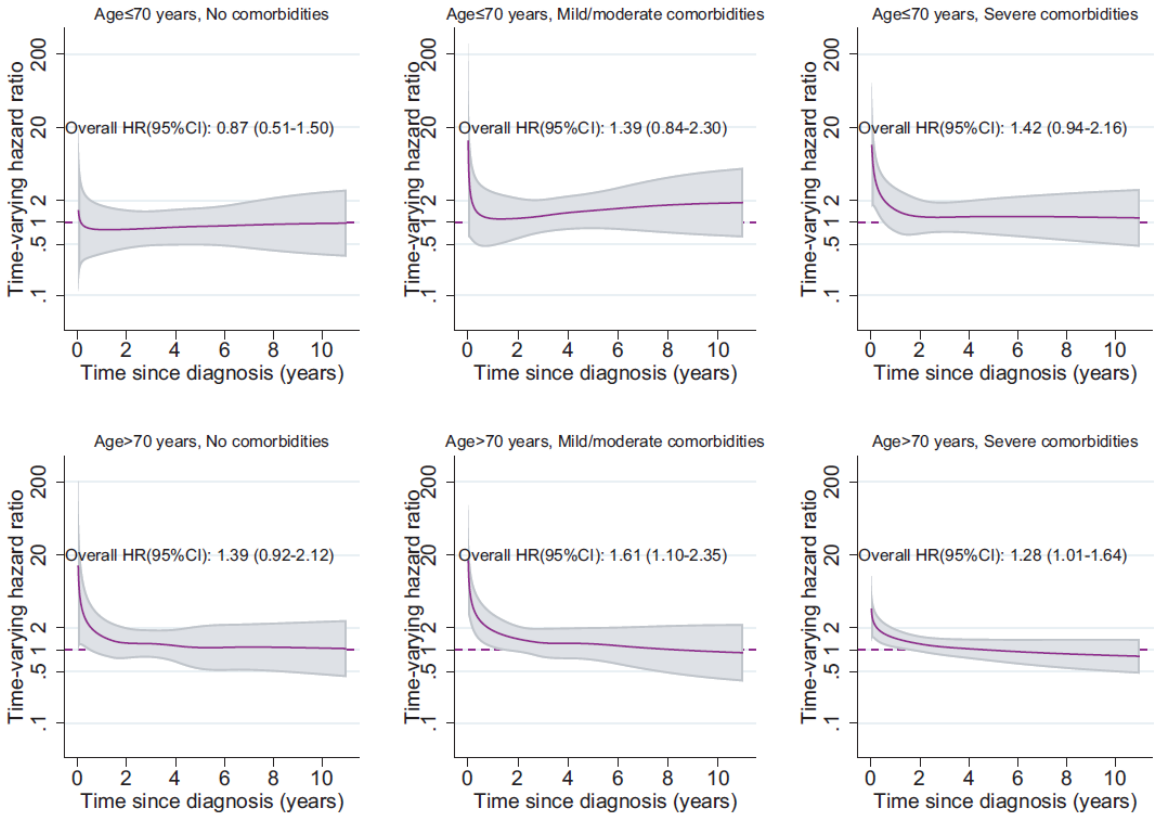


Figure 13. Time-varying hazard ratio (HR) and overall HR with 95% confidence intervals (CI) illustrating the relative risk of acute myocardial infarction (AMI) among curatively treated diffuse large B-cell lymphoma (DLBCL) patients compared to matched comparators from the general population stratified by age and comorbidities.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

All of the studies in this thesis are population-based observational studies and these types of studies of course have their strengths and weaknesses. The Swedish health care registers made it possible for us to include almost all DLBCL patients in Sweden. In doing so we also include the patients that would not be allowed in clinical trials because they have many comorbidities, are very old or maybe could not adhere to the standard treatment schedules. An advantage when including unselected patients from a whole population is that the results of the studies can be more generalisable to the real world, what is usually called external validity. Another advantage is that the large number of study participants provides conditions for good precision. A disadvantage when you are working with register data that is already collected is that some of the variables that you are interested in might not be available and there can be missing data. We tried to overcome some of this weakness by complementing the register data with a large collection of data from medical charts. Collected data was used to validate information regarding relapse and refractory disease; the most important outcome in study I and the basis for the following studies. We could also fill in some of the missing data in other register variables. However, the data collection from medical charts also had its weaknesses with examples of missing information.

