UPPSALA UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1682

Clinical and tumour biology studies of Diffuse Large B-cell Lymphoma

with emphasis on comorbidity, toxicity and outcome

CHARLOTT MÖRTH

ACTA UNIVERSITATIS UPSALIENSIS UPPSALA 2020

ISSN 1651-6206
ISBN 978-91-513-1014-5
urn:nbn:se:uu:diva-420746

Dissertation presented at Uppsala University to be publicly examined in H:son Holmdahl, Dag Hammarskjölds v 8, Ing 100/101 2tr, Uppsala, Thursday, 19 November 2020 at 09:00 for the degree of Doctor of Philosophy. The examination will be conducted in Swedish. Faculty examiner: Kristina Drott (Division of Hematology and Transfusion Medicine, Lund University.).

Abstract
Mörth, C. 2020. Clinical and tumour biology studies of Diffuse Large B-cell Lymphoma. with emphasis on comorbidity, toxicity and outcome. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1682. 85 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1014-5.

Diffuse large B-cell lymphoma (DLBCL) is curable in around 70% when treated with standard immunochemotherapy R- CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone). Treatment related toxicity affecting survivors is a clinical problem as well as comorbidities influencing the possibility to give adequate treatment. The relevance of follow-up time and follow-up measures can be debated, follow-up time in Sweden has been reduced to two years due to studies showing an OS survival comparable to standard population among DLBCL achieving EFS24.The main objective of this thesis is to study known side-effects of treatment and comorbidities and explore outcome in DLBCL in order to improve clinical treatment and follow-up decisions. In **Paper I** we explored whether the omission of vincristine (VCR) due to neurotoxicity affect outcome (DFS,OS). In 541 patients omission of VCR was made in 95 (17.6%). There was no impact on outcome measures in the whole cohort irrespective of treatment cycle for the omission nor was there a difference in the elderly (≥70 years) group. In **Paper II** we studied the occurrence of different autoimmune disorders (AID) in 612 DLBCL and analysed whether AID affects treatment outcome. We found a high occurrence of AID among DLBCL patients (17.3%) compared to the general population (3-10%). AID did not affect EFS/ LSS or OS in the whole cohort but women with B-cell response AID had a worse OS compared to other women (p=0.013). In **Paper III** we established event free survival at 24 months (EFS24) in a Swedish DLBCL cohort and analysed factors governing OS and compared OS with an age and gender matched standard population. 71.6% achieved EFS24 and OS was marginally lower than the standard population. Age was the only factor affecting OS in multivariate analysis and EFS24 patients<60 years had a comparable OS to the standard population. In older ages (>60years) there was a trend for worse OS driven by a significant difference in OS among those 60-69 years. Among DLBCL achieving EFS24, 22.4% died of cardiovascular disease and 16% from other malignancies. In **Paper IV** we explored whether advanced serum protein analysis can be used to measure doxorubicin (DXR) related cardiac/cardiovascular (CVD) disease in DLBCL patients. We found two proteins, SPON-1 associated with CVD at diagnosis and IL-1RT1 associated with emerging CVD after treatment. Compared to the general population and to a matched cohort DLBCL patients had a high occurrence of CVD at diagnosis, 33.4%. After treatment 22.6% developed CVD.

Keywords: DLBCL, autoimmune disease, cardiac toxicity, neurotoxicity, EFS, OS

Charlott Mörth, Department of Immunology, Genetics and Pathology, Experimental and Clinical Oncology, Rudbecklaboratoriet, Uppsala University, SE-751 85 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-513-1014-5
urn:nbn:se:uu:diva-420746 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-420746)

”Ibland bota, ofta lindra, alltid trösta”

This thesis is dedicated to my dear lymphoma mentors:
Late **Ulf Bandmann** and **Hans Hagberg**.
Both brilliant in mind and yet, so humble and wise. You both lead me steadily on my first trembling steps in the lymphoma universe, contaminated me with lymphoma fascination but most important you both as role models taught me the ways of an excellent, patient focused, ”doctorship”.

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Does the omission of vincristine in patients with diffuse large B cell lymphoma affect treatment outcome? Mörth C, Valachis A, Sabaa AA, Molin D, Flogegård M, Enblad G. *Ann Hematol*. 2018 Nov;97(11):2129-2135.

II Autoimmune disease in patients with diffuse large B-cell lymphoma: occurrence and impact on outcome. Mörth C, Valachis A, Abu Sabaa A, Marshall K, Hedström G, Flogegård M, Baecklund E, Enblad G. *Acta Oncol*. 2019 Aug;58(8):1170-1177.

III Age is the most important predictor of survival in diffuse large B-cell lymphoma patients achieving EFS24 - a Swedish population-based study. Amal Abu Sabaa*, Charlott Mörth*, Sverker Hasselblom, Gustaf Hedström, Max Flogegård, Mimmi Stern, Per-Ola Andersson, Ingrid Glimelius, Gunilla Enblad.* contributed equally. *Submitted.*

IV Plasma proteome profiling of cardiotoxicity in patients with Diffuse Large B-cell Lymphoma. Charlott Mörth, Amal Abu Sabaa, Eva Freyhult, Christina Christersson, Jamileh Hashemi, Nashmil Hashemi, Masood Kamali-Moghaddam, Daniel Molin, Martin Höglund, Anna Eriksson, Gunilla Enblad. *Submitted.*

Contents

1. Introduction 13
1.1 Epidemiology and etiology 13
1.2 Tumour biology 13
1.3 Prognostic factors/Clinical aspects 16
1.4 Autoimmune disease and DLBCL 19
1.5 Treatment 20
1.6 Treatment toxicity 22
1.7 Response evaluation 25
1.8 Treatment at relapse 26
1.9 Future visions 27

2 Aims 28
2.1 Overall aim 28
2.2 Specific aims 28

3 Patients and methods 29

4 Statistics 32

5 Results and Discussion 35
5.1 Paper I 35
5.2 Paper II 40
5.3 Paper III 48
5.4 Paper IV 54

6 Summary of results 65

7 Populärvetenskaplig sammanfattning på svenska 67
Bakgrund 67
Frågeställning 68
Metod 69
Resultat 69
Slutsatser 70

8 Acknowledgements 72

9 References 74

Abbreviations

aaIPI	Age adjusted international prognostic index
ABC	Activated B-cell like
ABVD	Bleomycin, dacarbazine, doxorubicin, vinblastine
ACE	Angiotensin converting enzyme
AID	Autoimmune disorder
ARB	Angiotensin II receptor blocker
ASCT	Autologous stem-cell support
ATP	Adenosine triphosphate
BCL-2	B-cell lymphoma-2 protein
BEAC	Carmustin, etoposide, cytarabine, cyclophosfamide
BEAM	Carmustine, etoposide, cytarabine, melfalan
BMI	Body mass index
CAR-T	Chimeric antigen receptor modified T-cell
CCL22	C-C motif chemokine 2
CEOP	CHOP with DXR exchanged for etoposide
CHOP	Cyclophosfamide, doxorubicin, vincristine, prednisone
CHOEP	CHOP plus etoposide
CI	Confidence interval
CLL	Chronic lymphatic leucemia
CNS	Central nervous system
COP	CHOP with DXR excluded
CPM	Cyclophosfamide
CR	Complete remission
CRu	Complete remission unconfirmed
CSR	Class switch recombination
CT	Computed tomography
CVAD	Cyclophosfamide, vincristine, DXR, methotrexate, cytarabine, prednisone.
CVD	Cardiovascular disease
DFS	Disease free survival
DHAP	Cisplatinum, cytarabine
DLBCL	Diffuse large B-cell lymphoma
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DoxoRDI	RDI for DXR
DXR	Doxorubicin
EBV	Epstein Barr virus

EC	Ethics committee
EF	Ejection fraction
EFS	Event free survival
EMA	European medicines agency
EPOCH	Etoposide, prednison, vincristine, cyclophosfamide, doxorubicin
FC	Fold change
FDA	Food and drug administration (USA)
FDG	Fluorodeoxyglucose
FISH	Fluorescense in situ hybridization
GC	Germinal center
GDP	Cisplatinum, gemcitabine
Gemox	Gemcitabine, oxaliplatine
GEP	Gene expression profiling
HGBL	High grade B-cell lymphoma
HIV	Human immunodeficiency virus
HR	Hazard ratio
Ig	Immunoglobulin
IHC	Immunhistochemistry
IKE	iIosfamide, carboplatine, etoposide
INCA	Informationsnätverk för Cancervården/ National Swedish Lymphoma Registry
IPI	International prognostic index
LDH	Lactate dehydrogenase
LSS	Lymphoma specific survival
LVEF	Left ventricular ejection fraction
MAPK	Mitogen activated protein kinase
MI	Multiple imputation
MIME	Metyl-GAG,Ifosfamide, methotrexate, etoposide
MMAE	Monomethyl auristatin
MR	Magnetic resonance imaging
MTX	Metotrexate
NCCN	national comprehensive cancer network
NF	Nuclear factor
NHL	Non Hodgkin lymphoma
NOS	Not otherwise specified
NPX	Normalized Protein Expression
NTproBNP	N-terminal pro-brain natriuretic peptide
O/O	Overweight/obesity
ORR	Overall response rate
OS	Overall survival
PCI	Percutaneous coronary intervention
PD	Progressiv disease
PEA	Proximity extension array
PET	Positron emission tomography

PFS	Progression free survival
PR	Partial response
PS	Performance status
pSS	Primary Sjögren's syndrome
R	Rituximab
RA	Rheumatoid arthritis
RDI	Relative dose intensity
RNA	Ribonucleic acid
SD	Stable disease
SEER	Surveillance, epidemiology and end results
SHM	Somatic hypermutation
SLE	Systemic lupus erythematosus
SMR	Standardized mortality ratio
U-CAN	Uppsala-Umeå Comprehensive Cancer Consortium
VCR	Vincristine
WHO	World health organisation

1. Introduction

1.1 Epidemiology and etiology

Diffuse large B-cell lymphoma (DLBCL), is the most common subtype of lymphoma and accounts for approximately 40% of all B-cell lymphomas [1–3]. DLBCL has in different studies a crude incidence of 3.81-8.31/100.000 in Europe [2–4] with around 600-700 newly diagnosed cases per year in Sweden [2] (Swedish lymphoma registry]. All age-groups are represented but incidence increases with age and the median age at diagnosis is 70 years [2,3]. More males than females are affected, with an observed male:female ratio of 1.2:1 [2].

The specific cause of DLBCL is unknown. Risk factors for developing DLBCL are family history of DLCBL with a 10-fold increase [5–7], autoimmune disorders (AID) [5,8–14] and immune suppression after organ transplant [15,16]. Chronic infection as HIV, EBV, helicobacter pylori and hepatitis C can cause DLBCL [5,11,17,18], and increased risk is seen for environmental factors such as hair dye and pesticides [5] and for high BMI [19,20]. Previous cancer and treatment of cancer may also increase the risk of DLBCL [21].

1.2 Tumour biology

Normal B-cells develop from precursor cells in the bone marrow in a process culminating in cells equipped with functional surface immunoglobulin (Ig) receptors. They then migrate to secondary lymphoid organs. After antigen stimulation creation of antibody diversity continues in germinal centers (GC) in the lymph node as the B-cells undergo somatic hypermutation (SHM) and class switch recombination (CSR). These processes demand double- stranded DNA breaks and the presence of activation-induced cytidine deaminase. B-cell differentiation through the germinal center is controlled by a number of key transcription factors. Failure of differentiation or the forming of autoantibodies lead to apoptosis but the B-cells developing Ig with a high-affinity for recognizing and binding antigen exits the GC and become either a mature plasma cell or a memory cell. However this is a complex process were oncogenic mutations may occur leading to DLBCL [22–25].

As the malignant transformation of a B cell occurs at a particular stage during normal differentiation, the malignant cell arrests in that differentiation stage. As so different lymphoma types represent a particular maturation step. The malignant B-cell and its subsequent clones carry the specific morphological, immunophenotypic and gene expression properties of that maturation step [25].

Morphologically, the entire or parts of the lymph node architecture is altered by diffuse growth of large lymphoid cells with a nuclear size equal to or exceeding normal macrophage nuclei. Reactive T-cells and histiocytes are present and there may be sclerosis and necrosis observed. There is high mitotic rate measured by Ki67. The WHO classification describes a number of morphological variants of DLBCL: centroblastic, immunoblastic, anaplastic variants and additionaly rare variants. DLBCL tumour cells express characteristic B-cell markers such as CD19, CD20, CD22, CD79a and PAX5 and a range of other markers in varying frequencies [26].

According to the WHO classification of lymphoid neoplasms 2008, DLBCL is divided into 15 different variants based on clinical, biologic and pathologic features where location has importance, for instance primary CNS and mediastinal DLBCL are considered separate diseases, divided by their gene expression, genomic profile and clinical characteristics [27]. In the latest WHO classification 2016 the distinction between activated B-cell like (ABC) and germinal center (GC) DLBCL is established as well as the new category of high-grade B-cell lymphoma (HGBL) [28]. The most common subgroup is still DLBCL not otherwise specified (NOS) and occurs in nodal and extra-nodal locations.

Table 1. High-grade B-cell lymphoma (2016WHO) Classification of Tumours of haematopoietic and Lymphoid Tissues

Table 1. High-grade B-cell lymphomas (2016WHO) Classification of Tumours of haematopoietic and Lymphoid Tissues
DLBCL, NOS
Primary DLBCL of the CNS
Primary cutaneous DLBCL leg type
EBV-positive DLBCL,NOS
Large B cell lymphoma with IRF4 rearrangement
Primary mediastinal (thymic) large B cell lymphoma
Intravascular large B cell lymphoma
DLBCL associated with chronic inflammation
HHV8 positive diffuse large B cell lymphoma
Primary effusion lymphoma
High-grade B-cell lymphoma
High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements
High-grade B-cell lymphoma, NOS
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and

Hodgkin lymphoma
Plasmablastic lymphoma
ALK-positive-large B cell lymphoma

Gene expression profiling (GEP) can be used to classify DLBCL in at least two subgroups according to the differentiation stage or cell of origin; GC (germinal center like) which is a malignant clone from an earlier maturation stage in the light zone of the germinal center and the ABC (Activated B-cell like) which derives from cells that have left the GC [24,25]. ABC is associated with a poorer prognosis in most studies [29–31]. In clinical practice the Hans algorithm (or similar ones), based on immunohistochemical (IHC) staining, is used to separate these entities [32] as the new 2016 WHO classification of lymphoid malignancies requires this distinction.

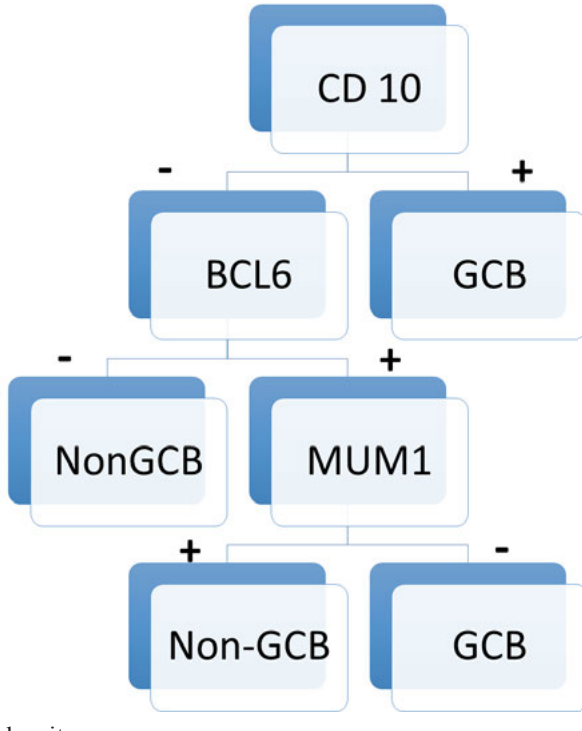


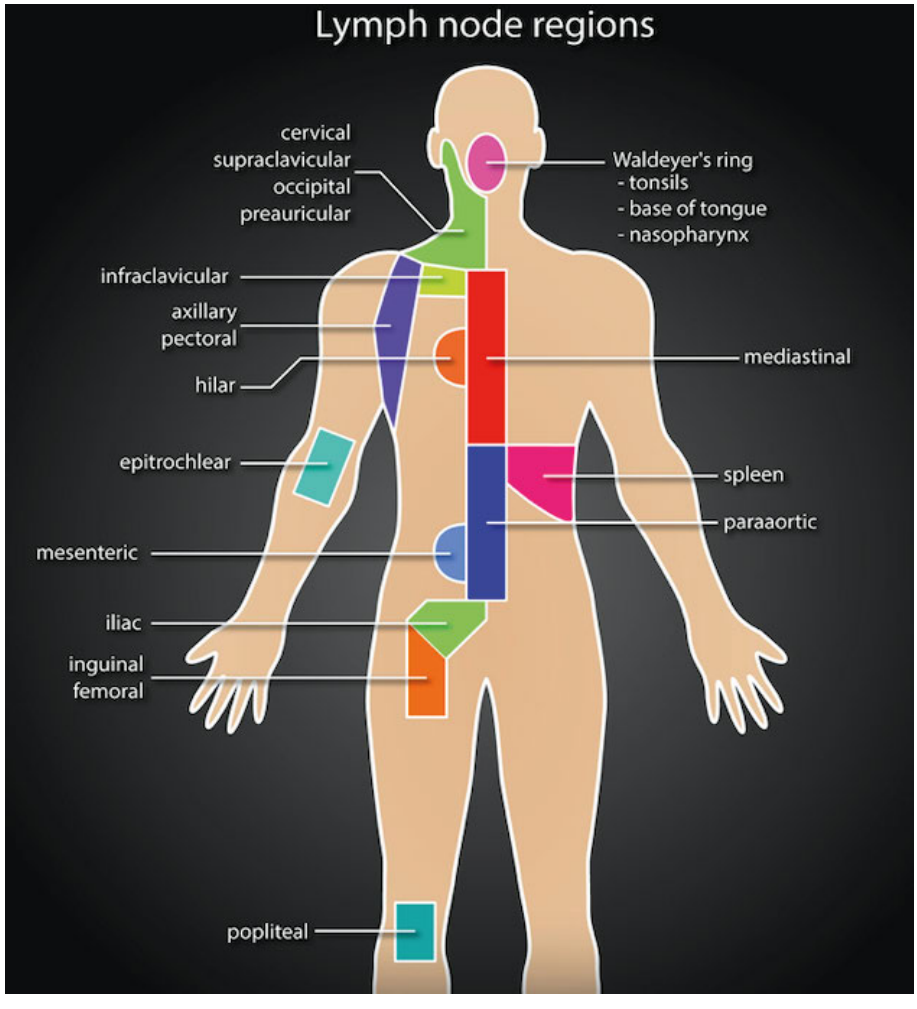
Figure 1. Hans algoritm.

But DLBCL is not as simple as two genetically different subtypes, it is a very heterogeneous group of tumours with varying biology and clinical course. It can form de novo or enhance from low-grade lymphomas. Over 700 mutated genes have been found in these tumors with 150 possible genetic drivers of the disease and with a median of 17 drivers/DLBCL case [23].

Simultaneous rearrangement of MYC and BCL-2 and/or BCL-6, so called double/triple-hit lymphoma is associated with high-risk clinical features and a dismal prognosis [33,34]. In the 2016 revision of the WHO classification of lymphoid neoplasms, DLBCL with these rearrangements became a new diagnostic category; high-grade B-cell lymphoma (HGBL), accounting for 5-10% of all DLBCL [28]. As FISH testing is not used primarily in the clinical setting screening for MYC, BCL-2 and BCL-6 with IHC can sort out the double and triple-expressors where further FISH analyses should be performed. The impact of double or triple expression is not clearly understood. IHC positive MYC and BCL-2 may occur due to increased protein expression or amplifications despite lack of rearrangement. They represent about 25% of DLBCL and are associated with inferior outcome [35–37].

1.3 Prognostic factors/Clinical aspects

There are other factors than tumour cell biology that affect prognosis in DLBCL. Age is crucial with a worsening of outcome for every 10 years addition of age [2,38]. Some studies indicate a gender difference in survival after standard treatment with women having better survival in general or in specific age groups [39–42]. DLBCL staging according to Ann Arbor for nodal involvement and to Musshoff in extra-nodal disease is used in studies and clinical practise and higher stage is associated with worse outcome [43,44].



Case courtesy of Dr Maciej Debowski, Radiopaedia.org, rID: 65530.

Figure 2. Ann Arbor stage;

I. Involvement of one lymph node region or structure.

II. Involvement of two or more lymph node regions on the same side of the diaphragm.

III. Involvement of lymph regions or structures on both sides of the diaphragm.

IV. Diffuse eller disseminated involvement of one or more extranodal locations/tissues with/without lymph node engagement.

Since 1993 the international prognostic index score (IPI) is used for dividing DLBCL in different prognostic groups. IPI consist of five factors; stage 3-4, elevated serum lactate dehydrogenas (S-LDH), performance status (PS) ac-

cording to world health organisation (WHO) 2-4, age>60 and involvement of more than one extra-nodal organ. 5-year OS for 0-1 factors is 73% compared to 26% for 4-5 factors.

Table 2. International prognostic index

International prognostic index
Stage III-IV
Elevated LDH
ECOG/WHO 2-4
Age>60 years
Involvement of >1 extra-nodal organ

The IPI score has since then been simplified to aaIPI where only three factors are used; stage 3-4, elevated S-LDH and PS [45,46]. A tool for prediction of later CNS relapse is also in clinical use; CNS-IPI which includes the five IPI factors and renal/adrenal involvement of disease [47]. New prognostic tools as the NCCN-IPI and R-IPI have been suggested but are not widely used in Sweden [48,49]. The existence of B-symptom's (fever>38, extensive night sweats, weight loss>10% in 6 months) are not considered as prognostic factors.

Standard investigation for adequate assessment of stage and prognosis includes CT scan or FDG-PET-CT scan, bone marrow biopsy, histopathological examination of tumour material and laboratory investigations. For diagnostic histopathological measurements a surgical excision/biopsy is preferred and needle biopsy should only be used in case this is not possible as the amount of good histopathologic material is essential for the possibility of making adequate diagnosis [50].

Thorough clinical examination and medical history is uttermost important for the decision on adequate therapy. In some cases with suspicion of engagement of CNS or high-risk features a spinal tap and MR of CNS is of value [51].

According to European guidelines a FDG-PET-CT scan is mandatory and it is more exact, and makes response evaluation safer compared to a CT scan but because of lack of availability this is not mandatory in Swedish guidelines [51–53](www.kunskapsbanken.cancercentrum.se (VP)).

One can refrain from bone-marrow biopsy in case FDG-PET-CT scan shows focal engagement but a negative FDG-PET-CT does not rule out low volume involvement or discordant lymphoma [51,54,55].

