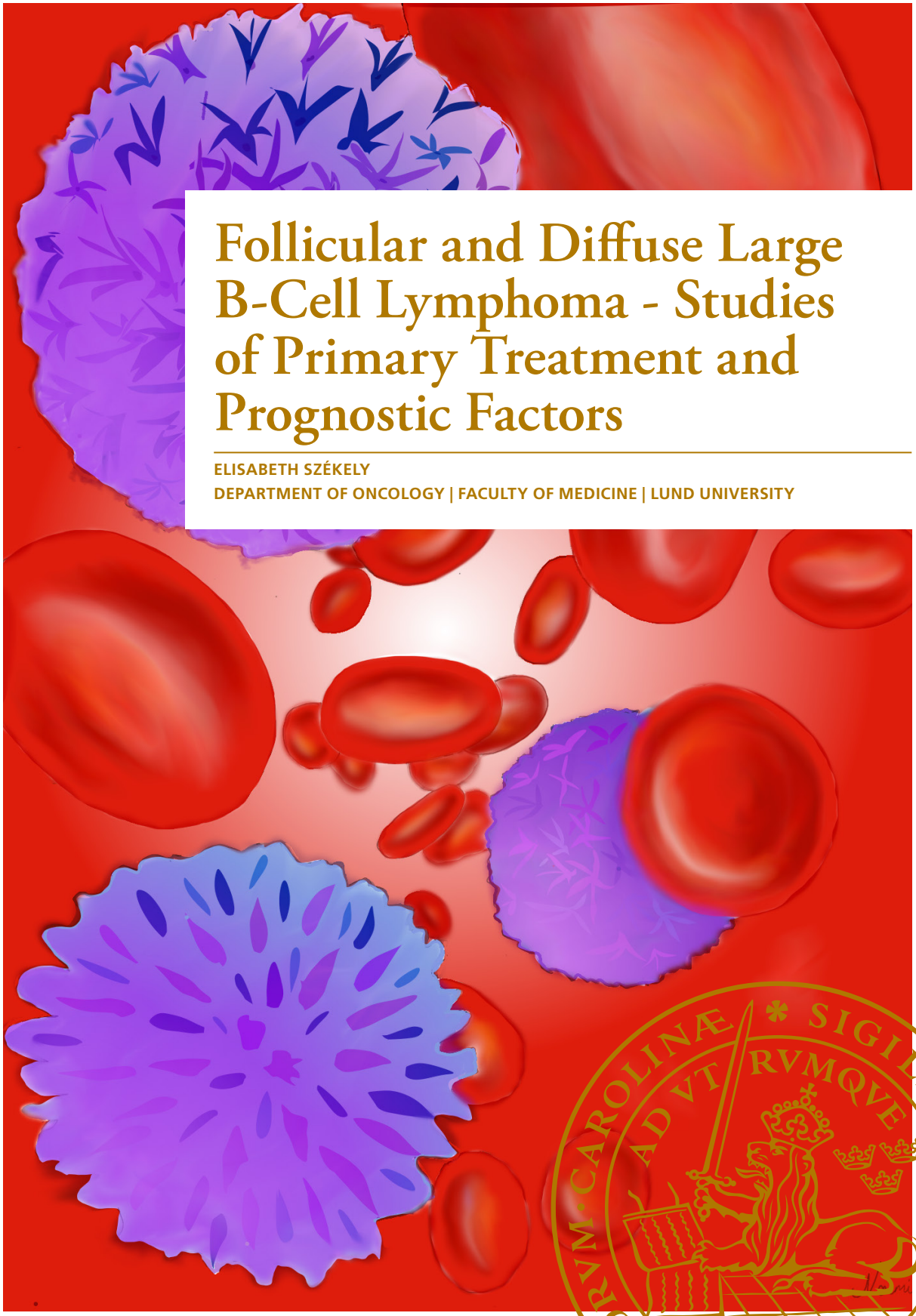

Follicular and Diffuse Large B-Cell Lymphoma - Studies of Primary Treatment and Prognostic Factors

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Elisabeth Székely, MD

DOCTORAL DISSERTATION

which, by due permission of the Faculty of Medicine, Lund University, Sweden, will be defended in the Lecture Hall, Radiotherapy Building, 3rd floor, Department of Oncology, Skåne University Hospital, Lund,
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Abstract: The most common types of B-cell lymphoma are diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL), which together make up 50% of all NHL (non-Hodgkin's lymphomas). DLBCL has a multifactorial aetiology. Patients with DLBCL are cured in approximately 50% of cases with adequate chemotherapy. FL is an indolent and usually incurable disease, and many different therapeutic approaches are used to maintain a good quality of life for a prolonged time. Data were obtained from the Swedish Lymphoma Registry and used to investigate prognostic factors, incidence, and treatment modalities of the population with DLBCL and FL in Sweden.