In all observational studies there are different types of systematic errors or bias and the levels of bias determine the internal validity. The study population for our studies was identified in the Swedish Lymphoma Register. Since this register has a coverage of 95% (compared to the mandatory National Cancer Register) we included most of the newly diagnosed DLBCL patients, but not all of them and it is possible that the patients that were not included in the register were not random. It is possible that patients with poor outcome, who perhaps only received one course of chemotherapy and then died or maybe were too sick to even start chemotherapy, were registered in the Swedish Lymphoma register to a lesser extent than those who fulfilled six rounds of chemotherapy and went to several doctors' visits before being cured. If this is the case, it is an example of selection bias and it would affect the results of study I so that an even larger proportion of patients did not receive curative treatment.

The large amounts of data may also lead to other disadvantages. For instance, it was not feasible to perform central pathological review of all cases in these studies. A few of the patients included in the study could be registered as having DLBCL, but might have another similar diagnosis, such as mantle cell lymphoma or Burkitt lymphoma. Also, the diagnostics and classification of lymphoma has changed over time so that a patient who received a diagnosis of DLBCL early during the study period might have received another diagnosis today (i.e. high-grade B-cell lymphoma with MYC and/or BCL-2 or BCL-6 translocation). These are examples of information bias or misclassification. Another example are the variables regarding the treatment regimens that patients received. Some patients only had free text variables describing their treatment and some of these patients had first one treatment

regimen and then another and we had to classify them as best as we could with the information that we had, and there could most certainly be cases of misclassification regarding treatment variables.

In study II we wanted to study selection to ASCT among relapsed/refractory DLBCL patients, but we also wanted to study survival for patients who had received ASCT. In most survival analyses in study II we followed the patients from the time of relapse/refractory disease until death. Since all patients who actually had an ASCT had to be alive to do it, they could not have died from the time of relapse until they had the transplant. Instead we had to follow them from the date of their transplant until death to get a more correct estimate and ensure that we did not include immortal time in the analysis.

Another common issue when working with observational studies is confounding. This is when an exposures effect on the outcome is affected by other known or unknown variables (confounders). Confounding variables are associated both with the exposure and has a causal effect on the outcome and as a consequence mix up with the effect of the exposure. For example, in study II we wanted to assess the effect of time to relapse on being selected to intensive second-line therapy among younger patients (≤70). The variable age is associated with time to relapse in the way that age could for instance have affected the chances of the patient to have tolerated primary treatment. Higher age is also likely to (on a group level) reduce the chance of being selected for intensive treatment and was thus considered a confounder that we needed to adjust for when analysing the association between time to relapse and selection to intensive treatment. Confounding can be minimised by different measures, for instance randomisation, matching or restriction. In an RCT, confounding is minimised by randomly assigning the patients to the different treatment arms at the beginning of the study. In all studies of this thesis we used multivariable regression models to minimise the effects of confounding in the analyses. There is however always a possibility that there are unknown confounders. Confounding by indication means that in observational studies every patient who receive a certain treatment is selected (or not selected) for this treatment for a reason (or various reasons). Even if we try to adjust for this in multivariable regression models we will very likely have some confounding left (residual confounding). Therefore, we cannot draw conclusions regarding for instance which treatment is the best second-line chemotherapy for relapsed/refractory DLBCL patients from study II.