Evaluation of cardiac function with clinical examination, ultrasound and NT-proBNP should be considered at least for patients with evident or suspected heart disease and higher age [51].

1.4 Autoimmune disease and DLBCL

Autoimmune diseases are a heterogeneous group consisting of more than 80 separate conditions. The prevalence in the general population ranges from 3% to 10% [4,9,56–59]. The risk of developing lymphoma in AID varies with each disease and has been most extensively studied in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) with increased risk of lymphoma 2-fold in RA, 5-fold in SLE, and in pSS up to 20-fold increase compared to the general population [6,8–14]. Increased lymphoma risk have also been reported in a number of other AIDs such as thyroiditis, diabetes type 1, inflammatory bowel disease, celiac disease, granulomatosis with polyangitis and psoriasis [8,10,12,60]. Several studies have described an association between AIDs and an increase in DLBCL [5,11,57,61]. Some studies categorise AIDs as primarily mediated by B-cell or by T-cell responses and recent studies have found a correlation between DLBCL and AIDs primarily mediated by B-cell responses such as RA, SLE and pSS [10,14,62–64]. The prevalence of AIDs in a DLBCL population remains, however, unclear [14,62,63].

The underlying causes for the association between AID and lymphoma development are not fully understood. Suspected treatment induced lymphoma of the immunosuppressive drugs used in AID has not been proved [65]. Rather, more severe AID and higher inflammatory activity in both RA and pSS are related to DLBCL and other lymphomas [65,66]. Genetic susceptibility to both AID and lymphoma could be a factor, however studies in patients with AID have not been able to show a significantly increased lymphoma risk among relatives [65]. In pSS studies of sequential biopsies demonstrate a multistep process with; inflammation-persistent chronic antigenic stimulation-B-cell activity-clonal expansions of B cells and the acquisition of genetic aberrations [65].

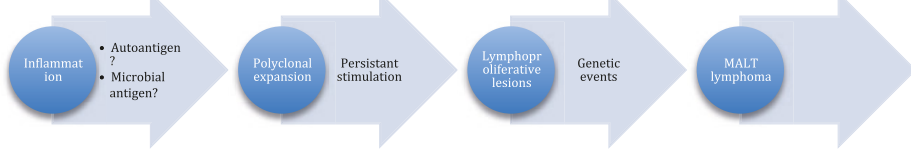


Figure 3. Development of MALT lymphoma

Further, the role of AIDs in DLBCL prognosis is still uncertain. One study, performed before the addition of rituximab (R) as standard treatment for DLBCL, indicated a poor overall survival (OS) in DLBCL patients with RA and widespread lymphoma already at diagnosis and correlation to severe RA disease [67] while another study including patients from the same period

found similar OS for DLBCL patients with RA compared to non-RA controls [68]. In one study including patients diagnosed with non-Hodgkin lymphoma (NHL) 1999–2002 self-reported AID was associated with an increased rate of all-cause death for all NHL combined but not for specific lymphoma subtypes [69]. Yet other studies indicate an association between the group of primarily B-cell mediated AIDs and inferior outcomes [62–64].

Most studies of AID and lymphoma are epidemiological and include cases before the introduction of Rituximab (R) to standard treatment. The introduction of R has improved DLBCL outcome and since it is also used to effectively curb disease progress in some AIDs updated outcome assessments of R-based treatment for AID- related DLBCL is of specific interest.

Gender-specific information about AID- related DLBCL is limited. Many of the AIDs are more common in women than in men [4,9,56–59] but studies suggest that the risk of developing lymphoma may be higher in men than women with AID [57]. Whether gender affects the outcome in AID-related DLBCL is not clear.

One clinical problem which potentially may influence prognosis in lymphoma is treatment-related neutropenic fever [70–73]. It is well known that patients with AIDs such as RA and SLE have an increased risk of infections and neutropenia may be a manifestation of some of the AIDs but it is not known if neutropenic fever is a more common problem in DLBCL patients with a concomitant AID than in DLBCL patients in general [70–74].

1.5 Treatment

Since the 1970's standard of care for DLBCL is a chemotherapy combination of CHOP (cyclophosphamide (CPM), doxorubicin (DXR), vincristine (VCR), and prednisone) [75,76]. CPM is an alkylating agent from the nitrogen mustard group that interferes with duplication of DNA [77].

Doxorubicin is an anthracyclin, an anti-tumour antibiotic, made by streptomyces bacteria. The main target for anthracyclines anti-tumour action is topoisomerase 2. Topoisomeras 2 is an adenosine triphosphate (ATP)-dependent enzyme. DXR inhibit topoisomerase 2 activity by formation of a complex, consisting of double-stranded DNA, topoisomerase 2 and DXR. The complex impedes DNA re-sealing, resulting in double- stranded DNA breaks leading to inhibited DNA replication and induced apoptosis. Furthermore DXR intercalates with DNA in the nucleus through hydrogen bindings with guanine inhibiting both ribonucleic acid (RNA) transcription and DNA replication. Activation of DNA damage responses induces cell death independent of topoisomerase 2. DXR has a complicated metabolism where reactive oxygen species are formed that may cause oxidative stress and reactions with metal ions resulting in DNA damage and cell death [78].

VCR is a vinca-alkaloid derived from the periwinkle plant that binds to beta-tubulin in the microtubules. Microtubules are essential in the spindle formation during cell division and are important for cellshape, secretion and intra-cell and axonal transport. VCR-induced inhibition of microtubule formation in the mitotic spindle of dividing cells inhibits cellular replication at the metaphase stage ultimately causing cell death. VCR can also affect cell membrane and synthesis of RNA and DNA [79–82].

Rituximab (R) a mouse/human monoclonal antibody against CD-20 has been a complement added after 2000 [51,83–85]. CD-20 is a surface marker on B-cells, expressed on >90% of malignant B-cell clones but it is not expressed on pre-B cells and mature plasma cells [86]. As so it provides an ideal target for immunotherapy in that normal B-cells restore after treatment and patients have intact memory- and plasma B-cells.

The introduction of R as a complement to CHOP has dramatically improved survival rates with an increase in overall survival (OS), progression free survival (PFS) and disease free survival (DFS) [83–85]. However, 30-40 % still have recurrent or treatment refractory disease. Gender differences in survival after R-CHOP treatment have been indicated in DLBCL patients in general with women having better survival overall [30,31] or in specific age groups [32,33].

Standard treatment includes six cycles of R-CHOP given in intervals of 14 or 21 days. Maintaining high relative dose intensity of CHOP, with or without R, has been associated with better progression free and overall survival in DLBCL [87–91]. Despite this, treatment schedules with 14 day cycles does not improve outcome compared to 21 days [92,93]. More intensive (R)-chemotherapy and high-dose treatment with autologous stem-cell rescue has not proven better than the conventional (R)-CHOP for DLBCL in general and is also prone with higher toxicity but may be considered in high-risk cases [94–98]. Population based studies indicate a better OS with the addition of etoposide to CHOP in younger patients [99,100].

For HGBL there is some evidence for giving more intense therapy [33,34,96] but no assured OS advantage [101]. Different strategies for this include dose-adjusted EPOCH, addition of etoposide to CHOP and treatment according to Burkitt-lymhoma protocols as hyper-CVAD.

Some clinical and pathological features are associated with higher risk of CNS involvement/relapse. HGBL (10% risk of CNS relapse), DLBCL with involvement in kidney/adrenal gland/uterus, primary testicular DLBCL, those with ≥3 extra-nodal sites engaged, CNS IPI≥4 (10% risk of CNS relapse) or intravascular DLBCL (25% risk of CNS relapse) should be treated with CNS-directed prophylaxis and considered for more dose intense regimens [47,102–108]. Addition of methotrexate (MTX) and cytarabine to standard treatment is recommended in Swedish national guidelines and early onset of MTX treatment seems to be better [108–110] (VP).

Table 3. Swedish guidelines treatment recommendations.

Swedish guidelines treatment recommendations for DLBCL patients with; * aaIPI=3 and age<80 or *CNS-IPI≥4 for age<70/CNS-IPI≥5 for age70-80 or *Involvement of ≥3 extranodal locations or *Involvement of testis/uterus/kidney/adrenal or intravascular LBCL
≤65 years; R-CHOP/MTX-21x2+R-CHOEP-14x4+R-ARA-Cx1
65-75 years;R-CHOP/MTX-21x2+R-CHOP-14x4+R-ARA-Cx1
75-80 years; R-CHOP-14x6
• Metotrexat (MTX) 3 000 mg/m^2 i v, on any of days 9-15 in the first 2 cycles. MTX is reduced to 2000 mg/m^2 >70 years. • Cytarabine (ARA-C) 3000mg/m^2 x2xII 14 days after end of R-CHO(E)P treatment. Patients 60-70 years 2000mg/m^2. Patients >70 years 1000mg/m^2.

Treatment with four cycles of R-CHOP followed by two courses of R is equal to treatment with six R-CHOP regarding PFS in young patients (≤60 years) with low stage (1-2) disease and aaIPI zero [111]. This result has led to new Swedish guidelines recommendations that also include older patients as there is no reason to believe that efficacy would be minor in the older population (VP).

The addition of R to CHOP does not improve the outcome of patients with skeletal involvement of disease [112]. Addition of radiation therapy to skeletal lesions is associated with better EFS [112]. For bulky disease with lymph nodes wider than 7.5 cm EFS and OS improves with the addition of radiation therapy but for both skeletal involvement and bulky disease there is no proof of better outcome with radiation therapy if PET-CT scan shows complete response (CR) after initial treatment [113,114]. Radiation therapy is also recommended to the contralateral testis in case of testicular DLBCL [115].

1.6 Treatment toxicity

The occurrence of dose-limiting toxicity with each of the chemotherapeutic agents can compromise the relative dose intensity and therefor the treatment outcome. Short-term side-effects of treatment include tumour lysis syndrome, cytokine release syndrome, pancytopenia including febrile neutropenia, nausea, hair loss and tiredness. Primary prophylaxis with granulocyte-colony-stimulating factor is a well-established strategy to decrease the risk and severity of neutropenia and maintain relative dose intensity and is recommended from current international guidelines and antibiotics, antiviral and antifungal prophylaxis may also be considered in the clinical setting [116,117]. In the long term R may cause late onset neutropenia [118].

Prednisone may cause oral fungal infection, diabetes mellitus, loss of bone mass and cause stomach ulceration [119].

DXR is known for dose-related, cumulative, heart toxicity. The cardiac (and hepatic) toxicity may be due to the one electron degradation to yet another active metabolite; doxorubicinol as well as topoisomerase 2 being the major form of topoisomerase in heart muscle. DXR affects the cardiomyocytes through oxidative damage and by direct, chemical, DNA damage through Topoisomerase 2 inhibition that may be reversible to a certain extent. However, beyond a certain point cell death occurs causing left ventricular ejection fraction (LVEF) reduction. It can cause acute onset failure but more often early and late problems and may present clinically after weeks to decades. The inter-individual tolerance to DXR differs and genetic factors, prior cardiac damage, tissue ischemia and other concomitant cardiac risk factors, such as radiation treatment, may affect the sensibility to DXR damage [120–126].

In clinical practice a maximum dose level of around 400 mg/m^2 is used to keep incidence of anthracycline induced heart failure below 5% as higher doses of 550mg/m2 rises the incidence to 7-26% [121,122,125,126].

A range of different approaches may be used in DLBCL and other cancer treatment in order to reduce cardiac toxicity;

1; Dose-limitation
2; Prolonged infusion
3; Liposomal or altered DXR
4; Cardiac protection treatment
5; Use of other drugs

1; Firstly, dose-limitation is an option which seldom is needed in DLBCL treatment as the standard CHOP treatment only includes 50mg/m2 DXR in 6 cycles and as so not exceeding dose -limits. However, in relapsed disease where you could suspect anthracyclin sensitive disease or for patients with earlier cancer treatment dose-limitation can be a trouble for giving the best anti-tumour treatment [126].

2; A prolonged infusion time gives a lower peak plasma level of DXR and is shown to be protective as treatment with prolonged infusion gives tolerance to higher accumulated doses of DXR. Schedules with up to 72 hours infusion time are used but there is evidence that infusion duration of 6 hours or more significantly lowers the rate of clinical heart failure. [122,126,127]. In the Swedish DLBCL treatment recommendations patients with a slightly reduced ejection fraction (EF)(not below 50%) are recommended prolonged infusion time >6 hours (VP).

3; Newer drugs as the liposomal pegylated doxorubicin (Caelyx in Sweden) has a longer plasma half life which lowers the peak plasma level giving the same protective effect as prolonged infusion time. It also has a greater dimension that only allows penetration through the more permeable tumour vasculature. Breast cancer studies demonstrate an equal treatment effect as for DXR. However, it is more expensive and neither in Sweden or US there are drugs approved for DLBCL treatment. Non-pegylated liposomal DXR (MyocetT) has a lower cardiac toxicity but is not available in Sweden and is not approved for DLBCL in US. Epirubicin is an antracyclin similar to DXR but with different spatial orientation and the same myelosuppressive dose causes less cardiac toxicity, approximately 0.7 times [122,125,126,128,129].

4; A spectrum of different drugs have been tested as cardiac protectors during DXR therapy. A meta-analysis showed 70% and 90% reduced risk for cardiac toxicity with B-blockers and ACE-antagonists as well as for statins but they are small studies with short follow up and critics query whether they really are cardioprotective or simply change hemodynamics. There is no recommendation on using them as protectors in Sweden [122,126,128]. Dexrazozane (Savene) changes Topoisomerase 2 beta configuration and prevents antracyclin effect on Topoisomerase 2. It is mostly analysed in breast cancer studies and clearly has an effect in preventing cardiotoxicity. Studies found no difference in response to tumour treatment. In Sweden it is only approved for treatment of anthracyclin extravasation. [121,122,126,129].

5;Some studies have shown similar treatment effect with DXR substituted for etoposide. It may be sure for DLBCL with GCB origin but more uncertain for ABC type. [130–132]. Swedish guidelines recommend R-CEOP for patients with EF<50% (VP).

In the clinical setting there are limited ways to predict the specific risk of each case to develop cardiotoxicity and even harder to measure ongoing cardiotoxicity during treatment. Anamnesis, clinical investigation, NTproBNP testing and heart ultrasound before start might help to select cases unsuitable for DXR or in need of protective strategies but there is no practical strategy to find those with early treatment associated damage before changes on ultrasound or symptoms appear. As so there is a need of better understanding of who is at risk before start and during on-going treatment

The dose-limiting toxicity of VCR is neurotoxicity caused by a high affinity to both mitotic and neuronal microtubules affecting axons of motor, sensory, autonomous nerve and c-fiber neurons causing both sensory and motor disturbances. VCR induced peripheral neuropathy is most common and pre-

dominantly sensory in nature. Early symptoms include numbness and tingling of the hands and feet while in its more severe form, muscle weakness develops, which is more marked in distal muscles of the hands and feet. Other manifestations of neurotoxicity include reduced motility of the intestines, resulting in constipation muscle cramps, ocular palsies, hoarseness, and autonomic neuropathy in the form of postural hypotension and atony of the urinary bladder. The incidence and severity is correlated to duration and dosing of therapy but there is a widespread inter-individual variation in the half-life, volume of distribution and drug clearance and sensitivity to VCR where some patients feature symptoms after a few treatment courses and others fulfil treatment unaffected [43,79–82]. Most studies in adults maximise given dose at 2 mg because attempts to give higher doses have resulted in significant neurotoxicity. There exist no prophylactic measures to avoid neurotoxicity. In the clinical setting it is common to reduce or omit VCR when neurotoxicity occurs. From prior studies we know the importance of keeping a high relative dose intensity of CHOP but only small studies have focused on the effect of reducing VCR dose [90].

Toxicity has led to attempts modify the drug or the drug delivery. These include making synthetic or semi-synthetic analogs, combinations with antibodies and to formulation of new drug delivery systems intended to enhance dosing with an improvement in antitumor activity without an increase in the toxicity. Vincristine sulphate liposome is a three- part formulation approved by FDA and EMA mainly for relapsed leucemia. The liposomes are nanoparticles that are composed of a phospholipid bilayer with an aqueous core which can be used to encapsulate drugs. They are taken up by the process of passive diffusion and may lead to higher concentrations as well as prolonged exposure of the drug in the tumour tissue. Pharmacokinetic and murine model studies have shown an increased half-life of vincristine, decreased free drug levels in the circulation which may result in less drug toxicity and increased efficacy [80–82].

Other late effects of cytotoxic treatment are infertility and the increased risk of secondary malignancies [133].

1.7 Response evaluation

Treatment results are measured according to the latest (2014) Lugano criteria based on FDG-PET-CT scan as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) [52]. FDG-PET-CT as evaluation is recommended since 2007 [134,135]. In clinical practice not all patients undergo FDG-PET-CT scan and older response criteria based on CT scan such as Cotswold criteria also include CRu (complete response unconfirmed) where residual mass on CT is considered fibrous tissue and not residual disease [44].

Event free survival (EFS) has become a new tool on how to predict risk of future disease after completion of first line therapy. It is shown that those who achieve EFS 24 (event free at 24 months after diagnosis) or PFS 24 (progression free at 24 months) had an OS equivalent to that of an age- and sex-matched general population [136,137]. These results regard patients included in clinical trials and other publications on population based cohorts could only confirm this for younger patients (<50 years) or found a remaining increased risk of death[138,139].

1.8 Treatment at relapse

The general 5-year OS after initiation of R-CHOP treatment is around 70% with better results in the younger population[83–85]. These results seems durable as the numbers are equal in the later EFS24 and PFS24 studies[136,137,139]. Most relapses occur early with 75% of relapses within 2 years among whom 10 to 15% of patients exhibit primary refractory disease (non-response or relapse within 3 months of therapy) and only approximately 7-8% relapse after 5 years [83].

Younger patients (<70 years) who relapse and are fit should be treated with platinum-based therapy followed, in responsive patients, by high-dose chemotherapy with autologous stem-cell support (ASCT) with curative intent [51](VP). A small study before the introduction of R indicated a 5 year OS of 53% for those treated with ASCT[140]. BEAM or BEAC are mostly used for high-dose treatment [140,141]. R-IKE (ifosfamide, carboplatin, etoposide) and R-DHAP (cisplatinum, cytarabine, dexamethasone) before ASCT have similar outcome with 3–year OS of 49% and R-GDP (cisplatinum, gemcitabine) is shown to have similar efficacy despite being less toxic than R-DHAP [141,142]. R is included in treatment in R sensitive cases meaning relapse>6 months after R containing therapy. Unfortunately, even among younger, fit patients a substantial number of patients never go to ASCT due progression or rapid deterioration during second line treatment. A recent study of relapsed/refractory DLBCL and HGBL show a 3-year OS of 24% and ORR in second line therapy of 43% and among those achieving CR/PR 49% went to ASCT and another 15% to allogenic stem cell transplant [143]. Allogenic stem cell transplant in chemosensitive disease is indicated in case of relapse after ASCT or in primary refractory disease [51](VP). Studies reveal a 1-year OS of 52% and 3-year survival probability of 37%[144,145].

CD-19 targeted chimeric antigen receptor modified T-cell (CAR T-cells) has become a new treatment of lymphoma and leukemia. T-cells from the patient are collected from the blood and modified in laboratory to express a chimeric antigen receptor (CAR) to better fight the tumour cells. Phase 2 clinical studies performed with satisfactory results in refractory/ relapsed

DLBCL with ORR up to 66% have resulted in the approval of two new drugs; axicabtagenciloceucel and tisagenlecleucel for treatment of DLBCL in second relapse or later. However, this treatment is toxic, very expensive and is only available in few oncology centers in Sweden [146,147].

For older or frail patients treatment is palliative and individualized and preferably platinum-based such as Gemox (gemcitabine, oxaliplatine) as it gives an ORR of 61% [51,148](VP). Other options are single bendamustin or combination therapy with MIME (metyl-GAG,Ifosfamide, methotrexate, etoposide) where studies demonstrate ORR of 50% and 56% respectively [149,150]. R should be added in R sensitive cases. Polatuzumab vedotin is a new drug consisting of a monoclonal antibody against CD79b covalently conjugated to the anti-mitotic cytotoxic agent monomethyl auristatin (MMAE). The antibody binds to CD79b on tumour cells and is internalised into the tumor cell and the linker between the antibody and the cytotoxic part is cleaved to release MMAE, which inhibits cell division and induces apoptosis of the tumour cell [151]. A recent study have presented the beneficial effects with addition of Polatuzumab vedotin to a bendamustin and R combination therapy in refractory/ relapsed disease showing better objective responses and median OS (12.4 vs 4.7 months) [152] although increased toxicity might restrain the possibility to give this therapy in frail cases. Efficacy of single Pixantrone, a less cardiotoxic antracyclin compared to various ”comparator drugs” was proved in a phase 3 study with ORR 26% vs 10% but disappointingly a combination study of pixantrone plus R compared to gemcitabine plus R did not improve outcome (PFS, OS) and the drug is at the moment not recommended for use in Sweden [153,154].

If possible patients should be included in clinical studies.

1.9 Future visions

As DLBCL still has a relapse rate of approximately 30% and most patients are older and not suitable for more toxic regimens or stem cell transplant there is a need for better first line and later therapies. As modern research has developed a deeper understanding of cell of origin and intracellular signalling function a lot of trials are focusing on finding new therapeutic possibilities in DLBCL. These include new CD-20 targeting antibodies, CD-30 and PD-L1/PD-1 antibodies, bi-specific antibodies, immunomodulation drugs as lenalidomide, targeting epigenetic regulators/ B-cell receptor cascade signalling/BCL-2/ NF-kB as well as new and modified CAR-T cells and more but has so far not shown results that change standard treatment [101,155].

2 Aims

2.1 Overall aim

The main objective of this thesis was to study known side-effects of treatment and comorbidities and explore outcome in DLBCL in order to improve clinical treatment and follow-up decisions.

2.2 Specific aims

I To explore if the omission of vincristine in R-CHO(E)P treatment of DLBCL in the clinical setting affects treatment outcome (DFS, OS).

II To study the occurrence of different AIDs in an unselected cohort of DLBCL. Furthermore to analyse if the occurrence of AID affects treatment outcome and to assess gender differences in occurrence and outcome.

III To establish EFS24 in a Swedish unselected DLBCL cohort. Furthermore to analyse factors governing overall survival and reviewing the causes of death and compare OS with the general Swedish population.

IV To explore whether advanced serum protein analysis can be used to measure DXR related cardiovascular toxicity in DLBCL patients treated with R-CHO(E)P.