The first study demonstrated an increased incidence of DLBCL during the period 2000-2010, especially among men. Extranodal involvement of bone marrow, the central nervous system, lung, and bone was associated with inferior outcome, and male sex also had a negative impact on overall survival (OS). The OS of DLBCL patients has improved markedly during the past decade, most prominently in the age group 60-78 years, and in patients with good performance status. No significant improvement was seen in OS in patients with poor PS or in patients older than 78 years, indicating that attention should be directed towards high-risk patients and elderly patients, who may require specially tailored treatment.

In the second study, the efficacy of three chemotherapy regimens was evaluated in terms of the OS of DLBCL patients. The addition of etoposide to primary chemotherapy was found to be associated with improved survival in DLBCL patients up to 65 years of age, when corrected for prognistic factors, indicating that this may be an effective treatment option for this patient population.

In the third study, the season of diagnosis was found to affect OS in male patients with DLBCL, showing improved survival of patients diagnosed during the summer months. Possible explanations of these results may be the higher vitamin D levels during this period and/or the immunomodulatory effects of sunlight.

In the final study, the initial management of FL was investigated by comparing the efficacy of different treatment options. The OS of the patients managed with the watch-and-wait approach was not significantly different from that of patients treated immediately. Patients treated with single-agent rituximab had a similar OS to patients treated with chemoimmunotherapy.

The studies described in this thesis were performed using population-based datasets, and the results contribute to previously reported findings on prognostic factors and the improvement of OS of patients with DLBCL over the past decade. The improved outcome of DLBCL patients under 65 years of age with etoposide addition suggests a beneficial effect of this strategy. Elderly patients and patients with high-risk factors are in need of customized and more aggressive treatment including novel agents. For the optimal management of patients with FL, the watch-and-wait strategy and rituximab monotherapy should both be considered as first-line treatment options. Vitamin D, activated by sunlight, could play an important role in immunomodulation and the enhancement of the effect of chemoimmunotherapy.

Key words: diffuse large B-cell lymphoma, follicular lymphoma, prognostic factors, etoposide, chemotherapy regimen, seasonal variation, gender, watch and wait, rituximab monotherapy

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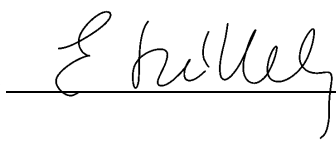
Signature  Date 2018-04-13

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It always seems impossible until it´s done

Nelson Mandela

To my gorgeous children, Anna, Balázs and Noémi

Content

Aims of this work

The general aim of the research presented in this thesis was to describe two large population-based cohorts of DLBCL and FL patients during the period 2000 to 2015, to evaluate risk factors, improvements in survival and to identify subgroups in need of novel treatment strategies. Studying population-based cohorts may contribute knowledge regarding the forms of treatment suitable for DLBCL and FL patients, and validate the general applicability of current therapeutic strategies in the whole patient population not fully represented in clinical trials.

The specific aims of the studies were:

- to describe a large population-based cohort of DLBCL patients from 2000 to 2010, in order to evaluate possible improvements in overall survival and to identify subgroups in need of novel treatment strategies (*Paper I*);
- to compare the chemotherapy regimens used in first-line treatment of patients with DLBCL in Sweden, and to evaluate the effect of the addition of etoposide to primary chemotherapy (*Paper II*);
- to investigate the outcome of patients with DLBCL and HL in relation to season of diagnosis (*Paper III*); and
- to describe a large population-based cohort of FL patients and compare the therapeutic efficacy of different therapeutic approaches (*Paper IV*).

List of papers

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

I. 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
Székely E, Hagberg O, Arnljots K, Jerkeman M

Leukemia & Lymphoma, 2014; 55(8): 1838-1843

II. Impact on survival of addition of etoposide to primary chemotherapy in diffuse large B-cell lymphoma: A Swedish Lymphoma Registry study
Wästerlid T, Hartman L, **Székely E**, Jerkeman M

Hematological Oncology, 2017; 35:151-157

III. Season of diagnosis is associated with overall survival in patients with diffuse large B-cell lymphoma but not with Hodgkin's lymphoma – A population-based Swedish Lymphoma Register study
Székely E, Lindén O, Peterson S, Jerkeman M

European Journal of Haematology, 2016; 97: 393-398

IV. First-line treatment of follicular lymphoma: A retrospective population-based study on data from the Swedish Lymphoma Registry
Székely E, Peterson S, Wahlin B, Jerkeman M

Manuscript

My contributions to the papers

Paper I.