6.2 INCIDENCE OF RELAPSED/REFRACTORY DLBCL

In study I of this thesis we found that relapsed/refractory DLBCL is less common than what is commonly stated in lectures and review articles regarding DLBCL, with a cumulative incidence of 23% at 5 years after diagnosis. One reason for this could be if the Swedish population was very different from other populations. That would represent an external validity or generalisability problem. However, the PFS estimates in study I were similar to what was reported in several clinical trials and register-based trials before, suggesting this was not the case (33, 34, 46). When estimating the cumulative incidence of relapsed/refractory disease we performed the analysis with a method that took the competing

risks of death into account. This is the method that is statistically most appropriate for understanding the real-world risk, but somewhat less familiar in clinical research. The findings in study I regarding the risk of having relapsed/refractory disease could be important to estimate the number of potential study participants when designing future clinical trials for relapsed/refractory DLBCL patients, for planning of resources for these patients in the hospitals and for risk communication with patients and their relatives.

Another important finding in study I is the large proportion (14%) of patients who were not selected to receive first line treatment with curative intent. We unfortunately did not have data to understand exactly why these patients were not selected for treatment with curative intent, but most of them were older. Further studies could be executed focusing on these patients and on older patients in general. One example of such an initiative is the ongoing Nordic Lymphoma Group trial POLARBEAR (NCT04332822, clinicaltrials.gov). This is an RCT for newly diagnosed DLBCL patients who are over 80 or over 75 and frail and the patients are randomised between 6 cycles of R-mini-CHOP or R-polatuzumab vedotin-mini-CHP.

6.3 TREATMENT FOR RELAPSED/REFRACTORY DLBCL

Much has happened in the field of relapsed/refractory DLBCL patients in the last years. Since we performed retrospective studies the patients that were included in study II had their relapse/refractoriness in 2007-2018 and none of the patients received any of the novel treatments such as CAR T-cell therapy or bispecific antibodies. In a way, study II is a description of what the treatment for relapsed/refractory DLBCL patients used to look like before the era of targeted and cellular therapies. On the other hand, at the time of the publication of this thesis we still do not have access to CAR T-cell therapy in second-line in Sweden (only in third line). The poor results for relapsed/refractory DLBCL patients underline the need of better treatment options and the results from study II can be used as a benchmark to compare with future clinical trials. It was evident that also younger patients (<70) where we would normally aim for cure at relapse did poorly. Surprisingly many were not selected for intensive second-line therapy or ASCT, and time to relapse was one of the most important prognostic factors both when it comes to selection to therapy and survival. The CORAL-study described similar patterns of around half of intensively treated patients being able to proceed to ASCT (65). I interpret this as mainly representing a biologically more aggressive less chemo-sensitive disease and it would be very interesting to correlate this to biological markers, which we unfortunately could not do in our studies.

The CAR T trials that have been published have shown impressive responses in DLBCL patients who with the standard treatment, as shown in study II, would have had very poor survival. But, CAR T still need to prove robust long-term survival benefits to really establish its superiority to standard chemoimmunotherapy for all relapsed/refractory DLBCL patients. Patients with a late relapse are likely to respond to second-line chemotherapy and perhaps be cured with a consolidative ASCT, as shown in study II, and it seems reasonable to prioritise CAR T-cell therapy for the relapsed/refractory patients with worse prognosis (those with

primary refractory disease or early relapse). On the other hand, in my experience from the clinic, the patients with early relapse who are less likely to respond to second line chemotherapy are also the patients that are most difficult to get to CAR T-cell therapy. These patients generally have more aggressive disease and it is difficult to achieve a good enough response to chemotherapy to keep the disease in control during the production time of the CAR T-cell product. It can also be more challenging among these patients to achieve a small tumour volume, which has been associated with better survival among patients receiving CAR T-cell therapy (167, 168).

In summary, we are certainly experiencing a very exciting time, when we can give immunotherapeutic treatment to some relapsed/refractory DLBCL patients who before would only receive palliative care. It is also very challenging to decide who benefits most from this expensive treatment and at what time point and this requires many more studies to sort out.