3 Patients and methods

In Paper I, II and III adult patients (≥18 years) registered in the National Swedish Lymphoma Registry (INCA, Informationsnätverk för Cancervården) from four different institutions in Sweden (Mälarsjukhuset, Eskilstuna, Uppsala University Hospital, Uppsala, Falu lasarett, Falun, and Gävle sjukhus, Gävle) diagnosed with DLBCL or subgroups of high-grade malignant B-cell lymphoma between 2000 and 2013 were included. INCA had coverage of 97.8% in the years 2008-2014 and at each clinic the level of coverage of INCA was checked by searching for DLBCL diagnose in the local data system not to miss any cases. Those who were primary treated with at least one course of R with either CHOP, CHOEP (CHOP plus etoposide), or mini-CHOP (reduced- dose CHOP) was identified. Patients with primary mediastinal, testicular lymphoma and with follicular lymphoma transformed to DLBCL were included if the only prior treatment given for follicular lymphoma was radiotherapy.

Patients with primary central nervous system lymphoma and Burkitt lymphoma were excluded. Those considered by the treating physician as too frail to receive any treatment or if treated with less intensive chemotherapy with no intention to cure or given combinations other than CHOP or CHOEP due to comorbidities (i.e., CEOP, liposomal doxorubicin, COP, bendamustine) were excluded as well. Omission of VCR was counted from the course number when no VCR was given. If a patient received less than six cycles of R-(mini)-CHO(E)P treatment due to other toxicity they were not included in the VCR omission group.

The initial histopatological diagnose, made by the local or regional departments of pathology using the 2008 WHO classification of lymphoma definitions, were not reviewed. All clinical data was retrieved retrospectively from medical records. The presence of any medical condition such at cardiac, vascular or autoimmune disorder was only determined by reading the medical charts and not confirmed by other means. A patient was considered to have an AID diagnosis in the presence of a medical condition commonly known to be caused by self-reactive antibodies or a disease that results when the immune system mistakenly attacks the body's own tissues. Hypothyroid conditions were included and grouped together. Diabetes mellitus was considered as an AID (type 1 diabetes) if the patient was treated with insulin only. Information on earlier or on-going treatment for AID was not collected. Cardiac disease was divided in the following categories; heart failure,

angina pectoris, cardiac arrest, fibrillation, vitium, pacemaker and PCI treatment. Vascular disease was categorized as; claudicatio, carotid stenosis, aortic aneurysm operation, deep vein thrombosis, pulmonary embolism, arterial thrombosis/ischemic stroke or cerebral haemorrhage, transient ischemic attack or operation for aneurysm. Cardiovascular disease (CVD) was defined as having a pre-existing or developing a diagnosis of any of cardiac or vascular disease. Several patients had more than one diagnosis. An emerging new diagnosis was counted for regardless if the patient already had a CVD.

The treatment outcome was defined as CR, PR, SD or PD based on the disease status at the oncology clinic visit immediately following the end of treatment and was assessed either from clinical and/or radiologic findings according to Cotswolds or Lugano criteria [44,52,134,135]. Patients were followed according to the now out-dated Swedish lymphoma group guidelines with physical examination and laboratory tests every 3-4 months the first 2 years, half annually year 3 and annually years 4 and 5.

In total, 612 patients were included.

In **Paper I** the analysed cohort consisted of those 541 cases whom we had knowledge about VCR treatment doses and the occurrence of omission of VCR. All 612 were included in **Paper II**. In **Paper III** the cohort was merged with a similar group of DLBCL diagnosed between 2004-2012 in another Swedish county, West Gotaland, resulting in 1169 cases.
The studies were approved by the local review board in Uppsala, Sweden (Dnr 2014/233).

Paper IV consist of another cohort of 95 patients aged ≥18 years included in the Uppsala-Umeå Comprehensive Cancer Consortium (U-CAN) bio-bank 2010-2015 at the time of DLBCL diagnosis. The U-CAN is a bio-bank with the sequential collection of clinical data as well as blood and tissue samples from cancer patients [156]. Samples were taken before start of treatment and in some cases during and after. Seven patients in the cohort were never treated with DXR.

Proteomics
The PEA technology was utilized to assess plasma samples (1µl) using the Olink™ multiplex protein panel Cardiovascular III (CVDIII) and Oncology II (ONCII).

Each panel consists of 92 human proteins where CVDIII includes cardiovascular- and inflammatory-related markers as well as some exploratory human proteins believed to be associated with cardiovascular disease. In multiplex PEA, each target protein is recognized by a pair of proximity probes consisting of an antibody conjugated to a single stranded DNA oligo-

nucleotide that in proximity are hybridized to each other allowing enzymatic DNA polymerization and subsequent DNA amplification [157].

The NTpro-BNP and CCL22 measurement in CVDIII did not meet the quality requirements and the results were disregarded, leaving 182 proteins for analysis.

Since NTpro-BNP is a key cardiovascular protein, supplementary analysis was performed on additional frozen samples at the Department for Clinical Chemistry, Uppsala university hospital together with Troponin I according to standard clinical procedure at the department. CRP was analysed with standard clinical procedure at the patients' local laboratory. With CRP included, a total of 185 proteins were analysed. NTproBNP and Troponin I samples were available for a proportion of patients (133 samples in total).

Table 4. Number of protein samples in **Paper IV**

	PEA-CO[a]	NTproBNP Troponin I[b]	CRP
Before	96[c]	32	92
During	30	25	0
After	60	76	0
Total	186	133	92

[a] PEA-CO, proximity extension assay of 182 proteins. [b]NTproBNP and Troponin I [c] 96 samples before start of treatment
from 94 patients. Two patients had two samples before and for one patient there was no sample and for one patient date of starting treatment was uncertain

For comparison, plasma samples from 60 non-DLBCL controls (30 male, 30 non-pregnant females) were obtained from the EpiHealth biobank. EpiHealth is an open-access, multi-center, longitudinal, cohort study investigating the interaction between genes and life-style factors possibly related to the development of common diseases in the adult population [158].

The U-CAN project, including this study, has been approved by the Regional Ethics Committee (EC) of Uppsala-Örebro (Ups 2012/198, 210/198/1, 2014/233). Data collection in the EpiHealth study and usage of the material in this project has been approved by the EC of Uppsala (Dnr 2010/402: 2010-12-01, 2011-11-17, 2015/179).

4 Statistics

Categorical variables were expressed as numbers (%) and continuous variables as median (range). For bivariate comparisons between groups, the t-test (in case of normally distributed variables) or the non-parametric Mann–Whitney test (in case of non-normally distributed variables) was used for continuous variables and the Chi-square or Fisher's exact test was used for categorical variables.

All time intervals were measured in months. Event-free survival (EFS) and disease free survival (DFS) were defined as the time between the date of diagnosis (date of histopatological sampling) and last follow up or death in the absence of relapse. Patients in complete remission (CR) and unconfirmed complete remission (CRu) at end of treatment were included. If a relapse occurred, EFS/DFS was set to time from diagnosis to date of relapse (date of clinical/radiology finding or biopsy). If the patient never reached CR or PR and subsequently died from lymphoma the EFS/DFS time was set to zero. Overall survival (OS) was defined as time from diagnosis to the date of death from any cause. Patients that were alive to the date of last follow-up were censored. Lymphoma-specific survival (LSS) was defined as the time between initial date of diagnosis and death from lymphoma. Treatment-related deaths were included in LSS. Time-to-event outcomes (EFS/DFS, LSS and OS) were analysed by using the Kaplan-Meier and the log-rank test was used to test statistical significance. A two-sided p value of ≤ 0.05 was regarded as cut-off for statistical significant results in comparisons between groups.

Any variables significantly associated with outcome variables in bivariate analyses were considered for entry into a multivariate Cox proportional hazards regression analysis.

In **Paper I** we performed two separate multivariate analyses for DFS and OS respectively with omission of VCR included in both models as an independent variable of interest. The main analyses were performed using the complete case analysis approach to handle missing data. A sensitivity analysis was performed using the multiple imputation (MI) method. The rates of missing values from potential predictors for DFS or OS ranged from 0 to 27%. We decided a priori to exclude variables with > 30% missing values. Missing data were imputed for the following variables (missing values are presented in parentheses): extra-nodal engagement of disease (20.1%), kid-

ney or adrenal involvement (20.1%), LDH level (20.9%), PS (23.8%), bulky disease (24.2%), and BMI (27%). The imputation was performed using the chained equations method and 10 multiple imputed datasets were created and used for the analyses.

Two subgroup analyses were performed, one restricted to patients ≥ 70 years old considering that older age has been associated with higher risk for reduced RDI [87]. An additional subgroup analysis investigated a dose-dependent relationship between cycle of VCR omission and treatment outcome as we calculated, using Cox proportional-hazards model, the adjusted hazard ratio for DFS and OS based on chemotherapy cycle number in which VCR was omitted (cycles 1–3, cycle 4, cycle 5, cycle 6) compared to no omission of VCR.

Statistical analyses were performed with the IBM statistics SPSS version 22.

In **Paper II** time-to-event outcomes (EFS,LSS,OS) were assessed for all AIDs together, and for the AIDs grouped as primarily mediated by B-cell responses or T-cell responses according to the classification of the Inter-Lymph Consortium (i.e., only the AID diagnoses classified by the Inter-Lymph Consortium were included in the categorization and outcome analyses of B- and T-cell mediated AIDs). Subgroup analyses were performed with and without the patients with thyroid disease due to uncertainty of AID origin based on the information in the medical records.

Statistical analyses were performed using the IBM statistics SPSS version 22.

In **Paper III** patients who died before reaching the mile stones (EFS 24/EFS12) were excluded from the analysis. The study population was divided into two groups; patients who achieved EFS24 and those who did not. Swedish life tables from Statistics Sweden (www.scb.se) state the historical individual risk of death depending on gender and age per calendar year. A standard population was generated that matched the study population in terms of age and gender. For each individual, probabilities matched on gender, age and index year for each subsequent year was calculated into a cumulative product of a time-series of likely survival for each individual. Subsequently, the survival of the standard population was compared to the survival of the study population. Overlapping confidence intervals (CI) were used to examine survival disparities between different cohorts of the study population and the survival of the derived standard population. Survival at different EFS milestones (12 and 24 months) was calculated (including only patients achieving the specified milestone in the analysis). Standardized mortality ratio (SMR) was calculated for 1 and 5 years after achieving the landmark time-point.

Statistical analyses were performed using the R statistical program version 3.4.3 (www.r-project.org).

In **Paper IV** missing BMI values were imputed by the median value of the patient group. The difference in protein level between two groups was assessed using linear regression, adjusting for age (at diagnosis), gender and BMI. Significance was determined using linear regression t-test.

To investigate if the protein level or change in protein level over time differ between two groups before, during or after treatment, we studied 87 patients treated with DXR using mixed effects linear regression with protein level as dependent variable, patient id as random effects variable and time point, age, gender, BMI and cardio or CVD or hypertension before as well as the interaction group:time point as fixed effects variables. The association between group and protein level was assessed using a likelihood ratio test. Significant associations were further investigated in post hoc tests. Benjamini-Hochberg's false discovery rate method for multiple testing correction was applied and a difference was considered significant if the q-value (the adjusted p-value) was ≤ 0.10.

Statistical analyses were performed with R version 3.6.3 and the IBM statistics SPSS version 22.

5 Results and Discussion

5.1 Paper I

Does the omission of vincristine in patients with diffuse large B cell lymphoma affect treatment outcome?

In total, 541 DLBCL cases in who we had knowledge about VCR treatment doses and the occurrence of omission of VCR were included. Relative dose intensity (RDI) for DXR (DoxoRDI) was calculated according to Yamagushi et al. [91].

In 95 of 541 (17.6%) patients, VCR was omitted due to toxicity and omission was more common during the last three cycles (n=86, 90.5%). Patients with VCR omission were older (p= 0.003) with higher IPI (p= 0.006) and higher amount of ≥ 1 extra-nodal involvement (p= 0.003). Bivariate analysis revealed nine predictors possibly associated with DFS and 10 predictors were associated with OS.

Table 5. Baseline characteristics in **Paper I**.

	VCR omission (%)	**VCR full dose (%)**	**p-value**
Number of pts	95	446	
PS			
0-1	81 (85.3)	281 (63.0)	0.376
2-4	14 (14.7)	36 (8.1)	
Missing	0	129 (28.9)	
Stage			
1-2	29 (30.5)	158 (35.4)	0.354
3-4	66 (69.5)	287 (64.4)	
Missing	0	1 (0.2)	
IPI			
0-2	38 (40.0)	234 (52.5)	0.006
3-5	57 (60.0)	186 (41.7)	
Missing	0	26 (5.8)	
LDH			
> ULN	65 (68.4)	216 (48.4)	0.520
≤ ULN	30 (31.6)	117 (26.2)	
Missing	0	113 (25.3)	
Bulky[a]			
Yes	20 (21.1)	73 (16.3)	0.665
No	75 (78.9)	242(54.3)	

Missing	0	131 (29.4)	
AI			
Yes	18 (18.9)	82 (18.4)	0.898
No	77 (81.1)	364 (81.6)	
Extranodal[b]			
>1	30 (31.6)	60 (13.5)	0.004
≤ 1	65 (68.4)	277 (62.1)	
Missing	0	109 (24.4)	
Kidney/adrenal[c]			
Yes	3 (3.2)	18 (4.1)	0.382
No	92 (96.8)	319 (71.5)	
Missing	0	109 (24.4)	
BMI, median (16,2-44,10)	25.7(16.2-41.4)	25.8 (16.9-44.1)	0.850
Missing	3 (3.2)	300 (67.3)	
Treatment			
CHOP	79 (83.2)	378 (84.8)	0.697
CHOEP	16 (16.8)	68 (15.2)	
DoxoRDI			0.396
≤70%	6 (6.3)	28 (6.3)	
>70%	83 (87.4)	261 (58.5)	
Missing	6 (6.3)	157 (35.2)	

Abbreviations: Pts, patients; PS, performance status; IPI, International prognostic index; LDH, lactate dehydrogenase; ULN, upper limit normal; AI, autoimmune disease; BMI, body mass index; DoxoRDI, doxorubicin dose intensity.
[a] Tumor mass>7,5 cm. [b] Involvement of extranodal organ. [c] Kidney or adrenal involvement.

Omission of VCR was not significantly associated with worse OS in Kaplan-Meier analyses.

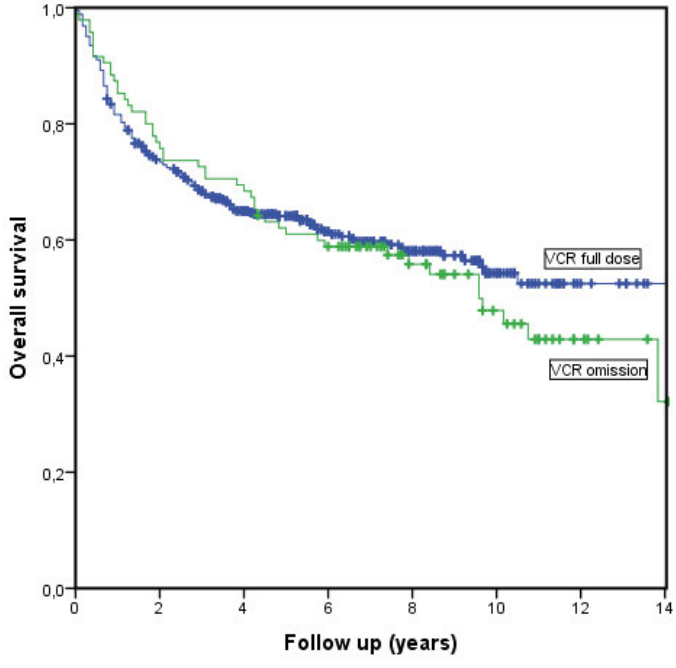


Figure 1. Kaplan Meier for survival comparing full dose VCR and omission of VCR. p=0.572

Figure 4. Kaplan Meier for survival comparing full dose VCR and omission of VCR. P=0.572

In multivariate Cox regression analyses for DFS only advanced stage at diagnosis was found to be significantly associated with worse outcome (HR: 2.04, 95 % CI: 1.01 - 4.00). In respect of OS, kidney/adrenal involvement (HR: 2.45, 95% CI: 1.20 - 4.98), DoxoRDI (relative dose-intensity of DXR) ≤ 70% (HR: 2.04, 95% CI: 1.15 – 3.61), age ≥ 60 years old (HR: 1.94, 95% CI: 1.09 - 3.48), and bulky disease (HR: 1.58, 95% CI: 1.03 - 2.42) were significant associated with worse survival.

Omission of VCR was not associated with either DFS or OS in multivariate analyses (HR for PFS: 1.21, 95% CI 0.76 - 1.95; HR for OS: 1.13, 95% CI: 0.75 - 1.71).

Table 6. Multivariate Cox regression analysis of Disease-free Survival (DFS) and Overall Survival (OS).

	DFS		OS	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age ≥ 60	Not included		1.94 (1.09-3.48)	0.025
Treatment[a]	Not included		1.76 (0.90-3.43)	0.096
PS ≥ 2	1.62 (0.74-3.57)	0.235	1.77 (0.84-3.74)	0.134
Stage > 2	2.04 (1.01-4.00)	0.047	1.59 (0.88-2.88)	0.127
IPI > 2	1.33 (0,70-2.50)	0.385	1.14 (0.60-2.16)	0.686
LDH > ULN	1.09 (0.63-1.89)	0.778	1.03 (0.63-1.69)	0.893
Bulky[b]	1.30 (0.81-2.10)	0.283	1.58 (1.03-2.42)	0.037
Oncovin omission[c]	1.21 (0,76-1.95)	0.421	1.13 (0.75-1.71)	0.571
Extranodal[d] >1	1.02 (0.59-1.78)	0.932	Not included	
Kidney/Adrenal[e]	1.72 (0.78-3.85)	0.171	2.45 (1.20-4.98)	0.014
BMI ≥ 25	0.89 (0.58-1.37)	0.591	0.98 (0.67-1.43)	0.904
DoxoRDI ≤ 70%	1.88 (0.97-3.67)	0.063	2.04 (1.15-3.61)	0.014

In the sensitivity analysis using the MI method to handle missing values, the lack of association between omission of VCR and DFS (HR: 1.20, 95% CI: 0.81-1.78) or OS (HR: 1.06, 95% CI: 0.76-1.48) remained unchanged and non-significant. The lack of association between VCR omission and survival was evident irrespective of the number of the cycle in which VCR was omitted.

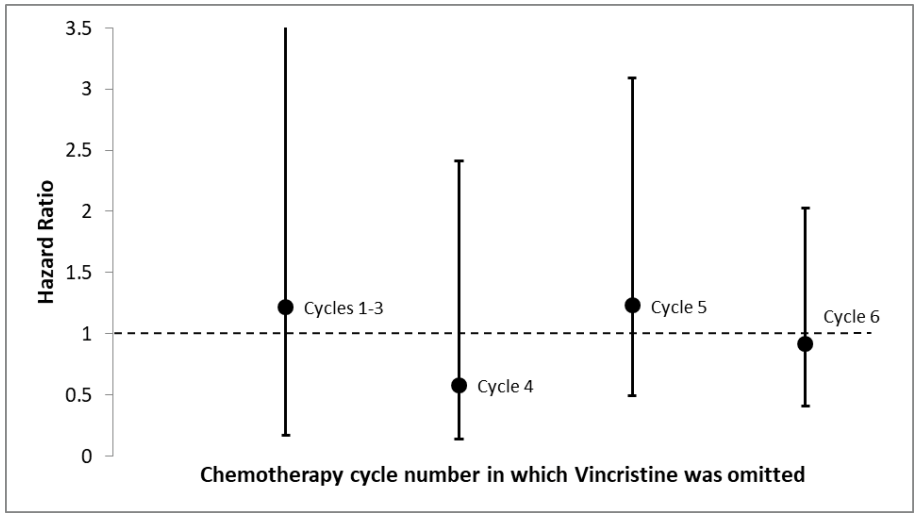


Figure 5. Hazard ratio for omission of VCR in cycle 1-3, 4, 5, 6 vs no omission.

Compared to patients treated with 6 VCR cycles, those who received only 1-3 cycles showed comparable survival (HR for DFS: 0.30, 95% CI: 0.07-1.30; HR for OS;1.22, 95% CI:0.17-9.01).

When the analysis was restricted to patients ≥ 70 years old (n = 204), the omission of VCR was not found to be associated to survival either (HR for DFS 1.43, 95% CI 0.53–3.83; HR for OS 1.30, 95% CI 0.53–3.16).

The potential effect of reduced VCR dose on DLBCL prognosis was previously investigated only in smaller study [90] including 86 patients treated with R-CHOP-21 due to DLBCL. They found a lower survival rate for RDI VCR < 85% despite adequate CPM and DXR doses. In the present large cohort of patients we could not confirm that omission of VCR affects prognosis of DLBCL in terms of DFS or OS. The lack of association remained unchanged in a sensitivity analysis with multiple imputation and when analysis was restricted to the elderly population (≥70years) and irrespectively of whether VCR was omitted early or late in the treatment course. We used more wide inclusion criteria and included patients with more diverse chemotherapy regimens in an effort to better reflect the daily clinical practice. Furthermore, we used a more pragmatic and clinically relevant approach for reduced VCR dose, namely the omission of VCR at a specific treatment course instead of RDI that is more complicated to interpret.

A potential explanation for our findings of the lack of association between VCR omission, even early during the treatment course, and survival could be that the presence of neurotoxicity is correlated to higher intra-cellular VCR bioavailability and as so might be associated with a better response to treatment.

Our results on the positive association between maintaining high RDI for DXR and treatment outcome are in accordance with prior studies and suggest that DXR might be a more important chemotherapeutic agent than VCR in the treatment of DLBCL [87–91]. Surprisingly, higher IPI was not correlated to OS but only age. The substantial lack of information on LD (20.9% missing values) and PS (23.8% missing values) might influence the result but we found no difference after multiple imputation analysis.

The retrospective nature of the study is prone to well-described bias. Second, there were missing values in some variables; however, our results remained stable even when we dealt with missing values by using MI methodology. In addition, unlike other studies investigating RDI and treatment outcome, the RDI CPM was not analysed. Finally, the number of cases was limited for calculation of the effect of omission of VCR after the first, second, or third treatment course separately.

In conclusion, the omission of VCR does not affect either DFS or OS in patients with DLBCL treated with R-CHOP/ CHOEP/mini-CHOP. As a result, clinicians can safely decide to omit VCR in case of severe neurotoxicity due to VCR. Considering the association of bulky disease and kidney/ adrenal manifestation of lymphoma on survival, further studies should focus on whether the treatment options for these subgroups need to be individual-

ized. Finally, clinicians should be aware of the importance of giving adequate doses of DXR during treatment given the growing body of evidence on the role of dose intensity on survival.

5.2 Paper II

Autoimmune disease in patients with diffuse large B-cell lymphoma: occurrence and impact on outcome.

In total, 612 patients were included in the study. Median follow-up time was 59 months. A male predominance was observed with a 1.4:1 male:female ratio The number of patients with AID was 106 (17.3%) with the distribution of different AIDs divided in primary B or T-cell response disease displayed in Table 7.