I was responsible for collection and analysis of the data, and for writing the paper.

Paper II.

I participated in the design of the study, in analysing the data, and writing the paper.

Paper III.

I was responsible for the design of the study, the collection and analysis of the data, and writing the paper.

Paper IV.

I was responsible for the design of the study, the collection and analysis of the data, and writing the paper.

Abbreviations

ABC	activated B-cell
alloSCT	allogeneic stem cell transplant
ASCT	autologous stem cell transplant
BCR	B-cell receptor
CHOP	cyclophosphamide, doxorubicin, vincristine and prednisone
CHOEP	cyclophosphamide, doxorubicin, vincristine, etoposide and prednisone
COO	cell of origin
CR	complete remission
DA-EPOCH	dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin
DLBCL	diffuse large B-cell lymphoma
DE	double expressor
DH	double hit
EFS	event-free survival
FL	follicular lymphoma
FLIPI	Follicular Lymphoma International Prognostic Index
GC	germinal centre
GCB	germinal centre B-cell
GEP	gene expression profiling
HL	Hodgkin's lymphoma
HT	histological transformation
IFRT	involved field radiation therapy
IPI	International Prognostic Index
LDH	lactate dehydrogenase
NHL	non-Hodgkin's lymphoma
ORR	overall response rate
OS	overall survival
PET	positron emission tomography
PFS	progression-free survival
PR	partial remission
R-ACVBP	rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone
RB	rituximab-bendamustine
R-CVP	rituximab- cyclophosphamide, vincristine, prednisone
TH	triple hit
VDR	vitamin D receptor
WHO-PS	WHO-performance status

Background

Malignant lymphoma

Malignant lymphomas are a heterogeneous group of tumours of lymphoid origin in which cells of the lymphatic system become abnormal and start to proliferate uncontrollably. Non-Hodgkin's lymphomas (NHLs) are derived from B-lymphocytes, T-lymphocytes and natural killer cells. B-cell lymphomas comprise over 85% of NHLs. The two most common subtypes, diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL), account for over 50% of all NHLs. Over 70 different entities of lymphoid malignancies have been identified based on the results of histopathological examination, immunohistochemical staining, gene expression profiling (GEP) and clinical characteristics. The classification of NHL has been modified several times as new scientific and clinical knowledge become available. The 4th edition of the World Health Organization (WHO) Classification has recently been revised and updated.[1,2] The correct identification of the lymphoma subtype is extremely important for planning patient care.

Clinically, malignant lymphomas are divided into indolent, aggressive or very aggressive lymphomas. FL is the most common form of indolent lymphoma, and accounts for approximately 10-20% of all lymphomas in Western world. Indolent lymphomas are generally slowly progressive but not curable. Aggressive and very aggressive lymphomas are potentially curable, but without modern treatment show rapid clinical progression. DLBCL is an aggressive lymphoma, and some subtypes of DLBCL have a very aggressive course.

The incidence of NHL is increasing steadily. During the 1970s and 1980s, the rate of increase was 3-4% per year, which stabilized in the 1990s at an annual rate of increase of 1-2%. This rise has been observed particularly in those aged more than 55 years and among men.[3]

Numerous infectious agents, various occupational, environmental and chemical agents and immunosuppression have been identified and analysed as risk factors for NHL. Malignant lymphoma often arises as a result of chronic immune stimulation or immunoregulatory imbalance.

Diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma is the most common subtype of lymphoma and over 80% of all aggressive lymphomas.[4] DLBCL is associated with an aggressive natural history and the median survival is less than one year in untreated patients.

The incidence of DLBCL in Western countries is approximately 4 to 7 per 100,000 person-years, corresponding to approximately 30% of all malignant lymphomas. The incidence of DLBCL increased up to the year 2000, especially among men.[4,5] The reason for this increase may be due to the increase in the median age of the population, more sensitive diagnostic methods, improvements in cancer reporting, changes in classification systems and the increase in DLBCL associated with human immunodeficiency virus (HIV). The median age at diagnosis is 70 years and it is slightly more common in males.