6.4 CNS RELAPSE

In study I we could report that the incidence of CNS relapse among curatively treated DLBCL patients overall was generally low with a cumulative incidence of 3% at 2 years after diagnosis. When estimated in different risk groups according to the CNS IPI score, the high-risk patients with CNS IPI 4-6 had an 8% cumulative incidence of CNS relapse. In the publication by Schmitz et al, where the CNS IPI risk score was first described, the high-risk patients had a probability of CNS relapse of 12% (104) estimated with the Kaplan-Meier method. The main purpose of the study by Schmitz et al was to create a risk score to separate patients into different risk groups to decide what patients could be candidates for prophylactic measures. For that the methods used were correct. There is however a risk that the interpretation made by many clinicians reading that study was that the absolute risk for patients with CNS IPI 4-6 to have CNS relapse is as high as 12% and this might affect the incentive to give prophylactic treatment that could potentially have other risks.

Even though we conclude in study I that CNS relapse is a rare event, the outcome for the individuals who experience it is extremely poor as shown in study III. It is important to mention that the CNS relapse patients in study III were treated in 2007-2018 and since then more intensive treatment protocols including high-dose methotrexate in combination with cytarabine and thiotepa and consolidating ASCT are probably more widely used (107). Study III was also a lot smaller than the other studies of this thesis, with only 145 included patients in total (118 with CNS involvement at first relapse). It is however hard to collect large cohorts of CNS relapse patients and the results were similar to what was reported in other previous trials (95).

With these poor outcomes it is an appealing strategy to try to prevent CNS relapse of ever occurring. However, the current state of knowledge regarding CNS prophylaxis is complicated. The use of systemic high-dose methotrexate as CNS prophylaxis has become a widespread approach, but recent publications have questioned its use (101, 103). Thus, at the moment we know that there is no strong evidence for any prophylactic approach, but all of

the available studies are retrospective observational studies and an advantageous effect of CNS prophylaxis among extremely high-risk patients could have been missed.

Another problem with the prophylactic approach is that the CNS IPI has limited specificity as demonstrated in study II of this thesis where more than half (58%) of the patients experiencing CNS relapse had low CNS IPI (0-3) at the time of primary diagnosis. There is thus a need for prognostic biomarkers. Ongoing research regarding cell free circulating tumour DNA (ctDNA) has shown interesting findings. For instance, a small series of newly diagnosed DLBCL patients reported a 29% cumulative incidence of CNS relapse among patients where ctDNA was detected in the cerebrospinal fluid (CSF) at diagnosis and 0% among patients where it was not (169).

6.5 CARDIOVASCULAR COMPLICATIONS

In study IV we studied the risk of cardiovascular complications among DLBCL patients compared to the general population and we found that DLBCL patients had a 33% excess rate of AMI. There are not many previous studies looking at this specific outcome but a study by Tsai et al also report an increased risk of AMI among older DLBCL patients. The key question is of course what we could do in the clinic to minimise the risk for patients to have a myocardial infarction. With the study design that we used we cannot really say anything about the causal effects. There are however known well established risk factors for cardiovascular disease in general. In the study we could also show that having hypertension or diabetes was associated with an even higher risk of AMI among older DLBCL patients, as compared to the general population. It seems reasonable that targeting these risk factors also among DLBCL patients could reduce the risk of cardiovascular events. When designing the study, I had expected an increased long-term risk for myocardial infarction as has been previously described regarding heart failure. I thought that maybe the oxidative stress caused by the anthracyclines could cause injuries on the coronary arteries damaging them so that the patient would have a higher risk of ischaemic heart disease later on. It turned out I was wrong and we saw no long-term elevated risk with up to 10 years of follow-up. This is of course good news for the patients that just as DLBCL relapse being very uncommon after two years also the risk of having a heart attack in our study is comparable to the general population after two years.

7 CONCLUSIONS

With the results of these large population-based studies of DLBCL patients we can conclude that relapsed/refractory disease including CNS relapse is less common than previously described. However, outcomes for relapsed/refractory patients are generally poor with standard immunochemotherapy regimens and ASCT and new treatment options in the relapse setting are urgently needed. Patients with primary refractory disease or early relapse have the worst prognosis whereas patients with a late relapse who respond to second-line therapy and go through ASCT have better long-term outcomes. We further conclude that curatively treated DLBCL patients have an increased risk for acute myocardial infarction during and shortly after treatment. This calls for cardiovascular risk assessment at the time of DLBCL diagnosis and a closer management of known cardiovascular risk factors such as hypertension and diabetes among these patients.