Table 7. Distribution of 122 different autoimmune disorders in 612 R-CHOP/R-CHOP-like treated patients with diffuse large B-cell lymphoma divided inte autoimmune disorders primary B-cell och T-cell responses using the InterLymph classification.

	AID	Patients with AID[a] n= 106 (%)	Females n=59	Males n=47
B-cell responses	Thyroid disease	33 (31.1)	24 (72.7)	9 (27.3)
	Rheumatoid arthritis	24 (22.6)	16 (66.7)	8 (33.3)
	SLE and DLE	6 (5.7)	3 (50.0)	3 (50.0)
	Primary Sjögren's syndrome	5 (4.7)	5 (100)	0 (0.0)
	Autoimmune hemolytic anemia	3 (2.8)	1 (33.3)	2 (66.7)
	Myasthenia gravis	1 (0.9)	1 (100)	0 (0.0)
T-cell responses	Inflammatory bowel disease[b]	14 (13.2)	7 (50.0)	7 (50.0)
	Psoriasis	13 (12.3)	4 (30.8)	9 (69.2)
	Diabetes mellitus	6 (5.7)	2 (33.3)	4 (66.7)
	Celiac disease	1 (0.9)	1 (100)	0 (0.0)
Not classified	Other[c]	16 (15.1)	7 (41.2)	10 (58.8)

AID: autoimmune disorder; SLE: systemic lupus erythematosus; DLL: discoid lupus erythematosus.[a] Fifteen patients had two autoimmune disorders (12 females and three males) and one male had three autoimmune disorders.[b] Ulcerative colitis/ Crohn's disease.[c] Including 2 primary biliary cirrhosis, 2 polymyalgia rheumatica, 2 vasculitis, 1 myositis, 1 Guillain- Barré syndrome, 1 demyelinating axonal neuropathy, 1 sclerosing cholangitis, 1 pemphigoid, 1 iritis, 1 ankylosing spondylitis, 1 Lambert Eaton disease, 1 autoimmune hepatitis, 1 dermatitis. These AIDs have not been categorized as primarily mediated by B-cell or T-cell responses by the InterLymph Consortium (ref 12).

Fifteen of the 106 patients had two AIDs (12 females and three males) and one male patient had three AIDs, resulting in totally 122 AID diagnoses in 106 patients.

Thyroid disease dominated (n=33, 31.1%) followed by RA (n=24, 22.6%). Overall the proportion of AID was significantly higher in females (n=59 of 254, 23.2%) than in males (n=47 of 358, 13.1%) (p=0.001). Except for gender (p=0.001) there were no detectable differences in prognostic factors for DLBCL outcome between the no AID and the AID group. Patients with AID had a higher frequency of febrile neutropenia after the first course of chemotherapy; 16.0% vs. 8.7% (p=0.034) compared to those without AID.

Table 8. Baseline characteristics of DLBCL patients with and without autoimmune disease.

	AID n=106 (%)	**No AID n=506 (%)**	**p-value**
Age median, years (range)	66.5 (18-88)	66 (26-91)	0.188
Sex			
Male	47 (44.3)	311 (61.5)	0.001
Female	59 (55.7)	195 (38.5)	
Performance status			
0-1	73 (68.9)	356 (70.4)	0.783
2-4	10 (9.4)	44 (8.7)	
Missing	23 (21.7)	106 (20.9)	
Stage			
1-2	39 (36.8)	182 (36.0)	0.883
3-4	67 (63.2)	323 (63.8)	
Missing	0	1 (0.2)	
IPI			
0	3 (2.8)	34 (6.7)	0.579
1	26 (24.5)	114 (22.5)	
2	28(26.4)	114 (22.5)	
3	26 (24.5)	146 (28.9)	
4	15 (14.2)	65 (12.8)	
5	2 (1.9)	13 (2.6)	
Missing	6 (5.7)	20 (4.0)	
LDH			
>ULN	53 (50.0)	260 (51.4)	0.701
≤ULN	34 (32.1)	152 (30.0)	
Missing	19 (17.9)	94 (18.6)	
Bulky disease[a]			
Yes	20 (18.9)	91 (18.0)	0.809
No	63 (59.4)	307 (60.7)	
Missing	23 (21.7)	108 (21.3)	
Extranodal[b]			
>1	21 (19.8)	83 (16.4)	0.381
≤1	66 (62.3)	333 (65.8)	
Missing	19 (17.9)	90 (17.8)	
Treatment			
CHOP	87 (82.1)	437 (86.4)	0.253

CHOEP	19 (17.9)	69 (13.6)	
Febrile neutropenia[c]			
Yes	17 (16.0)	44 (8.7)	0.034
No	65 (61.3)	326 (64.4)	
Missing	24 (22.6)	136 (26.9)	
B-symptoms[d]			
Yes	40 (37.7)	233 (46.0)	0.103
No	66 (62.3)	269 (53.2)	
Missing	0	4 (0.8)	
BMI			
≥25	45 (42.5)	193 (38.1)	0.571
<25	38 (35.8)	187 (37.0)	
Missing	23 (21.7)	126 (24.9)	
Kidney/Adrenal involvement			
Yes	4 (3.8)	20 (4.0)	0.933
No	83 (78.3)	396 (78.3)	
Missing	19 (17.9)	90 (17.8)	

Abbreviations: DLBCL: diffuse large B cell lymphoma; AID: autoimmune disease; IPI: international prognostic index; LDH: lactate dehydrogenase; ULN: upper limit normal; BMI: body mass index.
[a] Tumor mass>7.5cm; [b] Involvement of extra nodal organ; [c] Febrile neutropenia after first course of treatment; [d] Fever/night sweat/weight loss

In the whole cohort EFS, LSS and OS at 5 years were 70%, 77% and 69% respectively. In total, 243 (39.7%) patients died during the follow-up period. The cause of death was in 173 (71.2%) cases due to lymphoma or its treatment. Bivariate analysis revealed 5 variables associated with EFS, 5 with LSS and 6 with OS.

Table 9. Multivariate Cox regression analysis of prognostic factors for event-free survival (EFS) and overall survival (OS) in 612 patients with diffuse large B-cell lymphomatreated with R-CHOP regimens.

	EFS		**OS**	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Bulky disease[a]	1.29 (0.85-1.97)	0.231	1.45 (0.99-2.11)	0.057
Treatment[b]	NA[C]		2.31 (1.33-4.02)	0.003
Kidney/adrenal involvement	1.53 (0.76-3.05)	0.231	2.07 (1.09-3.94)	0.027
IPI	1.36 (1.13-1.62)	0.001	1.20 (1.03-1.40)	0.022
B-symptoms	1.46 (0.96-2.20)	0.074	1.53 (1.06-2.22)	0.023
BMI≥25	1.01 (0.69-1.48)	0.965	0.86 (0.61-1.21)	0.380
AID	1.40 (0.89-2.22)	0.147	1.21 (0.79-1.87)	0.378

NA: not analyzed; CI: confidence interval; IPI: international prognostic index; BMI: body mass index; AID: autoimmune disease. [a] Tumor mass > 7.5cm, [b] CHOP vs. CHOEP [c] Not analyzed due to lack of significance in bivariate analysis.

When all patients were included in a multivariate analysis with these factors and the presence of AID as a separate factor, there was no significant difference in EFS (HR 1.40, 95% CI: 0.89–2.22, p= .147), LSS (HR 1.46, 95% CI: 0.89–2.38, p=0.130) or OS (HR 1.21, 95% CI: 0.79–1.87, p=0.378) between patients with or without AID. Known risk factors as higher IPI, bulky disease, kidney/adrenal involvement and also choice of cytostatic treatment affected OS.

Thyroid disorders have been handled in different ways in previous studies and we decided to perform outcome analyses with and without this group of patients. A Kaplan–Meier analysis with non-thyroid AID vs all others showed a borderline worse OS for the non-thyroid AID patients (p=0.047).

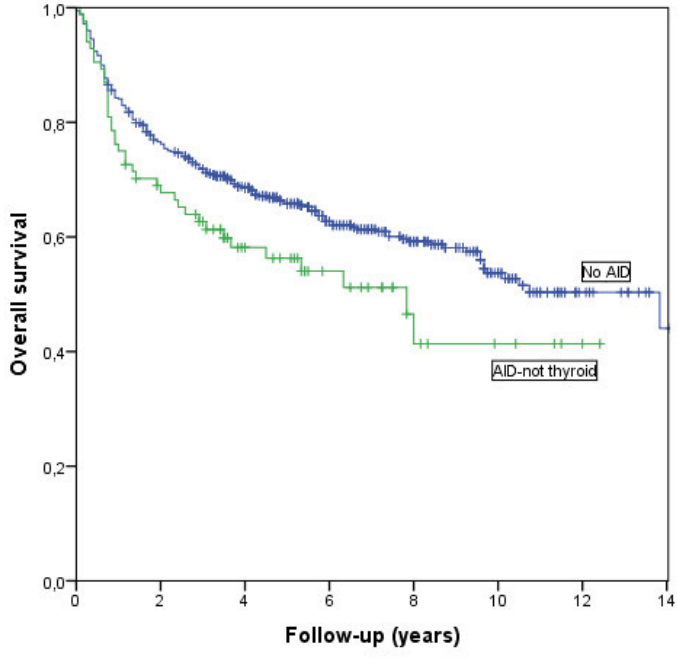


Figure 1a. Overall survival for AID-not thyroid vs others. p= 0.047

Figure 6. Overall survival for AID-not thyroid vs others. p=0.047

A multivariate analysis using the same variables as before including non-thyroid AID as a separate factor could not confirm any significant differences in outcome between these groups (HR for EFS 1.40, 95% CI: 0.85–2.31, p= 0.184, HR for LSS 1.58, 95% CI: 0.93–2.68, p= 0.090, HR for OS 1.43, 95% CI: 0.91–2.27, p= 0.125).

Overall survival was analysed twice, separately for B-cell response AIDs vs. all others, including and excluding thyroid disorders in the AID group. In B-cell response AIDs excluding the cases with thyroid disorder vs. all others

there was a worse OS (p=0.037) in Kaplan-Meier analysis not confirmed in multivariate analysis (HR for OS 1.43, 95% CI: 0.74–2.74, p= 0.29).

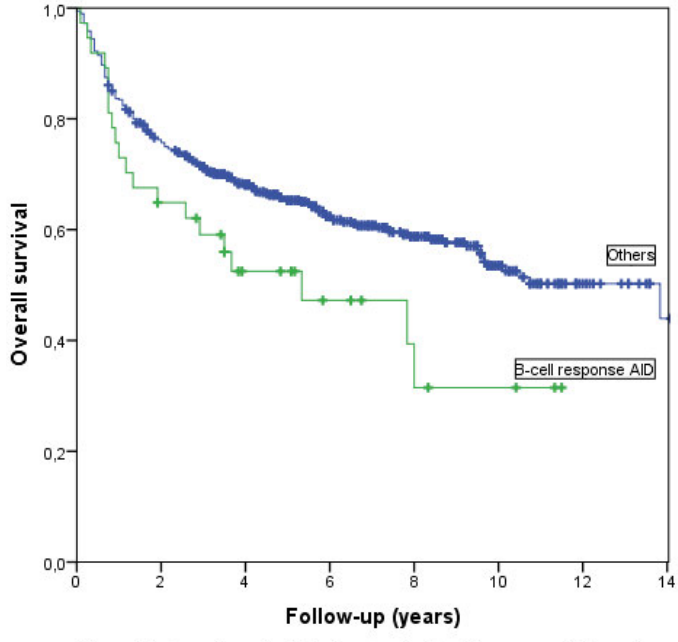


Figure 1b. Overall survival of patients with B-cell response AID vs others (No B-cell response AID). p=0.037

Figure 7. Overall Survival of patients with B-cell response AIDvs others (No B-cell response AID). P=0.037

There was no significant difference in LSS (HR for LSS 1.42, 95% CI: 0.65–3.08, p=0.38 or EFS (HR for EFS 1.17, 95% CI: 0.54–2.54, p= 0.690). Patients with AIDs primarily mediated by T-cell responses had no significant difference in OS in Kaplan-Meier analysis (p=0.244) vs. all others. In a multivariate analysis, these patients had a significantly worse LSS (HR=2.11, 95% CI 1.09–4.08 p=0.028, and EFS (HR=1.99, 95% CI 1.09–3.63 p=0.026) but not OS (HR=1.71, 95% CI 0.92–3.19, p=0.09).

Among all 612 patients in the cohort, OS was similar in men and women (p=0.448). However, a separate analysis of the AIDs primarily driven by B-cell responses with thyroid disorders excluded revealed a gender difference. OS was worse for women in this group compared to all other women (p=0.008).

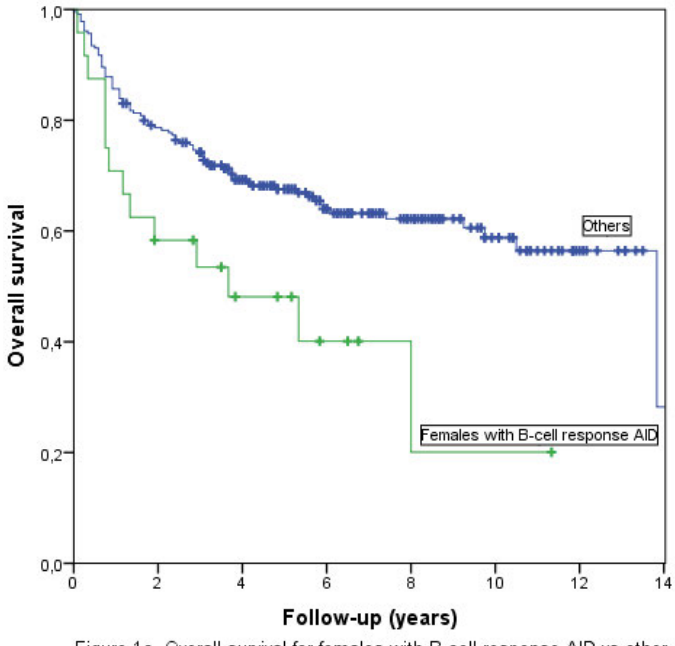


Figure 1c. Overall survival for females with B-cell response AID vs other women (No B-cell response AID). p= 0.008

Figure 8. Overall Survival for females with B-cell response AID vs other women (No B-cell response AID). p=0.008

In this group of 24 females there were 10 deaths related to lymphoma or its treatment (41.7%), four unrelated deaths and ten patients alive at the end of the follow up period. Cox regression analysis confirmed a worse OS (HR 2.90, 95% CI: 1.25–6.70, p=0.013). For the 13 men with B-cell response AID without thyroid disorders OS was similar compared to all other men (p =0.819) in Kaplan Meier analysis.

Patients with neutropenic fever after first treatment course had a worse OS in Kaplan–Meier analysis compared with those without neutropenic fever (p=0.026).

In this large study of patients with DLBCL and AID in the era of R-based lymphoma treatment we found a higher occurrence of AID, 17.3%, compared to the 3–10% of AID found in the general population [4,9,56,57,59]. As in previous studies, the occurrence of AID was higher than reported in general populations both in the women (23.2%) and in the men (13.1%) with DLBCL [9,57]. In other studies of AID in DLBCL populations varying figures between 3.7%-22.5% have been reported [5,14,60,62–64,159]. However, the definition of eligible AID diagnoses is not clear in all studies and may be different from the definition we have used. One reason for the high occurrence of AIDs in our study is that we included all pre-existing AID diagnoses reported in the medical records at lymphoma diagnosis thereby identify-

ing 16 additional diagnoses compared to the patient-reported diagnoses included in studies by the InterLymph Consortium.

The most common specific AIDs in this study population are consistent with previous studies as rheumatoid arthritis, SLE and pSS were the most common AIDs categorised as primarily B-cell mediated (apart from thyroid disorders), and inflammatory bowel disease and psoriasis were the most common T-cell mediated AIDs. Thyroid disorders have been handled in different ways in previous studies and as so we performed outcome analyses with and without this group of patients. As there is some evidence that a majority of thyroid diseases in non-iodine deficient areas are autoimmune [160] we grouped all thyroid disorders together.

Some differences in outcome were suggested in analysis separately for patients with AIDs categorized as primarily driven by B- or T-cell responses. B-cell response AIDs (with thyroid disorders excluded) were associated with worse OS in Kaplan–Meier analysis and T-cell response AIDs were associated with inferior LSS and EFS in multivariate analysis. This result is in accordance with similar analyses in previous studies of DLBCL and AID. In one study including 41 DLBCL cases with AID, a history of B-cell-mediated AIDs was associated with shorter relapse-free survival and OS compared to patients without AID [63]. The prospective study of 8 pre-defined AIDs by Kleinstern et al. reported a non-significant trend toward inferior OS in DLBCL patients with AIDs primarily mediated by B-cell responses, while AIDs primarily mediated by T-cell responses were not associated with OS or EFS in any lymphoma subtype [62]. The study based on SEER-data showed a trend toward deceased lymphoma-related survival in patients with SLE and DLBCL compared to DLBCL patients with other B-cell mediated AIDs [64]. Our study and other previous studies have involved too small numbers of patients with SLE to be able to confirm these findings.

Previous studies of AID-associated DLBCL have not reported gender-specific information on outcome. We analysed OS separately for women and men and found a significant difference. Only women and not men with AIDs primarily driven by B-cell responses (with thyroid disorders excluded) had a significantly worse OS both in Kaplan Meier analysis and multivariate analysis compared to all other women and men, respectively. We cannot explain the reason for the worse OS in women from the analyses possible to perform in this study setting, and e.g., there could be differences in comorbidities, disease severity or earlier and ongoing treatment for AID that we were unable to control for. As the number of investigated cases in this study is limited larger studies addressing gender differences in outcome with more detailed knowledge of the above mentioned factors would be necessary to confirm these findings.

In summary, in the literature, and in this study there is some support for a worse OS associated with AIDs primarily mediated by B-cell responses, mainly driven by women with RA, SLE and pSS in this study. The finding

of a worse OS but not LSS may indicate that factors linked to the underlying AID are of importance for the prognosis. This is also in line with the study of Mikuls et al. which showed that RA patients with NHL (43% with DLBCL) had a higher risk of deaths unrelated to lymphoma or its treatment and were more susceptible for coronary artery disease and stroke than non-RA lymphoma controls [68]. Studies have shown that RA patients at increased lymphoma risk are characterized by longstanding, severe RA, factors which are known to predispose to increased mortality and comorbidity per se [66].

The finding of inferior LSS and EFS, but not OS in patients with T-cell response AIDs needs to be further explored in larger studies and has so far no support in the literature. The categorization of AIDs primarily driven by B- or T-cell responses is not exact and overlap in immune effector mechanisms exists between the groups which may affect results when using this categorization.

We found a higher rate of febrile neutropenia after the first treatment course in the AID-group compared to patients without AID, and an inferior OS in those with febrile neutropenia. This has not been reported before in AID-associated DLBCL, and could be one explanation for the worse OS in groups of AID patients, but needs to be further explored in coming studies.

Our retrospective design based on data in medical records may result in missed historical data. Although recall bias is avoided, we cannot rule out that some AID diagnoses may have been overlooked or that some patients not fulfil current diagnostic criteria. We grouped all thyroid diseases together as it was often uncertain whether the condition was a consequence of Hashimoto's disease or not. This may have resulted in the inclusion of patients with non-autoimmune thyroid disorders in the study. Our definition of diabetes type 1 (treatment with insulin only) may have led to erroneous inclusion of some patients with diabetes type 2. Cases were selected as we made a cohort of only DXR treated patients and as so frail patients (were there might be an even higher frequency of AID cases) were excluded. Previous treatment of the AIDs was not available in detail and neither was information about duration and severity of the AIDs. We also had limited knowledge of concomitant diseases or lifestyle factors that may affect outcome. Subtype classification of DLBCLs into germinal centre (GC) B-cell like and activated B-cell (ABC)-like subtypes is also relevant for studies of prognosis in DLBCL as patients with the ABC-subtype in most studies have a worse survival [29–31,161]. We did not have this subtype information in this study, which would have been interesting as some studies indicate that the ABC-subtype may be overrepresented in DLBCL patients with RA and SLE [67,162]. Although this is a large study the individual AID diagnoses constitute small groups.

In conclusion in patients with DLBCL, as much as up to a quarter of female patients may have a history of AID. Female patients with B-cell re-

sponse AID seem to have a worse OS and further studies are needed to confirm the results and investigate the background for this potential association. Patients with AID may have an increased risk of febrile neutropenia that warrants raised awareness in the clinical care of these patients. Future studies should consider gender aspects and include detailed data of the underlying AIDs and the specific causes of death to better understand the drivers of prognosis in this patient group.

5.3 Paper III

Age is the most important predictor of survival in diffuse large B-cell lymphoma patients achieving EFS24 - a Swedish population-based study.

Among 1169 patients included the median age was 64.6 years (range 18-91), 56.6% were men and the median follow-up was 82.3 months (range: 0.4-213). IPI score was ≥3 in 44.2%. Bulky disease was detected in 23.9%. Extra-nodal disease was found in 46.4%.

More than two thirds of the patients (n=837, 71.6%) achieved EFS24 and those had a better OS compared with those who did not.

Patients not achieving EFS 24 (n=332, 28.4%) were older (67.6 vs 63.4 years, p=0.003), tended to have higher IPI score (3-5) (62% vs 34.2%, p<0.001) and were more likely to have B-symptoms (56.6% vs 38.6%, p<0.001), bulky disease (31.9 % vs 20.7%, p<0.001), and extra-nodal involvement (55.7% vs 42.9%, p<0.001). There was no significant differences in sex, treatment regimen (R-CHOP vs R-CHOEP) or the addition of radiotherapy (RT) following treatment between patients achieving EFS24 and those who did not.

OS for DLBCL patients was statistically only marginally worse when compared with an age- and gender-matched standard population once EFS 24 was reached. SMR at 5 years after EFS24 was 1.23 (95%-CI: 1.02 - 1.44).