Aetiology

The results of several studies suggest that DLBCL has a multifactorial aetiology. Factors associated with increasing risk of DLBCL are B-cell activating auto-immune diseases, seropositivity for hepatitis C virus, first-degree family history of NHL, high BMI as a young adult, and employment as a vegetable farmer or hairdresser.[6] In patients who have undergone a solid organ transplant or have HIV/AIDS, the risk of lymphoma is thought to be related to infection with, or reactivation of, the Epstein-Barr virus.[3] Findings from several epidemiological studies suggest that chronic (continuous) exposure to the sun is associated with a reduced risk of colorectal, breast and prostate cancer and NHL.[7] Most studies on the effects of sunlight have focused on the seasonal variation in vitamin D levels. However, some previously published studies[8-10] have failed to find evidence supporting the hypothesis that an elevated level of vitamin D is associated with a reduced risk of NHL.

Cell of origin

DLBCLs are divided into two main subgroups based on GEP: germinal centre B-cell-like- (GCB) and activated B-cell like- (ABC) DLBCL. These molecular phenotypes have prognostic value; (ABC) DLBCL having a more aggressive clinical course and unfavourable outcome than (GCB) DLBCL.[11] Germinal centres consist of a dark zone, including highly proliferating B-cells that undergo immunoglobulin somatic hypermutation, and a light zone, where the B-cells are selected based on their affinity for the antigen and perform class-switch recombination.[12,13] (Figure 1).

FL and the GCB-like subtype of DLBCL resemble light zone B-cells, while ABC-like DLBCLs are derived from GC cells arrested during the early stage of post-GC plasma-cell differentiation.[13] (Figure 1)

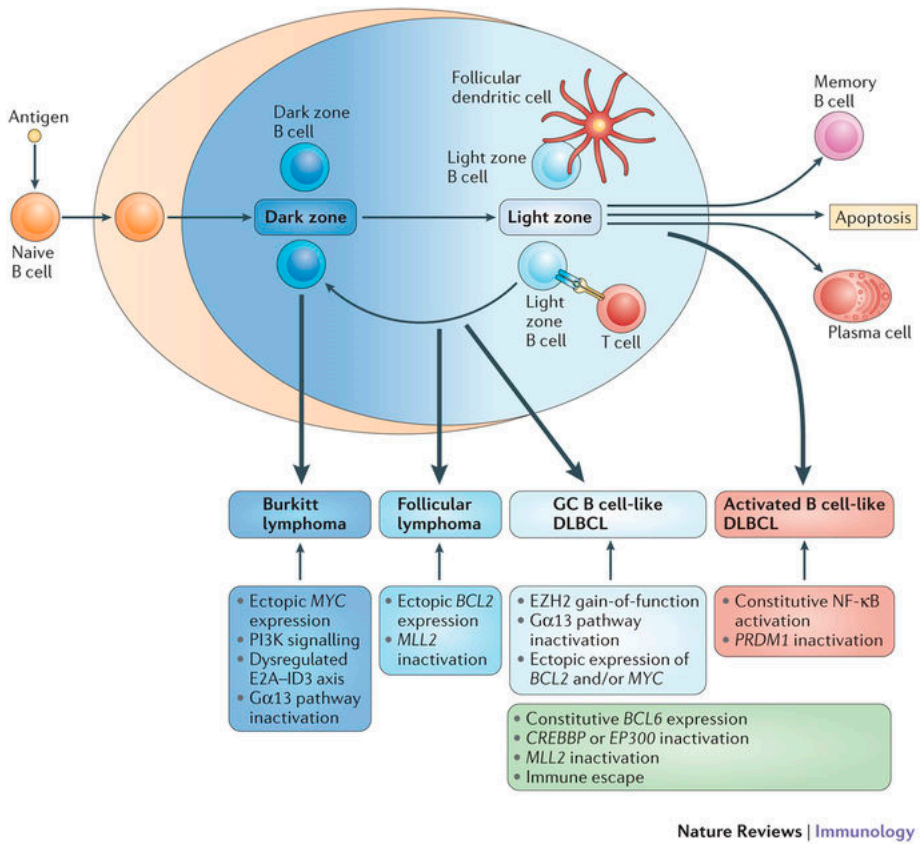

Figure 1:
Origin of GC-derived lymphomas and the main oncogenic pathways. (From[13], reprinted by permission from Springer Nature: Nature Reviews Immunology, copyright 2015)