8 POINTS OF PERSPECTIVE

When working with DLBCL in the clinic it becomes clear that the presentation and clinical behaviour of the disease is very diverse in different individuals. What we today group together as one disease is probably several diseases and there is a lot of ongoing research with the aim to better understand the molecular and genetic heterogeneity involving different biological pathways. Hopefully in the future we will also be able to connect the genetically distinct subtypes to targeted therapies. I believe that this type of precision medicine will gain importance and that we in the future will tailor the primary therapies for DLBCL patients not only based on clinical characteristics but to a much larger extent based on genetic characteristics in the tumours.

With improvement in primary treatment, relapsed/refractory disease and CNS relapse will hopefully be even more uncommon in the future. In a short perspective I think that we will have access to CAR T-cell therapy in second-line also in Sweden. However, the logistical and economical challenges remain. The bispecific antibodies are in an earlier stage of development and we are awaiting the results of the ongoing clinical trials of this immunotherapeutic approach. In a slightly longer perspective, I believe that bispecific antibodies will prove very efficient for relapsed/refractory patients and have the advantage of being more available (off the shelf) compared to CAR T and also have the advantage that they can be combined with chemotherapy or other therapies. It is likely that these therapies will also move into primary treatment of high-risk DLBCL patients in the coming years.

At the moment when this thesis is printed, knowing how to prevent and treat CNS relapse is probably one of the biggest challenges for haematologists and oncologists treating lymphoma patients around the world. We will hopefully see good effects of CAR T-cell therapy for patients with manifest CNS relapse in clinical trials very soon and be able to bring this treatment into clinical routine. The question regarding CNS prophylaxis would be better answered with a prospective randomised trial and perhaps the findings in the recently published observational studies can support conducting such a trial, also including prognostication with novel biological markers such as CSF ctDNA. Anyhow, I believe that the use of prophylactic high-dose methotrexate (and intrathecal methotrexate) will decrease in the coming years even if we will probably still use it in certain subgroups of patients (e.g. testicular and kidney involvement). Probably most important to avoid CNS relapse is to gain early general disease control and if we can achieve that with new treatment combinations, maybe we will not have to focus specifically on CNS prophylaxis in the future.

During the course of the work with my thesis the field of cardio-oncology has grown. With improvements in outcome not only for haematological cancers and lymphoma, but also in other cancer types the need to take care of complications and long-term consequences of the oncologic treatments have increased. Recently the European Society of Cardiology (ESC) guidelines on cardio-oncology were published in European Heart Journal (170). This is a joint effort between ESC, the European Haematology Association (EHA), the European

Society for Therapeutic Radiology and Oncology (ESTRO) and the International Cardio-Oncology Society (IC-OS). The recommendations cover not only the classical cardiotoxicities associated with anthracyclines and radiotherapies but also complications related to tyrosine kinase inhibitors and the cardiomyopathies seen with immunotherapeutic treatments such as check-point inhibitors. I think that all haematologists and oncologists working with lymphoma patients will have to be more aware of these issues in the years to come. To make risk assessments at the time of diagnosis/start of treatment and take decisions regarding preventive measures, sometimes in complicated cases in collaboration with subspecialised cardiologists. Specialised cardio-oncology units will probably be developed at least in the university hospitals. It will also be of importance to organise the follow-up for these patients. Since some of the cardiologic side effects will appear after many years when the lymphoma patients are no longer followed by the treating haematologist/oncologists it will probably be necessary to involve the primary health providers.

These measures to reduce serious side effects of the treatments in combination with evolving new and more efficient treatment alternatives will hopefully lead to an even larger proportion of DLBCL patients being cured in the future.

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