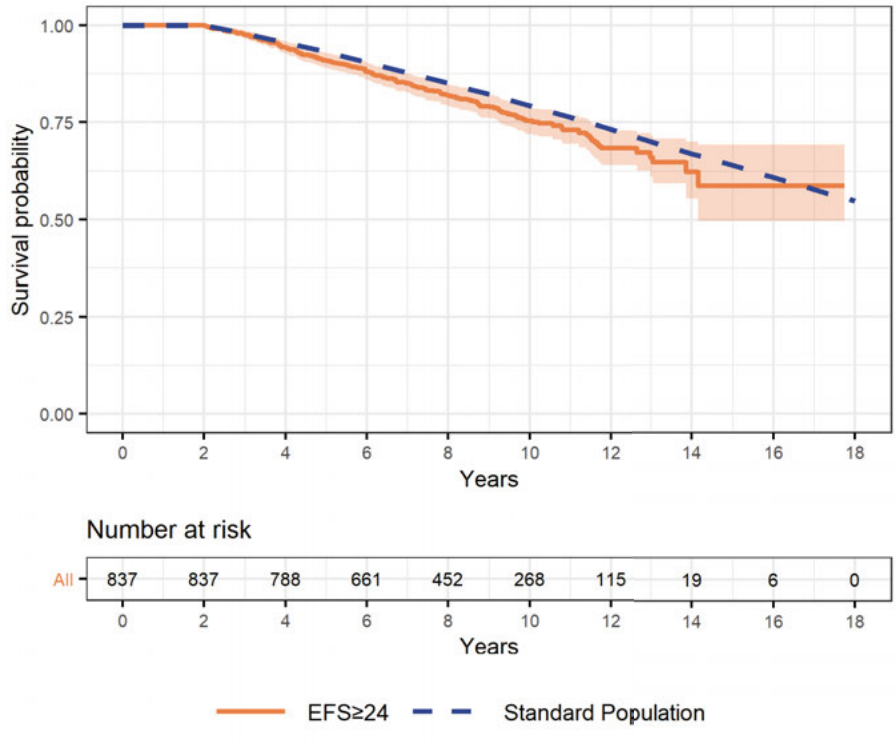


Figure 9. OS for DLBCL patients achieving EFS24 compared with an age-and-gender matched general population.
Standardized mortality ratio at 7 years (5 year past relapse free period) is 1.23 (95%-CI: 1.02-1.44).

When dividing the EFS24 cohort according to age (< or > 60 years), OS in patients younger than 60 years of age (n=266) was comparable to the standard population with only 9 events occuring up to 5 years after achievement of the EFS 24 mile stone. SMR at 5 years was 2.00 (95%-CI: 0.70 - 3.27).

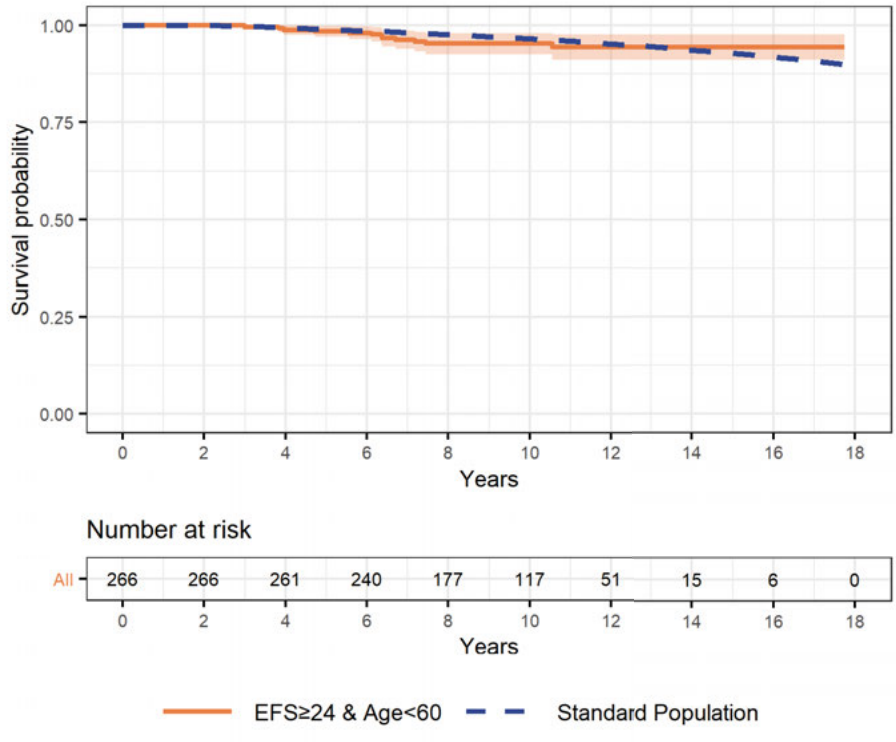


Figure 10. OS for DLBCL patients age<60 years who achieved EFS24 compared with an age-and-gender matched general population.
Standardized mortality ratio at 7 years (5 years past relapse free period) is 2.00 (95%-CI: 0.70-3.27).

In patients older than 60 years (n=571) there were 110 events at 5 years post EFS24 there was a trend to worse OS when compared to the standard population though statistically not significant, SMR 1.19 (95%-CI: 0.99 -

1.39).

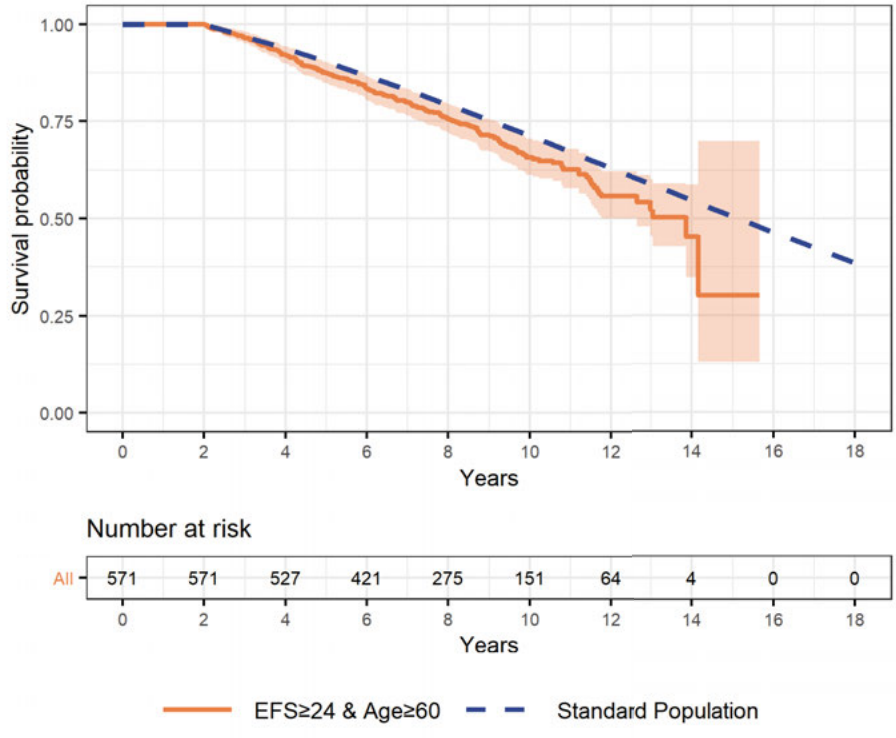


Figure 11. Overall Survival for DLBCL patients with age >60 years who achieved EFS24 compared with an age-and-gender matched general population. Standardized mortality ratio at 7 years (5 year past relapse free period) is 1.19 (95%-CI: 0.99-1.39).

Age-stratified analysis demonstrated that in younger patients groups (<29, 30-39,40-49 and 50-59) OS matched that of their peers in the standard population. OS for patients in the age interval 60-69 was worse compared to the standard population with SMR 1.65 (95%-CI: 1.09 - 2.18). The remaining older age groups (70-79 and >80 years) had an OS that almost matched the standard population.

Multivariate Cox regression analyses for patients achieving EFS24, considering risk factors identified by the IPI score revealed that age over 60 years is the only factor significantly affecting survival when compared to other risk factors after EFS24.

Table 10. Cox regression for different risk factors according to International prognostic index (IPI).

Variable	Hazard ratio	95% CI	P-value
Sex: Female (ref: Male)	0.78	(0.58-1.06)	0.117
Age≥60 (ref: Age<60)	9.27	(5.00-17.20)	<0.001
Tumor stage: 2 (ref: 1)	1.24	(0.78-1.96)	0.363
Tumor stage: 3 (ref: 1)	1.06	(0.62-1.81)	0.830
Tumor stage: 4 (ref: 1)	0.96	(0.57-1.64)	0.894
AB: Yes (ref: No)	0.93	(0.66-1.32)	0.688
Extranod: Yes (ref: No)	0.97	(0.64-1.48)	0.898
LD high: Yes (ref: No)	0.75	(0.54-1.03)	0.079
ECOG: 1 (ref: 0)	0.98	(0.69-1.41)	0.933
ECOG: 2 (ref: 0)	1.58	(0.95-2.60)	0.075
ECOG: 3 (ref: 0)	0.78	(0.34-1.79)	0.560
ECOG: 4 (ref: 0)	3.78	(1.12-12.78)	0.033

AB : Absence/Presence of B symptom.
Extranod: Extranodal Disease.
LD high: high Lactate dehydrogenase.
ECOG: performance status according to Eastern cooperative oncology group.

Patients with early stage lymphoma (stage I-II) had a worse OS after reaching EFS12 when compared to the matched standard population, SMR at 5 years post EFS12 was 1.35 (95%-CI: 1.07 - 1.62). OS was however better compared with patients not reaching EFS12.

Of all 1169 patients, 501 (42.9%) patients died. In the EFS 24 group, a total of 190 patients died, with 38 (20%) of death attributed to lymphoma. Causes of death for the remaining 152 patients was; cardiovascular disease 34 (22.4%), cancer 24 (16 %), dementia 5 (3.3%) others 6 (4%) and unclear 83 (54.6%).

Contradictory to Maurer et al, in our unselected patient cohort we could not confirm equal OS for DLBCL patients achieving EFS24 compared to a standard population [136]. This could only be confirmed for patients younger than 60 years of age. In older ages there was a trend for worse OS driven by a significant difference in OS among those 60-69 years. This resembles the findings of a Danish population-based study where they found that only patients younger than 50 years of age had a normalised OS comparable to an age- and gender-matched Danish population, regardless of other risk factors such as IPI score [138].

Interestingly, in another large population-based study where EFS24 was calculated from diagnosis, the five-year risk of relapse decreased after achieving EFS24 (33% to 11%), but OS for EFS24 patients remained worse than that of a matched local population regardless of age, IPI score and disease stage [139]. Pathological subtype analysis in that study revieled that

patients achieving EFS24 who had either GCB or primary mediastinal B-cell lymphoma did have an OS comparable to the standard population.

In yet another recent study from the Netherlands results supported findings of normalised OS after reaching PFS24 as patients in a population based cohort who achieved PFS24 (319 patients from a total of 585) had an OS that was similar to a large clinical trial cohort in which they had only marginally lower OS compared to a matched population [137,163]. The follow-up time was more than 10 years and PFS24 was calculated from the end of treatment. The causes of death were lymphoma relapse in 19%, cardiovascular death in 23%, and other malignancies in 25% of the patients.

The difference in survival for patients achieving EFS24/PFS24 between our cohort and the mentioned studies could partly be due to the different study populations, different standard populations but might be also partly due to different definitions of EFS (time from diagnosis vs time from end of treatment) and in inclusion criteria (patient who achieved CR or CRu).

Our finding of a worse OS in the specific age group 60-69 years is new. Further analysis to find out the reason for this excess mortality is of importance. One explanation may be an increased death rate due to treatment related toxicities as cardiovascular disease or secondary malignancies that may cause significant differences in OS for this age group but not for the older ages. Of patients achieving EFS24 about one fifth died from cardiovascular disease (CVD), a well described sequel after DXR based chemotherapy in NHL [164,165]. This number is in accordance with the 23% in the Netherlands study. Long-term follow-up of these patients is thus warranted both for clinical assessment and lifestyle counselling with regards to other risk factors such as smoking, obesity, hypertension, and hyperlipidemia. Early intervention should be considered as it has been shown to be crucial in reducing cardiovascular mortality and morbidity [166].

Patients treated for NHL are known to have an increased risk for secondary malignancies such as leukemia, lung cancer, renal cancer, and bladder cancer [133]. In our study 16% of the patients who died without lymphoma, died due to another cancer.

Established risk factors such as older age, poor performance status at diagnosis, presence of bulky disease, extranodal involvement, high LDH and IPI index, all increased the risk of never reaching EFS24. In part, this has previously been reported in a large study with over 7000 patients, which determined the loss of life expectancy and found that mainly IPI score >2 significantly had an impact on the outcome [167].

Unfortunately we did not account for histological subtypes in our analysis but doing this would be of great interest based on the findings of better outcome for GCB and primary mediastinal B-cell lymphoma.

In the Maurer study patients with stage I and II disease who achieved EFS12, had an overall survival similar to the age- and sex-matched standard

population but in our study they still had a worse prognosis in comparison to a matched healthy population [136].

The strengths of this study include the population-based design, the long follow-up time (17.8 years) and the relatively large number of patients with well documented data.

In conclusion, EFS24 appears to be an attractive end-point for follow-up as most lymphoma-related events occur before this milestone and the Swedish Lymphoma group considers two years of follow-up as satisfactory for relapse-free DLBCL patients. Yet, based on the findings of this study, prolonged follow-up for patients older than 60 years should be considered, at least at the primary care level, with regards to a possibly increased risk for cardiovascular disease and secondary malignancies.

5.4 Paper IV

Plasma proteome profiling of cardiotoxicity in patients with Diffuse Large B-Cell Lymphoma.

The 95 patients included in this study consisted of 55 (57.9%) men and 40 (42.1%) women with a median age of 65.0 years (range 27-87). Median follow up time was 69.0 months (1-109 months). Seven patients did not receive DXR containing treatment and were only included in base-line analysis.

Table 11. Demographic and clinical characteristics of 95 eligible patients.

Characteristic	Patients n(%)[c]	Missing n(%)
Gender[a]		
Male	55 (57.9)	
Female	40 (42.1)	
Age, years[b]	65.0 (27-87)	
IPI[a]		
0-2	62 (66.7)	2 (2.1)
3-5	31 (33.3)	
B-symtoms[a]		
Yes	32 (33.7)	
No	63 (66.3)	
DXR dose mg/m^2 [b]	292.6 (49.1-348.5)[d]	6 (6.3)
Smoking[a]		
Never	39 (52.7)	21 (22.1)
Former	24 (32.4)	
Current	11 (14.9)	
BMI[b]	25.0 (17.5-52.1)	4 (4.2)
eGFR[a]		
0-1	74 (82.2)	5 (5.3)
2-4	16 (17.8)	

CRP[a]		
Normal	32 (34.8)	3 (3.2)
Elevated	60 (65.2)	
Comorbidity[a]		
Heart disease	21 (22.6)	2 (2.1)
Vascular disease	10 (10.8)	2 (2.1)
Hypertension	34 (36.6)	2 (2.1)
DM	13 (14.0)	2 (2.1)
Medication[a]		
Metformin	9 (9.8)	2 (2.1)
Beta-blocker	18 (19.4)	2 (2.1)
ACE or ARB	26 (28.0)	2 (2.1)
Statins	23 (24.7)	2 (2.1)

Data are presented as an (%) or bmedian (range). IPI, international prognostic index; DXR, doxorubicin; BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, c reactive protein; DM, diabetes mellitus; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker. [c] Fever/night sweat/weight loss. [c] %; number of patients with X divided with total number of cases with X known. Missing cases are excluded, [d]Median dose for 82 patients.7 patients recieved no DXR. 6 patients where dose/m2 is missing.

At diagnosis, 21 (22.6%) had a pre-existing cardiac condition, 10 (10.8%) a vascular disease and 34 (36.6%) hypertension, whereas in the control group, corresponding numbers were 2 (3.3%), 5 (8.3%) and 14 (23.3%). There was no difference in age, gender or BMI between DLBCL and controls.

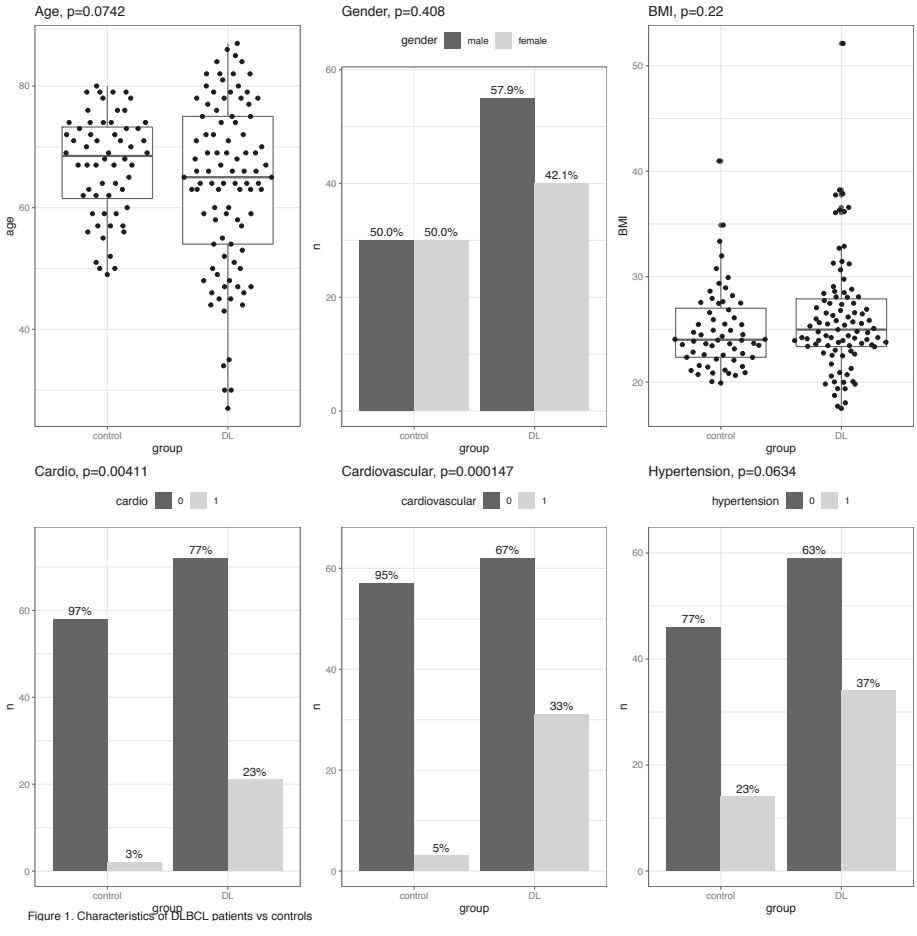


Figure 1. Characteristics of DLBCL patients vs controls

Figure 12. Characteristics of DLBCL patients vs controls.

Fifteen patients got a new diagnosis of a cardiac disease (15.8%) and 22 (23.2%) a new CVD (15 cardiac and 7 vascular) during follow up. Thirty-one patients (32.6%) died during follow up and causes of death were lymphoma in 20 (64.5%) patients and cardiovascular disease in 4 (12.9%) patients.

Patients with pre-existing CVD had a worse OS (p=0.027) in Kaplan-Meier analysis for OS regarding 87 patients treated with DXR, with or without pre-existing CVD.

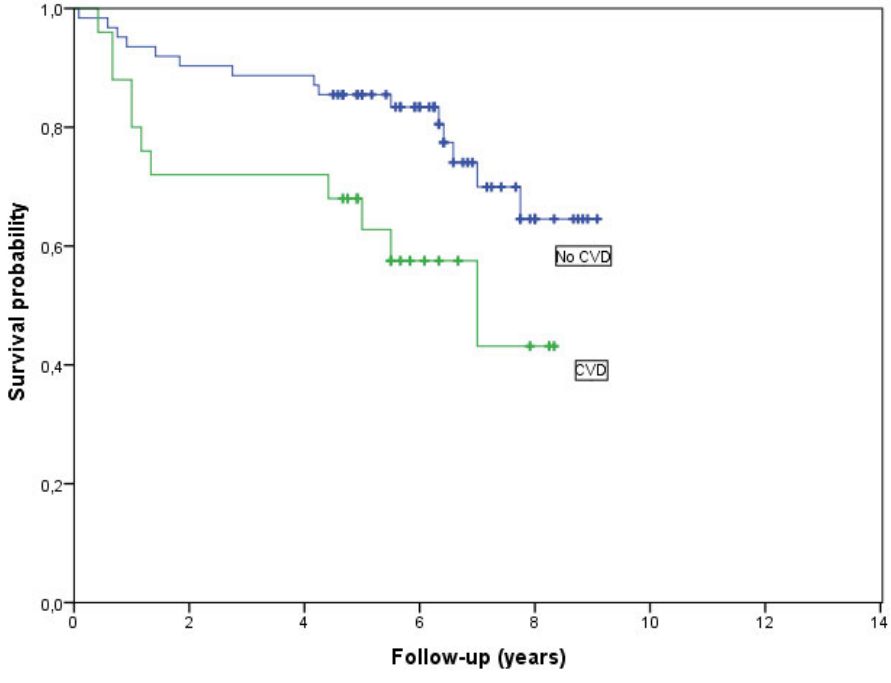


Figure 13. Kaplan-Meier for survival comparing patients treated with DXR (n=87) with cardiovascular disease (CVD, n=25) vs without CVD (n=62) at diagnosis (p=0.027).

However, Cox-regression analysis including known risk factors for CVD in the 80 cases for whom we had complete data (missing DXR dose, n=5, missing data on hypertension, n=1, missing IPI, n=1) and imputed missing BMI values (n=4, median value=25), showed no significant relation for pre-existing CVD and OS. Age (p=0.039), gender (p=0.014) and BMI (p=0.046) were associated to OS.

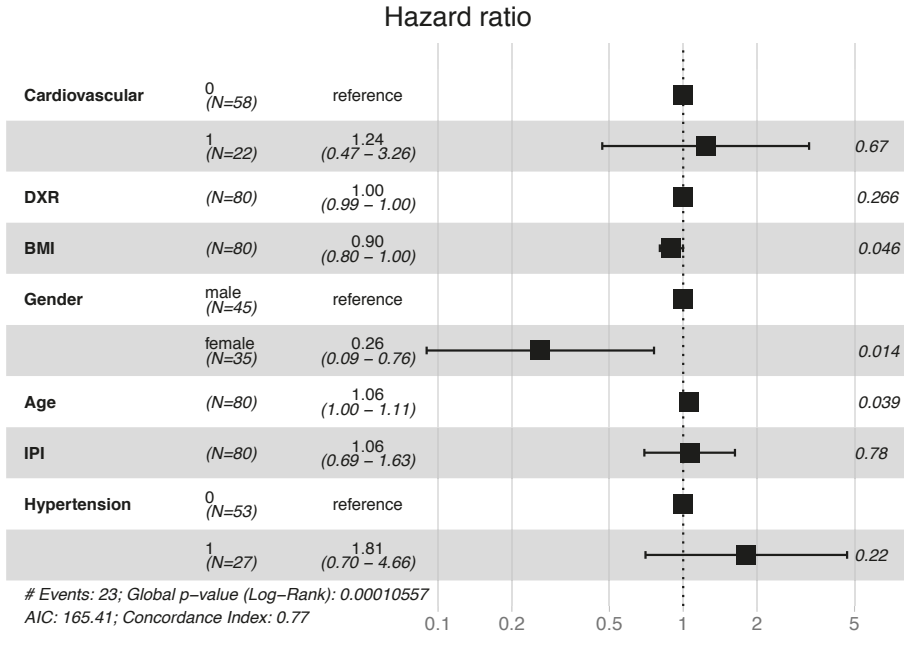


Figure 3. Cox regression analysis of risk factors for overall survival

Figure 14. Cox regression analysis of risk factors for Overall Survival.