Genetic aberrations

The genomes coding B-cell NHL carry genetic aberrations including amplifications, deletions and point mutations. Aberrant somatic hypermutation is associated with DLBCL, which leads to the aberrant targeting of multiple non-immunoglobulin foci. *MYC*, which has a specific bimodal expression during the germinal centre reaction, was the first gene to be linked to germinal centre derived lymphomagenesis. *MYC* expression is promptly silenced as a result of transcriptional repression by *BCL6*, which is a regulator of the germinal centre reaction. *BCL6* plays a critical role in lymphomagenesis by suppressing proper DNA damage responses, and by blocking terminal differentiation. The binding of BCL6 or IRF4 to the *BCL6* promoter can be impaired by mutations that contribute to the dysregulation of *BCL6* expression. Dysregulation of *BCL6* expression is also sustained by loss-of-function alterations in the acetyl-transferases CREBBP and EP300, which are involved in the acetylation-mediated inactivation of *BCL6* and acetyl-mediated activation of the *TP53* tumour suppressor. The microenvironment and its inflammatory process also affect DLBCL lymphomagenesis. In most cases of DLBCL, tumour cells appear to be invisible to both cytotoxic T cells and natural killer cell-mediated immune recognition.[14]

Chromosomal translocations involving *MYC* or *BCL2* are detected in approximately 10% and approximately 40% of (GCB) DLBCLs, respectively. The co-occurrence of lesions affecting *MYC* and *BCL2* and/or *BCL6* genes, in so-called "double-hit" (DH) and "triple-hit"(TH) lymphomas, is associated with poor prognosis. In the revised WHO classification from 2016, within the category "B-cell lymphoma unclassifiable (BCLU) with features intermediate between DLBCL and Burkitt lymphoma", it was decided to collect all DH/TH lymphomas into one group classified as "high-grade B-lymphoma with DH/TH". Cases with high-grade morphology but which lack *MYC*, *BCL2* or *BCL6* rearrangements were classified as high-grade B-cell lymphomas "not otherwise specified" (NOS).

In addition, the aberrant activity of the EZH2 histone methyltransferase and altered GC B-cell migration seem to affect (GCB) DLBCL pathogenesis. Mutation of the *EZH2* gene is seen in about 20% of (GCB) DLBCL cases, and encodes a methyltransferase, which contributes to germinal centre B-cell proliferation impairing differentiation. Several chemokines and their receptors are involved in modulating the cell migration occurring in the germinal centre. Approximately 30% of (GCB) DLBCLs have been shown to carry mutations that lead to the disruption of germinal centre architecture and the release of germinal centre B cells in the lymph and blood circulation.

The pathogenesis of (ABC) DLBCL is characterized by the activation of the pro-survival nuclear factor-kappa B (NF-κB) signalling pathway and the blockade of

terminal differentiation into plasma cells. NF-κB is a family of transcription factors that are activated by B-cell receptor (BCR) signalling and regulate various genes with proliferative and anti-apoptotic properties. In about 20% of (ABC) DLBCL cases, mutations of *CD79A* or/and *CD79B* contribute to chronic B-cell receptor (BCR) signalling. The activation of *CARD11* in 10% of (ABC) DLBCLs contributes to NF-κB upstream signals. Just over a third (35%) of (ABC) DLBCL cases carry a mutation in *MYD88*, which mediates IL-R1-associated kinase 2 (IRAK2) and NF-κB. Chronic stimulation of the BCR by antigens and autoantigens may contribute to the constitutive activation of NF-κB. The inability of (ABC) DLBCL cells to terminally differentiate seems to depend on two mechanisms of the negative regulation of the plasma cell master regulator BLIMP1. The bi-allelic inactivation of *PRDM1* is occurred in 30% of (ABC) DLBCL. The *BCL6* dysregulation by translocation also contributes to *PRDM1* inactivation. The repression of *PRDM1* by *BCL6* expression and activity is more common. The transcription factor SPIB can form a complex with IRF4 and also contributes to *PRDM1* inactivation. Inactive *PRDM1* contributes to NF-κB activation.[15]

GEP is considered the gold standard for differentiating between the two main subgroups of DLBCL, but it is not routinely available, and is not cost-effective for routine diagnosis. The Hans algorithm[16], based on a limited panel of antibodies to CD10, BCL6 and IRF/MUM1, has been widely used for this purpose in clinical trials.

In the revised WHO classification, the prognostic importance of simultaneous MYC and BCL2 protein expression, so-called double expression, has been emphasised. The recommended cut-off for MYC is >40% and for BCL2 expression >50%.[1] MYC and BCL2 "double-expressor" (DE) lymphoma has been reported in 19-34% of DLBCL patients. The prognosis for patients with DE lymphoma is worse than that for those who do not express any or only one protein, but better than the prognosis for DH or TH, which involve *BCL2, BCL6* and *MYC*. Cases of DE are more common in the ABC-like subtype.[17] The correlation between the COO and the presence of DH or DE lymphoma is presented in Figure 2.