We found no proteins in the PEA-CO or NTproBNP and Troponin I in DLBCL pre-treatment samples that significantly correlated with pre-existing cardiac disease. However, in patients with pre-existing CVD there was an association between higher levels of SPON-1 and CVD at diagnosis as estimated from 92 PEA-CO samples (Fold change (FC) 1.22, 95%CI 1.10-1.35, p=0.0002, q=0.046). We observed a significant association between higher level of protein IL-1RT1 in pre-treatment samples and upcoming CVD (adjusted for previous CVD and hypertension, 92 samples) (FC 1.24, 95%CI 1.10-1.39, p=0.0004, q=0.082).

We investigated if treatment affect the protein levels differently in patients developing a new cardiac disease or CVD as compared to patients who do not develop cardiac disease or CVD. The effect of treatment on protein level was studied by comparing levels in samples from before, during and after treatment in patients treated with DXR.

There was a significant difference related to new cardiac disease for IL-1RT1 (p=0.007, q=0.096) and SPON-1 (p=0.001, q=0.096). Post hoc analyses show that in IL-1RT1 this is due to a difference in protein level between patients with and without new cardiac disease mainly before, but also during treatment.

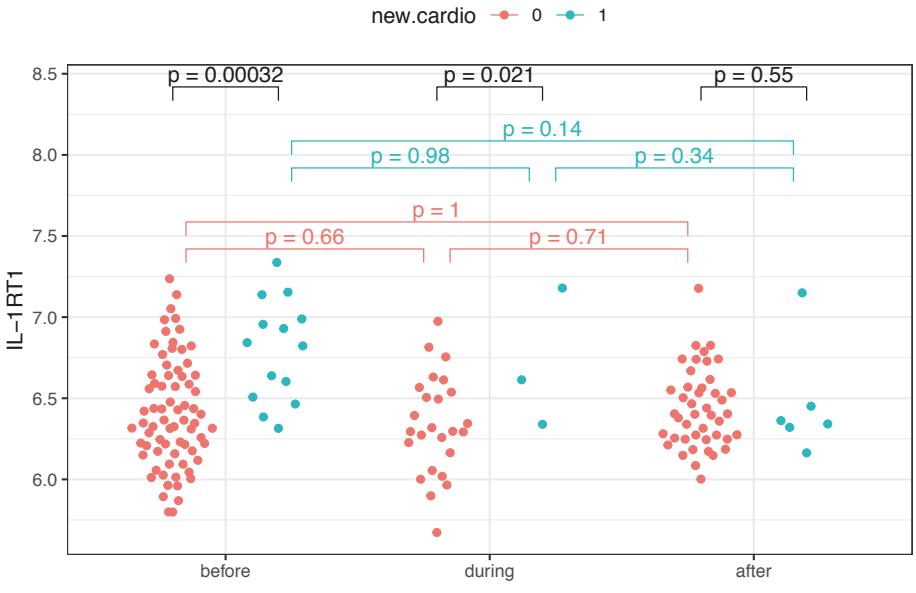


Figure 15. IL-1RT1 protein level difference in patients with (blue, 1) and without (red, 0) new cardiac disease after treatment in different time-points before, during and after treatment.

The difference in SPON1 is due to a group difference after treatment.

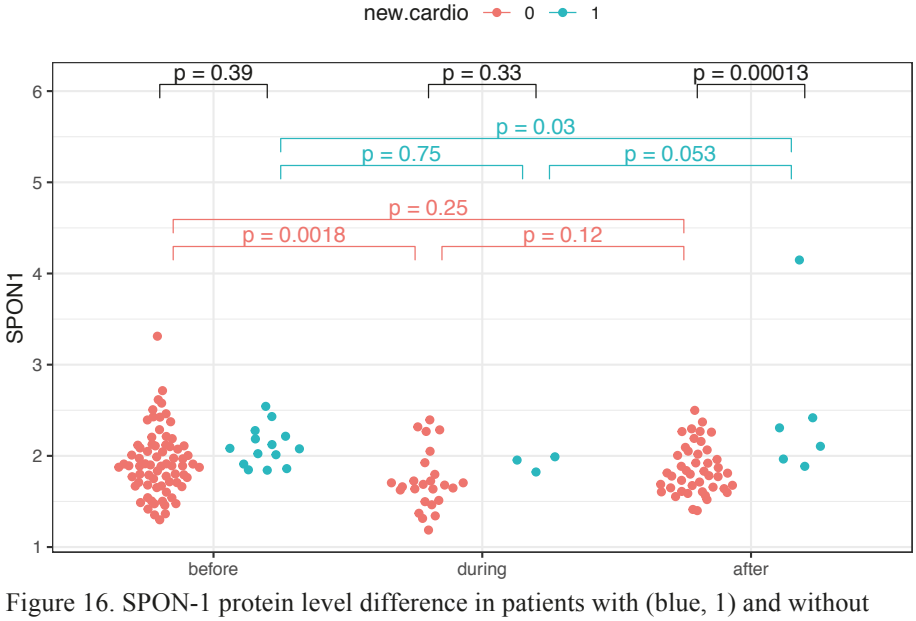


Figure 16. SPON-1 protein level difference in patients with (blue, 1) and without (red, 0) new cardiac disease after treatment in different time-points before, during and after treatment.

Emerging CVD was associated with change in IL-1RT1 (p=0.0003, q=0.056). Post hoc analysis to clarify time point for this protein level change revealed a difference between samples for new CVD vs no new CVD before and during treatment but not for samples after.

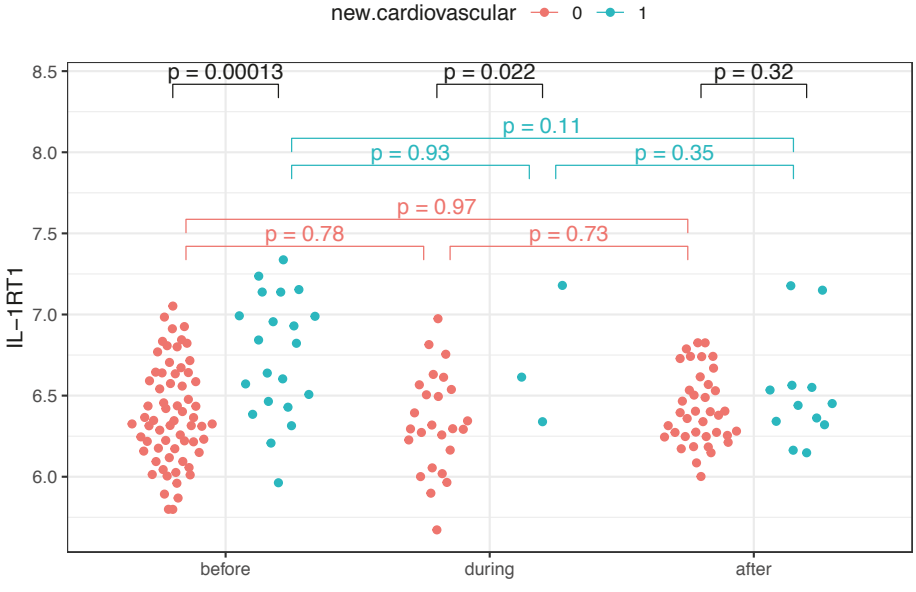


Figure 17. IL-1RT1 protein level difference in patients with (blue, 1) and without (red, 0) new cardiovascular disease (CVD) after treatment in different time-points before, during and after treatment.

Univariate tests for suspected CVD risk factors and drugs with possible association to CVD came up with one significant factor; age (p=0.003 q=0.057) pointing at a higher risk for CVD and three suspected risk factors: treatment with angiotensin converting enzyme/angiotensin II receptor (ACE/ARB) blockers (p=0.025, q=0.228), DXR (p=0.121, q=0.436) and hypertension (p=0.105, q=0.436) probably connected with CVD.

Table 12. Univariate tests for suspected CVD risk factors and drugs with possible association to CVD.

Variable	No CVD[b]	CVD[c]	Test[d]	p-value	q-value
Number[a]	71	22			
Age (mean)	61.4	71.1	Mann-W	0.003	0.057
Gender=1(%)	32(45.1)	7(31.8)	Chisq	0.393	0.885
DXR (mean)	493.4	417.9	Mann-W	0.121	0.436
Smoking (%)	24(43.6)	9(52.9)	Chisq	0.693	0.960
BMI (mean)	26.3	24.7	Mann-W	0.282	0.726
Cardiac (%)	14(20.0)	5(23.8)	Chisq	0.944	1.000
CVD (%)	21(30.0)	8(38.1)	Chisq	0.666	0.960
HT (%)	21(30.0)	11(52.4)	Chisq	0.105	0.436
DM(%)	9(12.9)	4(19.0)	Fisher	0.488	0.960
CRP (mean)	40.2(70.9)	39.1(53.6)	Mann-W	0.955	1.000
GFR (%)	11(16.2)	5(23.8)	Chisq	0.638	0.960
AB (%)	26(36.6)	6(27.3)	Chisq	0.583	0.960
IPIhigh (%)	22(31.9)	7(31.8)	Chisq	1.000	1.000
Metformin=1(%)	7(10.0)	2(9.5)	Fisher	1.000	1.000
B-block (%)	10(14.3)	6(28.6)	Chisq	0.237	0.712
ACE/ARB (%)	14(20.0)	10(47.6)	Chisq	0.025	0.228
B-block/ACE-ARB (%)	19(27.1)	11(52.4)	Chisq	0.058	0.350
Statin (%)	16(22.9)	6(28.6)	Chisq	0.806	1.000

Gender=1, females; DXR,doxorubicin; Smoking, ongoing or earlier smoker; BMI, body mass index; Cardiac, cardiac disease at diagnosis, CVD, cardiovascular disease at diagnosis; HT, hypertension at diagnosis; DM, diabetes mellitus at diagnosis; CRP, c-reactive protein; GFR, glomerular filtration rate <60 ml/min/1.72m^2; AB, B-symtoms (fever, night sweats, weight loss) at diagnosis; IPIhigh, International prognostic index score ≥3; B-block, treatment with beta-blocker; ACE/ARB, treatment with angiotensine converting enzyme/ angiotensine II receptor blocker; Statin, treatment with statins

Mann-W, Mann-Whitney test; Chisq, Chisquare test; Fisher, Fishers exact test

[a] 93 patients included. 2 patients excluded because of lack of information on emerging CVD

[b]No new CVD after treatment

[c]Emerging CVD after treatment

[d]type of test performed

All risk factors with a p-value below 0.20 were combined in a multivariate logistic regression, in which no factor showed significant association to CVD.

In this study we found evidence that high SPON-1 levels are associated with existing CVD in DLBCL patients. SPON-1, or Spondin-1, is a cell adhesion protein important for axons and a major factor for vascular smooth muscle cell activity, which might explain why it seems important in cardiac toxicity (www.uniprot.org). In recent studies on patients with [168] or without [169,170] pre-existing hearth failure, SPON-1 was associated with incident or deteriorating heart failure. The Interleukin-1 receptor type 1, IL-1RT1, was associated with the risk of developing cardiovascular disease both in

base-line test and in tests for protein changes during treatment. IL-1RT1 is a receptor for IL1A, IL1B and IL1RN that after binding mediates activation of NF-kappa-beta, MAPK and other pathways (www.uniprot.org). IL-1 blockade can reduce myocardial infarct size and injury by interrupting the inflammatory reactions also after DXR exposure which has been shown in animal studies [171,172]. To our knowledge, there are no reports on association between IL-1RT1 levels in plasma and heart toxicity in humans and our results might be the first to demonstrate a possible association between plasma IL-1RT1 levels and upcoming CVD after DXR treatment in humans.

Olink proteomic studies on deteriorating of, or emerging cardiac disease, have shown divergent results. 33-37. One previous study have reported the association of SPON-1 and incident heart failure in two community-based prospective cohorts of elderly without heart failure at baseline [169], further strengthening the implications of SPON-1 in CVD.

Studies on anthracycline treated cancer patients found correlations between Troponin I and cardiac toxicity without any connections with NTproBNP [166,173,174]. In the present study, neither NTproBNP nor Troponin I significantly co-varied with present or emerging cardiac or cardiovascular disease. The low sample frequency as well as the limited sample size may explain the lack of correlation, although NTproBNP was the protein with the closest to significant level on association with present cardiac disease (q=0.43).

There was a high incidence of emerging CVD, where almost one fourth (22.6%) of DLBCL patients receiving immunochemotherapy develop CVD and also a high proportion of patients with pre-existing CVD before treatment initiation, compared to a non-DLBCL cohort. A study from Khan et al regarding ischemic heart disease, reports a prevalence in Sweden of 3.858/100.000 which corresponds to 3.86% [175]. This number is similar to the prevalence in our control cohort (3.3%), making the almost seven times higher prevalence of 22.6% in the DLBCL group remarkable. In a recent study of chronic lymphocytic leukemia (CLL), a prevalence of CVD diagnosis, including hypertension, within 10 years prior to CLL, was found to be 32% [176]. When excluding hypertension in that material, 788 diagnoses of CVD in 2078 cases (37.9%) was observed, although the number might be overestimated due to patients having multiple diagnoses [176]. Nonetheless, this number, in a higher median aged cohort (71 years), is in accordance with our finding of 33.4% with CVD.

The occurrence of hypertension in the Swedish general population is not fully mapped, but data from the public health agency of Sweden (www.folkhalsomyndigheten.se) and the Swedish association of local authorities and regions (www.vardhandboken.se) indicate a prevalence of around 20-30%, hence similar frequencies as in our control group (23.3%). Again, higher prevalence was seen in our DLBCL cohort (36.6%, p=0.063).

Previous studies suggest that overweight/obesity (O/O) increase the risk of DLBCL. As O/O is a well-known risk factor also for hypertension and CVD, a possible explanation for the higher prevalence of CVD in our lymphoma cohort could be a higher proportion of O/O in the group [19,177,178]. We therefore compared BMI between the lymphoma patients and the normal controls from the EpiHealth study but no significant BMI-differences were observed between DLBCL and controls. Altogether our data reveals a high incidence of CVD and hypertension in DLBCL, a fact that may affect survival and sensitivity to treatment.

Previous studies show an incidence of emerging heart toxicity for anthracycline or DXR treated cancer and lymphoma patients of 10-20% [164,173,174,179–182]. Our result of 15.9%, with a quite long follow up time, fits into these results, despite the fact that our cases were only clinical evident ones, whereas in some studies they found a high amount of silent heart failure only observed in ultrasound measurements [179–181]. Moser et al have reported 12% and 22% new CVD 5 and 10 years respectively after treatment for NHL, well in accordance with our results of 23.2% [164]. Some of the mentioned studies have a rather low median age [164,181] and our older, population based cohort would be expected to be more prone to cardiac and cardiovascular diseases. Suspected risk factors for CVD was age, DXR dose, hypertension and ACE/ARB treatment. None of them was significant in our multivariate testing probably due to co-variation of these factors (at least for age, hypertension and ACE/ARB usage). Although it is plausible that other factors such as DM, renal function (GFR), BMI and smoking are associated, we could not observe such associations which might be due to missing data in patients records and a small cohort.

In conclusion, this study revealed two new proteins, SPON-1 and IL-1RT1, possibly related to pre-existing and emerging CVD respectively in DLBCL patients treated with DXR. If confirmed in larger study cohorts, IL-1RT1 may emerge as a very promising biomarker for the increased risk of developing CVD in DLBCL patients. In addition, we observed higher prevalence of cardiac disease and CVD in DLBCL patients compared to the average population. Larger epidemiological studies may confirm these results and possibly unravel the relation between CVD and DLBCL development – further studies are required to elucidate whether CVD increases the risk of DLBCL or vice versa. In clinical practice the results imply caution and attention in caring for DLBCL patients with CVD and use of more preventive strategies for optimized treatment for cardiac disease or CVD in these patients.

The cohort was relatively small and missing values for NTproBNP and Troponin I proteins at start were high. A larger investigation might lead to more significant results. Also time for evolvement of new cardiac or vascular disease after treatment is unknown, and the diagnosis is only based on

revision of medical records, not by any investigations. Furthermore the follow-up time is too short to see very late toxicities.

6 Summary of results

I The omission of vincristine due to neurotoxicity does not have an impact on DFS or OS neither in the whole cohort nor in the elderly (≥70 years) group of patients irrespective of at what treatment cycle the omission of vincristine was made.

II There is a high occurrence of AID among DLBCL patients compared to the general population. AID does not affect outcome (EFS/LSS/OS) in the whole cohort but women with B-cell response AID had a worse OS compared to other women. Patients with AID had a higher frequency of febrile neutropenia.

III 71.6% achieved EFS24 and OS was significant but marginally worse compared to a standard population. Age was the only factor affecting OS in multivariate analysis and EFS24 patients<60 years had a comparable OS to the standard population. In older ages (>60 years) there was a trend for worse OS driven by a significant difference in OS among those 60-69 years. Among DLBCL achieving EFS24 22.4% died of cardiovascular disease and16% from other malignancies.

IV Two proteins, SPON-1 associated with CVD at diagnosis and IL-1RT1 associated with emerging CVD after treatment was found. Compared to the general population and an age and gender matched cohort DLBCL patients had a high occurrence of CVD at diagnosis, 33.4%. After treatment 22.6% developed CVD.

In conclusion it seems safe to omit VCR due to neurotoxicity in the clinical setting. AID is common in DLBCL what warrants awareness, especially in women as they according to these findings have a worse OS although the reason for this is unclear. The achievement of EFS24 could in the younger (<60 years) DLBCL population open to short surveillance for two years but for the older this may not be safe due both to risk of late relapse and a possible excess death rate because of treatment. The occurrence of CVD in newly diagnosed DLBCL is higher than in the population and in matched controls and there is also a high occurrence of emerging CVD and CVD related deaths after treatment of DLBCL. More attention on CVD at diagnosis in order to optimise CVD treatment and to avoid worsening of CVD due to

treatment and eventually longer and CVD focused follow-up is desirable. The finding of two new proteins associated to CVD in DLBCL patients are promising but needs to be explored in further studies.

7 Populärvetenskaplig sammanfattning på svenska

Bakgrund

Diffust storcelligt B-cellslymfom (DLBCL) är en aggressiv form av lymfkörtelcancer som utgår från kroppens egna immunceller. Varje år insjuknar ca 600-700 personer i Sverige. Sjukdomen kan drabba människor i alla åldrar men medianålder vid diagnos är 70 år. Ålder är en prognostisk faktor med minskade chanser till bot med ökande ålder. DLBCL är något vanligare hos män (män:kvinnor 1,4:1). Orsaken till att vissa får DLBCL är i stort okänd men man vet att risken att insjukna ökar om man har en sjukdom där immunförsvaret angriper de egna cellerna (autoimmun sjukdom). Man vet dock inte hur många av de som drabbas av DLBCL som har en autoimmun sjukdom och om autoimmun sjukdom påverkar behandlingsresultat. De som har autoimmun sjukdom har vanligtvis fått många års behandling, ofta med celldödande ämnen (cytostatika), mot sin sjukdom vilket kan påverka deras individuella känslighet för cytostatikabehandling mot DLBCL. 60-70% av alla DLBCL botas med en kombinationsbehandling med antikroppar (Rituximab=R) riktade mot proteinet CD-20 på tumörytan och cytostatika som är celldödande. CHOP (cyklofosfamid, doxorubicin, vinkristin och prednison) är den vanligaste cytostatika kombinationen som ibland modifieras med till exempel tillägg av etoposid (CHOEP). Behandlingen ges i sex omgångar (kurer) med två eller tre veckors intervall emellan. De som inte botas kan vara primärt okänsliga (refraktära) mot behandlingen eller få senare återfall och de erbjuds om möjligt efterföljande behandlingar.

Biverkningar av behandlingen är ett kliniskt problem liksom att patienterna kan ha andra, samtidiga, sjukdomar (komorbiditet) som påverkar möjligheten till behandling. Cytostatikat vinkristin kan orsaka bestående nervskada som oftast drabbar de långa, perifera nerverna och yttrar sig som pirrningar, stickningar, nedsatt känsel och försämrad funktion i händer och fötter. Det finns en stor variation i känslighet för denna biverkan, vissa får problem redan efter ett par doser och andra får ingen biverkan alls, och det finns inga förebyggande (profylaktiska) metoder för att undvika biverkan. I klinisk praxis är det vanligt att man exkluderar vinkristin om patienten får kvarstående symtom som börjar påverka vardagsfunktioner till exempel att knäppa knappar.

Cytostatikat doxorubicin är hjärttoxiskt och ges inte till patienter med alltför påverkad hjärtfunktion från början. Kliniskt bedöms detta med undersökning, provtagning för markörer för hjärtskada (NTproBNP) och ibland med ultraljud av hjärtat. Man begränsar också den totala dosen av doxorubicin som får ges eftersom biverkningarna ökar med den ackumulerade dosen. Dock kan till synes helt hjärtfriska patienter drabbas av svår hjärtsjukdom inom månader till många år efter behandling.

Nya biotekniska landvinningar gör att man numer kan analysera ett stort antal proteiner i små mängder av blod. Man har grupperat en mängd olika proteinmarkörer i paneler som är associerade till olika sjukdomar, till exempel till hjärtskada. Studier visar att vissa proteiner, som kan mätas i patientens blod, är associerade med existerande och nytillkommen hjärtsjukdom samt till försämring av känd hjärtsjukdom. Om man kan hitta någon proteinmarkör hos DLBCL patienter som är kopplad till utveckling av hjärtsjukdom skulle man eventuellt kunna följa denna markör hos patienter för att kunna ändra behandlingen i tid innan hjärtsjukdom utvecklas.

Uppföljningen av patienter efter avslutad behandling för DLBCL har länge pågått i minst fem år då man har befarat att patienter kan drabbas av återfall. Nyare studier visar att färdigbehandlade DLBCL patienter som förblivit friska från DLBCL två år efter diagnos (de har uppnått ”event free survival 24”=EFS24=sjukdomsfri överlevnad efter 24 månader) har samma förväntade överlevnad som normalpopulationen, de dör alltså inte i högre utsträckning än den övriga befolkningen. Dock gjordes dessa studier på patienter som varit inkluderade i behandlingsstudier. Det är vanligt att patienter i behandlingsstudier inte speglar hela gruppen av patienter då de är utvalda och oftast är yngre och totalt sett friskare. Uppföljande populationsbaserade (som omfattar alla DLBCL patienter, inte bara utvalda) studier har inte kunnat bekräfta resultaten eller bara påvisat att patienter yngre än 50 år som uppnår EFS24 har samma överlevnad som övrig befolkning.