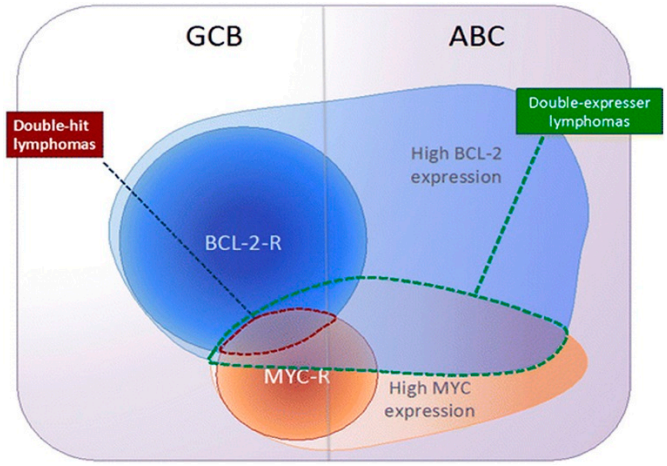

Figure 2:
Correlation between the COO and the presence of the DH or DE lymphoma. The majority of DH lymphoma, almost 91% belong to the GCB group. From[204](Reprinted by permission from Elsevier: Clinical Lymphoma Myeloma and Leukaemia, copyright 2017)

Clinical characteristics and prognostic factors

The clinical presentation of DLBCL is most commonly characterised by rapidly growing lymph nodes, but extranodal involvement, e.g. bone marrow, skeletal, gastric, lung, liver and central nervous system (CNS), also occurs in up to 40% of patients. Staging is based on the Ann Arbor classification, which was designed primarily for the classification of Hodgkin's lymphoma (HL)[18,19] [17,18]. A few years later, Rosenberg validated the Ann Arbor classification for NHL.[20](see Table1) Following the report from the Cotswolds meeting in 1989, computed tomography (CT) has been included in the staging of lymphoma for the evaluation of intrathoracic and intra-abdominal lymph nodes.[21]

Table 1:
Ann Arbor classification. *Lymph node regions include: right cervical (including cervical, supraclavicular, occipital, and preauricular lymph nodes), left cervical, right axillary, left axillary, right infraclavicular, left infraclavicular, mediastinal, hilar, periaortic, mesentery, right pelvic, left pelvic, right inguinal femoral, and left inguinal femoral

Stage	Definition
I	Involvement of a single lymph node region* or lymphoid structure
II	Involvement of two or more lymph node regions,* or localized involvement of one extranodal site and one or lymph node regions, all on the same side of the diaphragm
III	Involvement of lymph node regions* or structures on both sides of the diaphragm
IV	Diffuse or disseminated involvement of one or more extralymphatic organs, OR isolated extralymphatic organ involvement without adjacent regional lymph node involvement, but with disease in distant site(s), OR any involvement of the liver, bone marrow, pleura or CSF

Approximately 50% of DLBCL patients present with localized (stage I) and loco-regional disease (stage II). Another clinically important characteristic is the presence or absence of systemic B-symptoms: fever for 3 consecutive days, weight loss (without trying) exceeding 10% of body weight in 6 months, or drenching night sweats. B symptoms are most common in more rapidly growing lymphomas and occur in about 30% of cases.

The patient's performance status (WHO-PS) is thought to reflect the patient's response to a tumour and his or her ability to tolerate intensive therapy.[22] (see Table 2).

Table 2:
WHO performance status

Grade	Explanation of activity
0	Fully active, able to carry on all pre-disease activities without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Serum lactate dehydrogenase (s-LDH) concentration and bulky disease (maximum diameter >10 cm) are associated with tumour growth and invasive potential. The patient's ability to tolerate intensive therapy depends strongly on age.

The International Prognostic Index (IPI) is a clinical instrument used to predict the outcome for patients with DLBCL. It was introduced by Shipp et al. in the 1990s, and was based on treatment with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP)-like regimens, and overall survival (OS) as the endpoint. The IPI is based on five clinical factors: age>60 years, stage III-IV disease, elevated s-LDH level, WHO-PS ≥2, and more than one extranodal site of disease. The IPI score differentiates four prognostic groups based on the number of factors present: (0-1: low-risk group, 2: low-intermediate-risk group, 3: high-intermediate-risk group and 4-5 high-risk group). (see Table 3) The age-adjusted IPI (aaIPI) is a simplified prognostic index, comparing patients aged ≤60 or >60 years, and includes three prognostic factors: elevated s-LDH, stage III-IV and WHO-PS ≥2.