Frågeställning

I den här avhandlingen har jag tittat på fyra huvudfrågor:

Delarbete 1; Spelar det någon roll för behandlingsresultatet (möjligheten att bli botad) om man tar bort vinkristin från behandlingen när patienten utvecklar nervbiverkan?

Delarbete 2; Hur många DLBCL patienter har också en autoimmun sjukdom och påverkar autoimmun sjukdom behandlingsresultat och biverkningar av behandling?

Delarbete 3; Har svenska DLBCL patienter som förblir friska två år efter diagnos (uppnår EFS24) samma förväntade överlevnad som en svensk ”normalpopulation” och hur ser dödsorsakerna ut hos de som uppnår respektive inte uppnår EFS24?

Delarbete 4; Finns det proteinmarkörer i blodet hos DLBCL patienter som är relaterade till existerande eller debuterande hjärtsjukdom under behandlingen?

Metod

För att kunna besvara dessa frågor har vi samlat en databas med patienter som diagnosticerats och behandlats för DLBCL mellan 2000-2013 i fyra regioner; Dalarna, Uppland, Sörmland och Gävleborg. Kliniska data samlades från patienternas medicinska journaler. Totalt 612 patienter inkluderades. Hos 541 av dessa kunde vi hitta fakta om hur stor dos vinkristin de fått och om de avbrutit vinkristin behandlingen. Dessa 541 utgör materialet till delarbete ett. I delarbete två analyserades alla 612. För delarbete tre slog vi ihop vår databas med en liknande grupp av patienter (kohort) från Västra Götaland och fick då en grupp med 1169 DLBCL patienter att analysera. I det fjärde delarbetet användes en annan grupp av DLBCL patienter. Det finns i Sverige en biobank som heter U-CAN där tumörmaterial och blod från bland annat DLBCL patienter samlas. 95 patienter som fått diagnosen mellan 2010-2015 kunde hittas. Hos dessa har vi sedan gjort proteinanalyser från blodprover tagna före och hos vissa under och efter behandling och tittat om några proteiner är associerade med hjärtsjukdom.

Resultat

Delarbete 1; Av 541 DLBCL patienter, med känd dosering av vinkristin, sattes läkemedlet ut hos 95 (17.6%) personer på grund av biverkningar. Dessa personer var något äldre och hade fler riskfaktorer gällande sjukdomen än de där vinkristin inte sattes ut. Hos majoriteten, 86 patienter (90.5%), sattes läkemedlet ut vid någon av de sista tre behandlingarna (av totalt sex). Vi kunde inte se att utsättningen av vinkristin påverkade sjukdomsfri eller total överlevnad, det vill säga det spelade ingen roll för behandlingsresultatet om vinkristin sattes ut. Det spelade inte heller någon roll om vinkristin sattes ut tidigt (under kur 1-3) eller senare i behandlingsserien.

Delarbete 2; 106 DLBCL patienter av 612 (17,3%) hade en autoimmun sjukdom. Det är ett högt antal om man jämför med normalpopulationen där studier pekar på en frekvens på 3-10%. Hos kvinnor med DLBCL var antalet

59 av 254, det vill säga 23,2%. För hela gruppen kunde vi inte finna någon skillnad i överlevnad efter behandling beroende på om man hade en autoimmun sjukdom eller inte. Dock var överlevnaden sämre för kvinnor som hade någon av de autoimmuna sjukdomar som är mest kopplade till DLBCL; ledgångsreumatism (RA), systemisk lupus erytematosus (SLE) eller Sjögrens sjukdom jämfört med hur det gick för övriga kvinnor. Det var vanligare att patienter med autoimmun sjukdom fick en kraftig påverkan på immunförsvaret efter sin första behandling.

Delarbete 3; Av 1169 DLBCL patienter var det 837 (71,6%) som uppnådde EFS24 det vill säga de hade inga lymfomrelaterade problem 24 månader efter diagnos. De som inte uppnådde EFS24 var äldre och hade fler lymfomrelaterade riskfaktorer men det var ingen skillnad gällande kön, vilken cytostatikakombination som givits eller om patienten fått strålning som del i behandlingen. I jämförelse med en köns- och åldersmatchad svensk normalpopulation hade patienterna som uppnådde EFS24 en sämre överlevnad men för patienter <60 år var överlevnaden dock samma.

Median uppföljningstid var 82 månader. För de som inte uppnådde EFS24 avled 311 (93,7%) och dödsorsaken var hos 84.8% (264 stycken) lymfom medan för de som uppnådde EFS24 avled 190 (22,7%) stycken och bara 20,5% av lymfom. Hos de patienter där man kände till dödsorsaken var 31,4% hjärtkärlsjukdom och 34,3% annan cancer men hos många var dödsorsaken oklar (annan orsak=10,5%, oklar orsak=19%).

Delarbete 4; För 95 DLBCL patienter hade vi U-CAN blodprover som vi kunde analysera med proteinanalyser. Totalt testades 185 olika proteiner. Som jämförelsegrupp testades 60 stycken ålders och könsmatchade personer utan DLBCL. Vid analys av prover tagna före start av cytostatikabehandling var proteinet SPON-1 associerat med existerande hjärt-kärlsjukdom. Ett annat protein, IL-1RT1, var kopplat till utveckling av hjärt-kärlsjukdom. Vi fann inget protein som under pågående behandling förändrades och kunde förutsäga uppkommande hjärtsjukdom. Hos DLBCL patienterna hade 31 (33,3%) någon hjärt-kärlsjukdom redan vid diagnos av DLBCL vilket är högt jämfört med kontrollgruppens 5% och det är också en hög siffra om man jämför med befolkningsstatistik. 22 patienter (23,2%) utvecklade hjärtsjukdom efter behandlingen.

Slutsatser

Det verkar säkert att sätta ut vinkristin behandlingen hos patienter som utvecklar biverkningar. Sannolikt har de som utvecklar biverkningar fått en tillräckligt stor biologisk dos och därmed också effekt mot sjukdomen. Detta

betyder dock inte att man enligt denna studie kan avstå helt från vinkristin behandlingen från början.

Det är vanligare med autoimmun sjukdom hos DLBCL patienter än hos normalbefolkningen och framför allt gäller detta kvinnor. För kvinnor som har DLBCL och någon av de autoimmuna sjukdomar som är vanligast vid DLBCL verkar det dessutom gå sämre än för övriga DLBCL. Detta är ett viktigt observandum som bör studeras ytterligare och om det bekräftas kan denna patientgrupp behöva individuellt anpassad behandling. Hos patienter med autoimmuna sjukdomar förekom fler med kraftig påverkan på immunförsvaret efter första behandlingen och man bör överväga intensifierad, skyddande behandling mot infektioner hos dessa individer.

I en oselekterad grupp av DLBCL patienter kunde vi inte bekräfta att de som uppnår EFS24 har samma förväntade överlevnad som en normalbefolkning annat än för patienter <60 år. De som uppnår EFS24 har en hög dödlighet i hjärt-kärl sjukdom. Nuvarande svenska riktlinjer rekommenderar uppföljning i två år. Man bör överväga förlängd kontroll, framför allt gällande hjärt-kärlsjukdom, för de över 60 år.

Två proteiner i blodet tagna före start av behandling, SPON-1 och IL-1RT1, var associerade till existerande respektive nytillkommen hjärt-kärlsjukdom hos DLBCL patienter. Om detta kan bekräftas i större studier kan dessa eventuellt användas som riskmarkörer inför beslut om hjärttoxisk cytostatika kan ges eller bör sättas ut (seponeras). Jämfört med normalpopulationen och jämförelsekohorten har en stor andel av DLBCL patienterna i detta material existerande hjärt-kärlsjukdom. Detta fynd är inte beskrivet i aktuella studier och bör analyseras ytterligare i större material.

8 Acknowledgements

I would like to express my gratitude to all you wonderful people in my universe and in particular I want to thank;

Gunilla Enblad, my supervisor, for inviting me to her research and for always staying calm, supportive and believing in me in times of great pressure.

My co-supervisor, **Antonis Valachis**. Like a modern Sisyfos you have rolled me up the hill of knowledge and understanding so many times, just to face the question again; “but what about….”.

My co-supervisor,**Gustaf Hedström,** for hard work with statistics and even harder work making it understandable.

To all my co-authors, for wise and nice advise and feedback.

Special thanks to **Amal**, queen of dead-lines and night-work, **Eva B** for patient and meticulous writing when I almost gave in and **Eva F** for conjuring structured data and figures out of Excel chaos and giving priority to my work and my bunch of questions.

My dear friends and fellows in “the **Uppsala-Örebro lymphoma community**” including the hematopathology department at Akademiska. To work with you is indeed a favour.

Colleagues and staff at **Mälarsjukhuset** for years of hard work, cooperation, laughs and tears together. I miss you.

My student friends from medical school; ”**Bluff, Spark, Tork and Kvark**”. Without you I would not have passed my medical exam or worse ended up with the ”tipp-ex girls”.

My two sisters and best friends, **Mari** and **Kristin**. You are my greatest supporters and comforters. With you by my side I can endure anything and everything.

Kristin, special thanks for contribution with free, 24-7, data support in times of need.

My mother, **Ann-Charlott**. With your everlasting love embracing me since I was born I feel I have nothing to fear in the world.

My late father, **Björn.** You inspired me to be curious, fear-less and believe that anything is possible and so, here I am!

The **Falcons**, my BFF's. There is nowhere I would rather be than in your company anywhere in the world.

Lowe, **Linn** and **Vidar**, you are the meaning of my life and my greatest successes.

Ricard, for eternal love and "markservice". *"Högt över havet och bort emot land, stämmer frekvensen så räcker jag fram, plus når minus och lyckan är gjord, sanna mina ord".*

This work was supported by Centre for Clinical Research, Sormland, Uppsala University and by non-graduated researchers grants from Faculty of medicine, Uppsala University as well as grants from Erik, Karin och Gosta Selanders stiftelse.

9 References

[1] Campo E, Swerdlow SH, Harris NL, et al. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117:5019–5032.

[2] Székely E, Hagberg O, Arnljots K, et al. Improvement in survival of diffuse large B-cell lymphoma in relation to age, gender, International Prognostic Index and extranodal presentation: a population based Swedish Lymphoma Registry study. Leukemia & Lymphoma. 2014;55:1838–1843.

[3] Smith A, Crouch S, Lax S, et al. Lymphoma incidence, survival and prevalence 2004–2014: sub-type analyses from the UK's Haematological Malignancy Research Network. British Journal of Cancer. 2015;112:1575–1584.

[4] Sant M, Allemani C, Tereanu C, et al. Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. Blood. 2010;116:3724–3734.

[5] Cerhan JR, Kricker A, Paltiel O, et al. Medical history, lifestyle, family history, and occupational risk factors for diffuse large B-cell lymphoma: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. J Natl Cancer Inst Monographs. 2014;2014:15–25.

[6] Goldin LR, Landgren O. Autoimmunity and lymphomagenesis. International Journal of Cancer. 2009;124:1497–1502.

[7] Altieri A, Bermejo JL, Hemminki K. Familial risk for non-Hodgkin lymphoma and other lymphoproliferative malignancies by histopathologic subtype: the Swedish Family-Cancer Database. Blood. 2005;106:668–672.

[8] Anderson LA, Gadalla S, Morton LM, et al. Population-based study of autoimmune conditions and the risk of specific lymphoid malignancies. International Journal of Cancer. 2009;125:398–405.

[9] Cuttner J, Spiera H, Troy K, et al. Autoimmune disease is a risk factor for the development of non-Hodgkin's lymphoma. J Rheumatol. 2005;32:1884–1887.

[10] Ekstrom Smedby K, Vajdic CM, Falster M, et al. Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph Consortium. Blood. 2008;111:4029–4038.

[11] Morton LM, Slager SL, Cerhan JR, et al. Etiologic Heterogeneity Among Non-Hodgkin Lymphoma Subtypes: The InterLymph Non-Hodgkin Lymphoma Subtypes Project. JNCI Monographs. 2014;2014:130–144.

[12] Smedby KE, Askling J, Mariette X, et al. Autoimmune and inflammatory disorders and risk of malignant lymphomas - an update. Journal of Internal Medicine. 2008;264:514–527.

[13] Vanura K, Späth F, Gleiss A, et al. Prevalence and clinical impact of autoimmune diseases and chronic infections in malignant lymphomas at diagnosis. Annals of Hematology. 2011;90:947–954.

[14] Wang SS, Vajdic CM, Linet MS, et al. Associations of Non-Hodgkin Lymphoma (NHL) Risk With Autoimmune Conditions According to Putative NHL Loci. American Journal of Epidemiology. 2015;181:406–421.
[15] Quinlan SC, Morton LM, Pfeiffer RM, et al. Increased Risk for Lymphoid and Myeloid Neoplasms in Elderly Solid-Organ Transplant Recipients. Cancer Epidemiology Biomarkers & Prevention. 2010;19:1229–1237.
[16] Clarke CA, Morton LM, Lynch C, et al. Risk of lymphoma subtypes after solid organ transplantation in the United States. Br J Cancer. 2013;109:280–288.
[17] Coté TR, Biggar RJ, Rosenberg PS, et al. Non-Hodgkin's lymphoma among people with AIDS: incidence, presentation and public health burden. AIDS/Cancer Study Group. Int J Cancer. 1997;73:645–650.
[18] Dal Maso L, Franceschi S. Epidemiology of non-Hodgkin lymphomas and other haemolymphopoietic neoplasms in people with AIDS. Lancet Oncol. 2003;4:110–119.
[19] Larsson SC, Wolk A. Body mass index and risk of non-Hodgkin's and Hodgkin's lymphoma: a meta-analysis of prospective studies. Eur J Cancer. 2011;47:2422–2430.
[20] Hidayat K, Li H-J, Shi B-M. Anthropometric factors and non-Hodgkin's lymphoma risk: systematic review and meta-analysis of prospective studies. Crit Rev Oncol Hematol. 2018;129:113–123.
[21] Chattopadhyay S, Sud A, Zheng G, et al. Second primary cancers in non-Hodgkin lymphoma: Bidirectional analyses suggesting role for immune dysfunction. Int J Cancer. 2018;143:2449–2457.
[22] Natkunam Y. The biology of the germinal center. Hematology Am Soc Hematol Educ Program. 2007;210–215.
[23] Pasqualucci L. Molecular pathogenesis of germinal center-derived B cell lymphomas. Immunological Reviews. 2019;288:240–261.
[24] Basso K, Dalla-Favera R. Germinal centres and B cell lymphomagenesis. Nat Rev Immunol. 2015;15:172–184.
[25] Küppers R. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer. 2005;5:251–262.
[26] Li S, Young KH, Medeiros LJ. Diffuse large B-cell lymphoma. Pathology. 2018;50:74–87.
[27] Sabattini E, Bacci F, Sagramoso C, et al. WHO classification of tumours of haematopoietic and lymphoid tissues in 2008: an overview. Pathologica. 2010;102:83–87.
[28] Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127:2375–2390.
[29] Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403:503–511.
[30] Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:1937–1947.
[31] Rosenwald A, Staudt LM. Gene Expression Profiling of Diffuse Large B-Cell Lymphoma. Leukemia & Lymphoma. 2003;44:S41–S47.

[32] Visco C, Li Y, Xu-Monette ZY, et al. Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Leukemia. 2012;26:2103–2113.
[33] Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol. 2012;30:3452–3459.
[34] Oki Y, Noorani M, Lin P, et al. Double hit lymphoma: the MD Anderson Cancer Center clinical experience. Br J Haematol. 2014;166:891–901.
[35] Perry AM, Alvarado-Bernal Y, Laurini JA, et al. MYC and BCL2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with rituximab. Br J Haematol. 2014;165:382–391.
[36] Horn H, Ziepert M, Becher C, et al. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood. 2013;121:2253–2263.
[37] Hu S, Xu-Monette ZY, Tzankov A, et al. MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. Blood. 2013;121:4021–4031; quiz 4250.
[38] Hedström G, Hagberg O, Jerkeman M, et al. The impact of age on survival of diffuse large B-cell lymphoma - a population-based study. Acta Oncol. 2015;54:916–923.
[39] Hedström G, Peterson S, Berglund M, et al. Male gender is an adverse risk factor only in young patients with diffuse large B-cell lymphoma – a Swedish population-based study. Acta Oncologica. 2015;54:924–932.
[40] Horesh N, Horowitz NA. Does gender matter in non-hodgkin lymphoma? Differences in epidemiology, clinical behavior, and therapy. Rambam Maimonides Med J. 2014;5:e0038.
[41] Sarkozy C, Mounier N, Delmer A, et al. Impact of BMI and Gender on Outcomes in DLBCL Patients Treated with R-CHOP: A Pooled Study from the LYSA. Lymphoma. 2014;2014:1–12.
[42] Pfreundschuh M, Muller C, Zeynalova S, et al. Suboptimal dosing of rituximab in male and female patients with DLBCL. Blood. 2014;123:640–646.
[43] Carbone PP, Kaplan HS, Musshoff K, et al. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res. 1971;31:1860–1861.
[44] Lister TA, Crowther D, Sutcliffe SB, et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. J Clin Oncol. 1989;7:1630–1636.
[45] International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med. 1993;329:987–994.
[46] Ziepert M, Hasenclever D, Kuhnt E, et al. Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. J Clin Oncol. 2010;28:2373–2380.
[47] Schmitz N, Zeynalova S, Nickelsen M, et al. CNS International Prognostic Index: A Risk Model for CNS Relapse in Patients With Diffuse Large B-Cell Lymphoma Treated With R-CHOP. J Clin Oncol. 2016;34:3150–3156.

[48] Zhou Z, Sehn LH, Rademaker AW, et al. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood. 2014;123:837–842.
[49] Sehn LH, Berry B, Chhanabhai M, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood. 2007;109:1857–1861.
[50] Abdulla M, Laszlo S, Triumf J, et al. Core needle biopsies for the diagnosis of diffuse large B-cell lymphoma - a great concern for research. Acta Oncol. 2017;56:106–109.
[51] Tilly H, Gomes da Silva M, Vitolo U, et al. Diffuse large B-cell lymphoma (DLBCL): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2015;26:v116–v125.
[52] Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol. 2014;32:3059–3068.
[53] Barrington SF, Mikhaeel NG, Kostakoglu L, et al. Role of imaging in the staging and response assessment of lymphoma: consensus of the International Conference on Malignant Lymphomas Imaging Working Group. J Clin Oncol. 2014;32:3048–3058.
[54] Khan AB, Barrington SF, Mikhaeel NG, et al. PET-CT staging of DLBCL accurately identifies and provides new insight into the clinical significance of bone marrow involvement. Blood. 2013;122:61–67.
[55] Pelosi E, Penna D, Douroukas A, et al. Bone marrow disease detection with FDG-PET/CT and bone marrow biopsy during the staging of malignant lymphoma: results from a large multicentre study. Q J Nucl Med Mol Imaging. 2011;55:469–475.
[56] Cooper GS, Bynum MLK, Somers EC. Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases. Journal of Autoimmunity. 2009;33:197–207.
[57] Ansell P, Simpson J, Lightfoot T, et al. Non-Hodgkin lymphoma and autoimmunity: Does gender matter? International Journal of Cancer. 2011;129:460–466.
[58] Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol. 2014;35:347–369.
[59] Youinou P, Pers J-O, Gershwin ME, et al. Geo-epidemiology and autoimmunity. Journal of Autoimmunity. 2010;34:J163–J167.
[60] Engels EA, Parsons R, Besson C, et al. Comprehensive Evaluation of Medical Conditions Associated with Risk of Non-Hodgkin Lymphoma using Medicare Claims ("MedWAS"). Cancer Epidemiology Biomarkers & Prevention. 2016;25:1105–1113.
[61] Hemminki K, Liu X, Ji J, et al. Origin of B-Cell Neoplasms in Autoimmune Disease. PLoS ONE. 2016;11:e0158360.
[62] Kleinstern G, Maurer MJ, Liebow M, et al. History of autoimmune conditions and lymphoma prognosis. Blood Cancer J. 2018;8:73.
[63] Kleinstern G, Averbuch M, Abu Seir R, et al. Presence of autoimmune disease affects not only risk but also survival in patients with B-cell non-Hodgkin lymphoma. Hematol Oncol. 2018;36:457–462.
[64] Koff JL, Rai A, Flowers CR. Characterizing Autoimmune Disease-associated Diffuse Large B-cell Lymphoma in a SEER-Medicare Cohort. Clin Lymphoma Myeloma Leuk. 2018;18:e115–e121.

[65] Baecklund E, Smedby KE, Sutton L-A, et al. Lymphoma development in patients with autoimmune and inflammatory disorders – What are the driving forces? Seminars in Cancer Biology. 2014;24:61–70.
[66] Baecklund E, Iliadou A, Askling J, et al. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. Arthritis & Rheumatism. 2006;54:692–701.
[67] Baecklund E, Backlin C, Iliadou A, et al. Characteristics of diffuse large B cell lymphomas in rheumatoid arthritis. Arthritis & Rheumatism. 2006;54:3774–3781.
[68] Mikuls TR, Endo JO, Puumala SE, et al. Prospective Study of Survival Outcomes in Non-Hodgkin's Lymphoma Patients With Rheumatoid Arthritis. Journal of Clinical Oncology. 2006;24:1597–1602.
[69] Simard JF, Baecklund F, Chang ET, et al. Lifestyle factors, autoimmune disease and family history in prognosis of non-hodgkin lymphoma overall and subtypes. Int J Cancer. 2013;132:2659–2666.
[70] Gabriel SE, Michaud K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. Arthritis Res Ther. 2009;11:229.
[71] Lee YH, Choi SJ, Ji JD, et al. Overall and cause-specific mortality in systemic lupus erythematosus: an updated meta-analysis. Lupus. 2016;25:727–734.
[72] Gazitt T, Loughran TP. Chronic neutropenia in LGL leukemia and rheumatoid arthritis. Hematology Am Soc Hematol Educ Program. 2017;2017:181–186.
[73] Zheng P, Chang X, Lu Q, et al. Cytopenia and autoimmune diseases: a vicious cycle fueled by mTOR dysregulation in hematopoietic stem cells. J Autoimmun. 2013;41:182–187.
[74] Fragoulis GE, Paterson C, Gilmour A, et al. Neutropaenia in early rheumatoid arthritis: frequency, predicting factors, natural history and outcome. RMD Open. 2018;4:e000739.
[75] Yi PI, Coleman M, Saltz L, et al. Chemotherapy for large cell lymphoma: a status update. Semin Oncol. 1990;17:60–73.
[76] DeVita VT, Canellos GP, Chabner B, et al. Advanced diffuse histiocytic lymphoma, a potentially curable disease. Lancet. 1975;1:248–250.
[77] Povirk LF, Shuker DE. DNA damage and mutagenesis induced by nitrogen mustards. Mutat Res. 1994;318:205–226.
[78] Corremans R, Adão R, De Keulenaer GW, et al. Update on pathophysiology and preventive strategies of anthracycline-induced cardiotoxicity. Clin Exp Pharmacol Physiol. 2019;46:204–215.
[79] Carozzi VA, Canta A, Chiorazzi A. Chemotherapy-induced peripheral neuropathy: What do we know about mechanisms? Neuroscience Letters. 2015;596:90–107.
[80] Martino E, Casamassima G, Castiglione S, et al. Vinca alkaloids and analogues as anti-cancer agents: Looking back, peering ahead. Bioorg Med Chem Lett. 2018;28:2816–2826.
[81] Douer D. Efficacy and Safety of Vincristine Sulfate Liposome Injection in the Treatment of Adult Acute Lymphocytic Leukemia. Oncologist. 2016;21:840–847.
[82] Pathak P, Hess R, Weiss MA. Liposomal vincristine for relapsed or refractory Ph-negative acute lymphoblastic leukemia: a review of literature. Therapeutic Advances in Hematology. 2014;5:18–24.

[83] Coiffier B, Thieblemont C, Van Den Neste E, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010;116:2040–2045.

[84] Sehn LH, Donaldson J, Chhanabhai M, et al. Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. J Clin Oncol. 2005;23:5027–5033.

[85] Pfreundschuh M, Kuhnt E, Trümper L, et al. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. The Lancet Oncology. 2011;12:1013–1022.

[86] Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:235–242.

[87] Gutiérrez A, Bento L, Bautista-Gili AM, et al. Differential Impact of Relative Dose-Intensity Reductions in Diffuse Large B-Cell Lymphoma Treated with R-CHOP21 or R-CHOP14. PLoS ONE. 2015;10:e0123978.

[88] Hirakawa T, Yamaguchi H, Yokose N, et al. Importance of maintaining the relative dose intensity of CHOP-like regimens combined with rituximab in patients with diffuse large B-cell lymphoma. Ann Hematol. 2010;89:897–904.

[89] Terada Y, Nakamae H, Aimoto R, et al. Impact of relative dose intensity (RDI) in CHOP combined with rituximab (R-CHOP) on survival in diffuse large B-cell lymphoma. J Exp Clin Cancer Res. 2009;28:116.

[90] Utsu Y, Takaishi K, Inagaki S, et al. Influence of dose reduction of vincristine in R-CHOP on outcomes of diffuse large B cell lymphoma. Ann Hematol. 2016;95:41–47.

[91] Yamaguchi H, Hirakawa T, Inokuchi K. Importance of Relative Dose Intensity in Chemotherapy for Diffuse Large B-Cell Lymphoma. J Clin Exp Hematopathol. 2011;51:1–5.

[92] Cunningham D, Hawkes EA, Jack A, et al. Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles. Lancet. 2013;381:1817–1826.

[93] Delarue R, Tilly H, Mounier N, et al. Dose-dense rituximab-CHOP compared with standard rituximab-CHOP in elderly patients with diffuse large B-cell lymphoma (the LNH03-6B study): a randomised phase 3 trial. Lancet Oncol. 2013;14:525–533.

[94] Greb A, Bohlius J, Schiefer D, et al. High-dose chemotherapy with autologous stem cell transplantation in the first line treatment of aggressive non-Hodgkin lymphoma (NHL) in adults. Cochrane Database Syst Rev. 2008;CD004024.

[95] Récher C, Coiffier B, Haioun C, et al. Intensified chemotherapy with ACVBP plus rituximab versus standard CHOP plus rituximab for the treatment of diffuse large B-cell lymphoma (LNH03-2B): an open-label randomised phase 3 trial. Lancet. 2011;378:1858–1867.

[96] Wilson WH, Jung S-H, Porcu P, et al. A Cancer and Leukemia Group B multi-center study of DA-EPOCH-rituximab in untreated diffuse large B-cell lymphoma with analysis of outcome by molecular subtype. Haematologica. 2012;97:758–765.
[97] Stiff PJ, Unger JM, Cook JR, et al. Autologous Transplantation as Consolidation for Aggressive Non-Hodgkin's Lymphoma. N Engl J Med. 2013;369:1681–1690.
[98] Epperla N, Hamadani M, Reljic T, et al. Upfront autologous hematopoietic stem cell transplantation consolidation for patients with aggressive B-cell lymphomas in first remission in the rituximab era: A systematic review and meta-analysis. Cancer. 2019;125:4417–4425.
[99] Wästerlid T, Hartman L, Székely E, et al. Impact on survival of addition of etoposide to primary chemotherapy in diffuse large B-cell lymphoma: a Swedish Lymphoma Registry study: Etoposide addition in DLBCL treatment. Hematological Oncology. 2017;35:151–157.
[100] Gang AO, Strøm C, Pedersen M, et al. R-CHOEP-14 improves overall survival in young high-risk patients with diffuse large B-cell lymphoma compared with R-CHOP-14. A population-based investigation from the Danish Lymphoma Group. Annals of Oncology. 2012;23:147–153.
[101] Davies A. Tailoring front-line therapy in diffuse large B-cell lymphoma: who should we treat differently? Hematology Am Soc Hematol Educ Program. 2017;2017:284–294.
[102] Tai WM, Chung J, Tang PL, et al. Central nervous system (CNS) relapse in diffuse large B cell lymphoma (DLBCL): pre- and post-rituximab. Ann Hematol. 2011;90:809–818.
[103] El-Galaly TC, Villa D, Michaelsen TY, et al. The number of extranodal sites assessed by PET/CT scan is a powerful predictor of CNS relapse for patients with diffuse large B-cell lymphoma: An international multicenter study of 1532 patients treated with chemoimmunotherapy. Eur J Cancer. 2017;75:195–203.
[104] Villa D, Connors JM, Shenkier TN, et al. Incidence and risk factors for central nervous system relapse in patients with diffuse large B-cell lymphoma: the impact of the addition of rituximab to CHOP chemotherapy. Ann Oncol. 2010;21:1046–1052.
[105] Boehme V, Zeynalova S, Kloess M, et al. Incidence and risk factors of central nervous system recurrence in aggressive lymphoma--a survey of 1693 patients treated in protocols of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). Ann Oncol. 2007;18:149–157.
[106] Melén CM, Enblad G, Sonnevi K, et al. Chemotherapeutic intensity and survival differences in young patients with diffuse large B-cell lymphoma: a Swedish Lymphoma Registry study. Br J Haematol. 2016;175:614–622.
[107] Holte H, Leppä S, Björkholm M, et al. Dose-densified chemoimmunotherapy followed by systemic central nervous system prophylaxis for younger high-risk diffuse large B-cell/follicular grade 3 lymphoma patients: results of a phase II Nordic Lymphoma Group study. Ann Oncol. 2013;24:1385–1392.
[108] Savage KJ. Secondary CNS relapse in diffuse large B-cell lymphoma: defining high-risk patients and optimization of prophylaxis strategies. Hematology Am Soc Hematol Educ Program. 2017;2017:578–586.
[109] Abramson JS, Hellmann M, Barnes JA, et al. Intravenous methotrexate as central nervous system (CNS) prophylaxis is associated with a low risk of CNS recurrence in high-risk patients with diffuse large B-cell lymphoma. Cancer. 2010;116:4283–4290.

[110] Leppä S, Jørgensen J, Tierens A, et al. Patients with high-risk DLBCL benefit from dose-dense immunochemotherapy combined with early systemic CNS prophylaxis. Blood Adv. 2020;4:1906–1915.
[111] Poeschel V, Held G, Ziepert M, et al. Four versus six cycles of CHOP chemotherapy in combination with six applications of rituximab in patients with aggressive B-cell lymphoma with favourable prognosis (FLYER): a randomised, phase 3, non-inferiority trial. Lancet. 2019;394:2271–2281.
[112] Held G, Zeynalova S, Murawski N, et al. Impact of rituximab and radiotherapy on outcome of patients with aggressive B-cell lymphoma and skeletal involvement. J Clin Oncol. 2013;31:4115–4122.
[113] Held G, Murawski N, Ziepert M, et al. Role of Radiotherapy to Bulky Disease in Elderly Patients With Aggressive B-Cell Lymphoma. JCO. 2014;32:1112–1118.
[114] Pfreundschuh M, Ho AD, Cavallin-Stahl E, et al. Prognostic significance of maximum tumour (bulk) diameter in young patients with good-prognosis diffuse large-B-cell lymphoma treated with CHOP-like chemotherapy with or without rituximab: an exploratory analysis of the MabThera International Trial Group (MInT) study. Lancet Oncol. 2008;9:435–444.
[115] Vitolo U, Seymour JF, Martelli M, et al. Extranodal diffuse large B-cell lymphoma (DLBCL) and primary mediastinal B-cell lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2016;27:v91–v102.
[116] Aapro MS, Bohlius J, Cameron DA, et al. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. European Journal of Cancer. 2011;47:8–32.
[117] Smith TJ, Bohlke K, Lyman GH, et al. Recommendations for the Use of WBC Growth Factors: American Society of Clinical Oncology Clinical Practice Guideline Update. Journal of Clinical Oncology. 2015;33:3199–3212.
[118] Lai GGY, Lim S-T, Tao M, et al. Late-onset neutropenia following RCHOP chemotherapy in diffuse large B-cell lymphoma. Am J Hematol. 2009;84:414–417.
[119] Petta I, Peene I, Elewaut D, et al. Risks and benefits of corticosteroids in arthritic diseases in the clinic. Biochem Pharmacol. 2019;165:112–125.
[120] Shan K, Lincoff AM, Young JB. Anthracycline-induced cardiotoxicity. Ann Intern Med. 1996;125:47–58.
[121] Zamorano JL, Lancellotti P, Rodriguez Muñoz D, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur J Heart Fail. 2017;19:9–42.
[122] Vejpongsa P, Yeh ETH. Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities. J Am Coll Cardiol. 2014;64:938–945.
[123] Wojtacki J, Lewicka-Nowak E, Leśniewski-Kmak K. Anthracycline-induced cardiotoxicity: clinical course, risk factors, pathogenesis, detection and prevention--review of the literature. Med Sci Monit. 2000;6:411–420.
[124] Bernaba BN, Chan JB, Lai CK, et al. Pathology of late-onset anthracycline cardiomyopathy. Cardiovasc Pathol. 2010;19:308–311.
[125] Nebigil CG, Désaubry L. Updates in Anthracycline-Mediated Cardiotoxicity. Front Pharmacol. 2018;9:1262.

[126] Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments. Nat Rev Cardiol. 2015;12:547–558.
[127] van Dalen EC, van der Pal HJ, Kremer LC. Different dosage schedules for reducing cardiotoxicity in people with cancer receiving anthracycline chemotherapy. Cochrane Gynaecological, Neuro-oncology and Orphan Cancer Group, editor. Cochrane Database of Systematic Reviews [Internet]. 2016 [cited 2020 Apr 3]; Available from: http://doi.wiley.com/10.1002/14651858.CD005008.pub4.
[128] Kalam K, Marwick TH. Role of cardioprotective therapy for prevention of cardiotoxicity with chemotherapy: a systematic review and meta-analysis. Eur J Cancer. 2013;49:2900–2909.
[129] van Dalen EC, Caron HN, Dickinson HO, et al. Cardioprotective interventions for cancer patients receiving anthracyclines. Cochrane Database Syst Rev. 2011;CD003917.
[130] Rashidi A, Oak E, Carson KR, et al. Outcomes with R-CEOP for R-CHOP-ineligible patients with diffuse large B-cell lymphoma are highly dependent on cell of origin defined by Hans criteria. Leukemia & Lymphoma. 2016;57:1191–1193.
[131] De Lena M, Maiello E, Lorusso V, et al. Comparison of CHOP-B vs CEOP-B in “poor prognosis” non-Hodgkin’s lymphomas. A randomized trial. Med Oncol Tumor Pharmacother. 1989;6:163–169.
[132] Moccia AA, Schaff K, Hoskins P, et al. R-CHOP with Etoposide Substituted for Doxorubicin (R-CEOP): Excellent Outcome in Diffuse Large B Cell Lymphoma for Patients with a Contraindication to Anthracyclines. Blood. 2009;114:408–408.
[133] Tward JD, Wendland MMM, Shrieve DC, et al. The risk of secondary malignancies over 30 years after the treatment of non-Hodgkin lymphoma. Cancer. 2006;107:108–115.
[134] Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin’s lymphomas. NCI Sponsored International Working Group. J Clin Oncol. 1999;17:1244.
[135] Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25:579–586.
[136] Maurer MJ, Ghesquières H, Jais J-P, et al. Event-free survival at 24 months is a robust end point for disease-related outcome in diffuse large B-cell lymphoma treated with immunochemotherapy. J Clin Oncol. 2014;32:1066–1073.
[137] Maurer MJ, Habermann TM, Shi Q, et al. Progression-free survival at 24 months (PFS24) and subsequent outcome for patients with diffuse large B-cell lymphoma (DLBCL) enrolled on randomized clinical trials. Annals of Oncology. 2018;29:1822–1827.
[138] Jakobsen LH, Bøgsted M, Brown P de N, et al. Minimal Loss of Lifetime for Patients With Diffuse Large B-Cell Lymphoma in Remission and Event Free 24 Months After Treatment: A Danish Population-Based Study. J Clin Oncol. 2017;35:778–784.
[139] Srour L, Zheng YY, Gerrie AS, et al. EFS24 as a predictor of outcome in a population-based cohort of patients with DLBCL in British Columbia (BC). JCO. 2016;34:7569–7569.
[140] Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin’s lymphoma. N Engl J Med. 1995;333:1540–1545.

[141] Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol. 2010;28:4184–4190.
[142] Crump M, Kuruvilla J, Couban S, et al. Randomized comparison of gemcitabine, dexamethasone, and cisplatin versus dexamethasone, cytarabine, and cisplatin chemotherapy before autologous stem-cell transplantation for relapsed and refractory aggressive lymphomas: NCIC-CTG LY.12. J Clin Oncol. 2014;32:3490–3496.
[143] Ayers EC, Li S, Medeiros LJ, et al. Outcomes in patients with aggressive B-cell non-Hodgkin lymphoma after intensive frontline treatment failure. Cancer. 2020;126:293–303.
[144] Glass B, Hasenkamp J, Wulf G, et al. Rituximab after lymphoma-directed conditioning and allogeneic stem-cell transplantation for relapsed and refractory aggressive non-Hodgkin lymphoma (DSHNHL R3): an open-label, randomised, phase 2 trial. Lancet Oncol. 2014;15:757–766.
[145] Fenske TS, Ahn KW, Graff TM, et al. Allogeneic transplantation provides durable remission in a subset of DLBCL patients relapsing after autologous transplantation. Br J Haematol. 2016;174:235–248.
[146] Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. N Engl J Med. 2019;380:45–56.
[147] Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N Engl J Med. 2017;377:2531–2544.
[148] Mounier N, El Gnaoui T, Tilly H, et al. Rituximab plus gemcitabine and oxaliplatin in patients with refractory/relapsed diffuse large B-cell lymphoma who are not candidates for high-dose therapy. A phase II Lymphoma Study Association trial. Haematologica. 2013;98:1726–1731.
[149] Arcari A, Chiappella A, Spina M, et al. Safety and efficacy of rituximab plus bendamustine in relapsed or refractory diffuse large B-cell lymphoma patients: an Italian retrospective multicenter study. Leukemia & Lymphoma. 2016;57:1823–1830.
[150] Enblad G, Hagberg H, Glimelius B. Methyl-Gag, Ifosfamide, Methotrexate and Etoposide (Mime) as Salvage Therapy for Non-Hodgkin's Lymphomasa Swedish national prospective study. Acta Oncologica. 1996;35:165–170.
[151] Deeks ED. Polatuzumab Vedotin: First Global Approval. Drugs. 2019;79:1467–1475.
[152] Sehn LH, Herrera AF, Flowers CR, et al. Polatuzumab Vedotin in Relapsed or Refractory Diffuse Large B-Cell Lymphoma. JCO. 2020;38:155–165.
[153] Pettengell R, Coiffier B, Narayanan G, et al. Pixantrone dimaleate versus other chemotherapeutic agents as a single-agent salvage treatment in patients with relapsed or refractory aggressive non-Hodgkin lymphoma: a phase 3, multicentre, open-label, randomised trial. The Lancet Oncology. 2012;13:696–706.
[154] Pettengell R, Długosz-Danecka M, Andorsky D, et al. Pixantrone plus rituximab versus gemcitabine plus rituximab in patients with relapsed aggressive B-cell non-Hodgkin lymphoma not eligible for stem cell transplantation: a phase 3, randomized, multicentre trial (PIX306). Br J Haematol. 2020;188:240–248.
[155] Mondello P, Nowakowski GS. Treatment of Aggressive B Cell Lymphomas: Updates in 2019. Curr Hematol Malig Rep. 2020;15:225–234.

[156] Glimelius B, Melin B, Enblad G, et al. U-CAN: a prospective longitudinal collection of biomaterials and clinical information from adult cancer patients in Sweden. Acta Oncologica. 2018;57:187–194.
[157] Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS ONE. 2014;9:e95192.
[158] Lind L, Elmståhl S, Bergman E, et al. EpiHealth: a large population-based cohort study for investigation of gene-lifestyle interactions in the pathogenesis of common diseases. Eur J Epidemiol. 2013;28:189–197.
[159] Shih Y-H, Yang Y, Chang K-H, et al. Clinical features and outcome of lymphoma patients with pre-existing autoimmune diseases. Int J Rheum Dis. 2018;21:93–101.
[160] Bjoro T, Holmen J, Krüger O, et al. Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur J Endocrinol. 2000;143:639–647.
[161] Fu K, Weisenburger DD, Choi WWL, et al. Addition of Rituximab to Standard Chemotherapy Improves the Survival of Both the Germinal Center B-Cell–Like and Non–Germinal Center B-Cell–Like Subtypes of Diffuse Large B-Cell Lymphoma. Journal of Clinical Oncology. 2008;26:4587–4594.
[162] Löfström B, Backlin C, Sundström C, et al. A closer look at non-Hodgkin's lymphoma cases in a national Swedish systemic lupus erythematosus cohort: a nested case-control study. Ann Rheum Dis. 2007;66:1627–1632.
[163] van der Galiën HT, Hoogendoorn M, Kibbelaar RE, et al. Progression-free survival at 24 months (PFS24) and subsequent outcome for patients with diffuse large B-cell lymphoma (DLBCL) in the real-world setting. Annals of Oncology. 2019;30:151–152.
[164] Moser EC. Long-term risk of cardiovascular disease after treatment for aggressive non-Hodgkin lymphoma. Blood. 2006;107:2912–2919.
[165] Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. N Engl J Med. 1998;339:900–905.
[166] Cardinale D, Sandri MT, Martinoni A, et al. Left ventricular dysfunction predicted by early troponin I release after high-dose chemotherapy. J Am Coll Cardiol. 2000;36:517–522.
[167] Ekberg S, Jerkeman M, Andersson P-O, et al. Long-term survival and loss in expectancy of life in a population-based cohort of 7114 patients with diffuse large B-cell lymphoma. Am J Hematol. 2018;
[168] Klimczak-Tomaniak D, Bouwens E, Schuurman A-S, et al. Temporal patterns of macrophage- and neutrophil-related markers are associated with clinical outcome in heart failure patients. ESC Heart Fail. 2020;
[169] Stenemo M, Nowak C, Byberg L, et al. Circulating proteins as predictors of incident heart failure in the elderly: Circulating proteins as predictors of incident heart failure. Eur J Heart Fail. 2018;20:55–62.
[170] Dubin RF, Whooley M, Pico A, et al. Proteomic analysis of heart failure hospitalization among patients with chronic kidney disease: The Heart and Soul Study. Shimosawa T, editor. PLoS ONE. 2018;13:e0208042.
[171] Qian W, Zhao C, Li D, et al. Mechanism of interleukin-1 receptor antagonist protection against myocardial ischaemia/reperfusion-induced injury. Archives of Cardiovascular Diseases. 2018;111:545–554.
[172] Zhu J, Zhang J, Zhang L, et al. Interleukin-1 signaling mediates acute doxorubicin-induced cardiotoxicity. Biomedicine & Pharmacotherapy. 2011;65:481–485.

[173] Daugaard G, Lassen U, Bie P, et al. Natriuretic peptides in the monitoring of anthracycline induced reduction in left ventricular ejection fraction. European Journal of Heart Failure. 2005;7:87–93.
[174] Sawaya H, Sebag IA, Plana JC, et al. Early Detection and Prediction of Cardiotoxicity in Chemotherapy-Treated Patients. The American Journal of Cardiology. 2011;107:1375–1380.
[175] Khan MA, Hashim MJ, Mustafa H, et al. Global Epidemiology of Ischemic Heart Disease: Results from the Global Burden of Disease Study. Cureus [Internet]. 2020 [cited 2020 Aug 31]; Available from: https://www.cureus.com/articles/36728-global-epidemiology-of-ischemic-heart-disease-results-from-the-global-burden-of-disease-study.
[176] Larsson K, Mattsson M, Ebrahim F, et al. High prevalence and incidence of cardiovascular disease in chronic lymphocytic leukaemia: a nationwide population-based study. Br J Haematol [Internet]. 2020 [cited 2020 Aug 31];190. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/bjh.16859.
[177] Castillo JJ, Ingham RR, Reagan JL, et al. Obesity Is Associated With Increased Relative Risk of Diffuse Large B-Cell Lymphoma: A Meta-Analysis of Observational Studies. Clinical Lymphoma Myeloma and Leukemia. 2014;14:122–130.
[178] Sarkozy C, Camus V, Tilly H, et al. Body mass index and other anthropometric parameters in patients with diffuse large B-cell lymphoma: physiopathological significance and predictive value in the immunochemotherapy era. Leukemia & Lymphoma. 2015;56:1959–1968.
[179] Ferraro MP, Gimeno-Vazquez E, Subirana I, et al. Anthracycline-induced cardiotoxicity in diffuse large B-cell lymphoma: NT-proBNP and cardiovascular score for risk stratification. Eur J Haematol. 2019;ejh.13234.
[180] Limat S, Demesmay K, Voillat L, et al. Early cardiotoxicity of the CHOP regimen in aggressivenon-Hodgkin's lymphoma. Annals of Oncology. 2003;14:277–281.
[181] Hequet O, Le QH, Moullet I, et al. Subclinical Late Cardiomyopathy After Doxorubicin Therapy for Lymphoma in Adults. JCO. 2004;22:1864–1871.
[182] Lotrionte M, Biondi-Zoccai G, Abbate A, et al. Review and Meta-Analysis of Incidence and Clinical Predictors of Anthracycline Cardiotoxicity. The American Journal of Cardiology. 2013;112:1980–1984.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1682

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-420746

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020