Table 3:
IPI risk groups (based on age>60 years, elevated s-LDH level, stage III-IV disease, WHO-PS ≥2, more than one extranodal site of disease

IPI risk group	Score
Low-risk	0-1
Low-intermediate risk	2
High-intermediate risk	3
High risk	4-5

The National Comprehensive Cancer Network (NCCN) database contains clinical data collected during the rituximab era. NCCN-IPI is an enhanced IPI, based on five predictors (age, s-LDH, sites of involvement, Ann Arbor stage and WHO-PS). Compared with the IPI, the NCCN-IPI distinguishes better between low- and high-risk groups.[23]

Male sex has been found to have a negative impact on OS and progression-free survival (PFS) in DLBCL, also when patients are treated with CHOP and rituximab.[24-26]

Treatment of DLBCL

The standard treatment for DLBCL since the 1970s has consisted of combination chemotherapy with CHOP. Several attempts to intensify chemotherapy have failed to show any further benefit. CHOP remains the best available treatment, which was confirmed in a randomized phase III trial in 1993, in which CHOP was compared with third-generation regimens such as low-dose methotrexate with leucovorin rescue, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone (m-BACOD); prednisone, doxorubicin, cyclophosphamide and etoposide, followed by cytarabine, bleomycin, vincristine and methotrexate with leucovorin rescue (ProMACE-CytaBOM), and methotrexate with leucovorin rescue, doxorubicin, cyclophosphamide, vincristine, prednisone and bleomycin (MACOP-B).[27]

Table 4:
Randomized phase III trials on the treatment of DLBCL published since 2004

Regimen	Reference	Number of patients	Median age	OS (%)			Comments
CHOP 14 vs. CHOP 21	Pfreundschuh et al.[28]2004 (NHL-B2 trial)	831	>60	CHOP-14	5-year 53		Randomized prospective (high toxicity of etoposide)
				CHOP-21	40		
CHOEP 14/21 vs. CHOP 14/21	Pfreundschuh et al.[29]2004 (NHL-B1 trial)	866	≤60	CHOEP-14/21	5-year 85		Randomized prospective (aggressive lymphomas, better CR for CHOEP)
				CHOP-14/21	80		
R-CHOP 21 vs. CHOP 21	Coiffier et al.[30]2002 (GELA trial)	399	69	R-CHOP-21	2-year 70		Randomized prospective
				CHOP-21	57		
	Pfreundschuh et al.[31]2006 (MInT trial)	824	47	R-CHOP-21	3-year 93		Randomized prospective (no benefit of CHOEP over CHOP after adding rituximab)
				CHOP-21	84		
	Sehn et al.[32]2005 (British Columbia	292	64	R-CHOP-21	2-year 78		Population-based
				CHOP-21	52		
	Habermann et al.[33]2006	632	69	R-CHOP-21	3-year 67		Randomized prospective (no benefit of maintenance)
				CHOP-21	57		
R-CHOP 14 x 6 or 8 cycles vs. CHOP 14 x 6 or 8 cycles	Pfreundschuh et al.[34]2008 (RICOVER-60 trial)	1222	68	R-CHOP 14 x 6	3-year 78		Randomized prospective
				R-CHOP 14 x 8	72		
				CHOP-14 x 6	67		
				CHOP 14 x 8	66		
R-CHOP 14 vs. 21	Cunningham et al.[35]2013	1080	61	R-CHOP-14	2-year 83		Randomized prospective (8 cycles)
				R-CHOP-21	81		
	Delarue et al.[36]2013	602	70	R-CHOP-14	3-year 69		Randomized prospective
				R-CHOP-21	72		
R-CHOEP-14 vs. R-MegaCHOEP	Schmitz et al.[37]2012 (DSH NHL 2002-1)	264	50	R-CHOEP-14	3-year 85		Randomized prospective
				R-Mega CHOEP	77		
R-CHOP vs. R-ACVBP	Récher et al.[38]2011	379	47	R-CHOP	3-year 84		Randomized prospective(3 and 5 deaths, respectively)
				R-ACVBP	92		
R-CHOP vs. Obinutuzumab+ CHOP	Vitolo et al.[39]2017 (GOYA trial)	1418	62	R-CHOP	3-year 81		Randomized prospective
				Obinutuzumab-CHOP	81		
(R)-CHOP+ Consolidation ASCT vs. observation	Stiff et al.[40]2013	397	51	(R)-CHOP+ASCT	2-year 74 (82 high risk)		Randomized prospective
				(R)-CHOP+ observation	71 (64 high risk)		
R-CHOP Consolidation enzastaurin vs. placebo	Crump et al.[41]2016 (PRELUDE trial)	758	64	R-CHOP+ enzastaurin	2-year 87		Randomized prospective
				R-CHOP+ placebo	89		
R-CHOP vs. DA-EPOCH-R	Wilson et al.[42]2016 (CALGB 50303 trial)	524	56	R-CHOP	5-year No difference in EFS or OS		Randomized prospective (oral abstract ASH, 2016)
				DA-EPOCH-R			
R-CHOP + Consolidation lenalidomide vs. placebo	Thieblemont et al.[43]2017 (REMARC trial)	650	60-80	R-CHOP+ lenalidomide	2-year Estimated PFS 80	2-year estimated OS 87	Randomized prospective Medianfollow-up not reached
				R-CHOP+ placebo	75	89	

Rituximab addition

The most important change in therapy during the past decade is the addition of the anti-CD20 monoclonal antibody, rituximab, to chemotherapy. The benefit of the addition of rituximab to cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) has been proven in multiple randomized trials, and has resulted in an absolute improvement in survival of approximately 15% to 20% across all age groups. The first randomized study, performed by the French GELA group, demonstrated an improved 2-year OS rate of 70% for R-CHOP, compared to 57% for CHOP alone.[30] Additional randomized trials have established the benefit of adding rituximab to the conventional CHOP regimen for both younger and older patients.[31,33,34] In a large population-based study in British Columbia, Sehn et al. found that the addition of rituximab to CHOP resulted in a dramatic improvement in outcome for DLBCL patients of all ages.[32] These studies provide further evidence that R-CHOP is currently the best treatment for patients with DLBCL (see Table 4).

Dose intensification

Efforts to improve the efficacy of R-CHOP have included shortening the cycle length (14 days instead of 21 days), adding more cycles (8 cycles instead of 6), adding more cytotoxic agents (etoposide) or delivering chemotherapy as a long-term infusion regimen. (see Table 4)

In a randomized controlled study on a group of German lymphoma patients, in which they were randomly assigned to six or eight cycles of CHOP-14 (14-day cycle length) with or without rituximab, Pfreundschuh et al. found that 6 cycles of R-CHOP-14 was the preferred treatment for elderly patients.[34] However, two other randomized studies have subsequently reported that the R-CHOP-14 regimen was no more efficacious than the standard 3-week schedule.[35,36](see Table 4) They found that the 14-day regimen was associated with increased frequency of side effects, particularly in elderly patients.

Addition of etoposide

Before the introduction of rituximab, the addition of etoposide to CHOP (CHOEP) was shown to achieve better 5-year OS than CHOP in younger DLBCL patients (<60 years).[29] (see Table 4).In elderly patients, the CHOEP regimen was

associated with a higher degree and frequency of haematological toxicity, infection and mucositis.[28,44] The benefit of the more intensive CHOEP regimen over that of CHOP was not present after the addition of rituximab.[31]

Etoposide is also included in the EPOCH regimen (etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin), in which the agents are administered as a continuous infusion. EPOCH has been found to be well tolerated and highly effective in relapsed/refractory DLBCL.[45] In the dose-adjusted EPOCH regimen (DA-EPOCH), the doses of etoposide, cyclophosphamide and doxorubicin are adjusted 20% in each cycle to achieve a nadir absolute neutrophil count below 0.5 x 10^9/L.[46] Several single centres and multicentre phase II trials have indicated higher PFS and OS among patients given DA-EPOCH-R than found in historical results with R-CHOP.[47,48] The CALGB 50303 phase III randomized study compared R-CHOP with DA-EPOCH-R. After evaluating 524 patients no difference was found in the primary endpoint, event-free-survival (EFS).[42] Schmitz et al. compared R-CHOEP-14 to high-dose chemotherapy (R-Mega-CHOEP) in a randomized trial. They were unable to show that R-Mega-CHOEP was superior to conventional R-CHOEP therapy, but it was associated with significantly more toxic effects. In a randomized study in which the efficacy of dose-intensive rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin and prednisone (R-ACVBP) was compared to standard R-CHOP, DLBCL patients receiving R-ACVBP showed significantly improved survival.[38] (see Table 4)

Despite efforts to improve CHOP, by shortening the cycle length (from 21 to 14 days), adding more cytotoxic agents, or delivering chemotherapy as an infusion regimen (DA-EPOCH), the benefit of these efforts disappeared once rituximab was introduced. The major improvement in the treatment of DLBCL thus appeared to be the addition of rituximab to CHOP. Despite the good efficacy of R-CHOP, a large number of patients with high IPI and elderly patients are not cured; the cure rates in these groups being only approximately 50%.[49,50]

Other well-designed and well-intentioned trials were unable to improve the outcome of patients with DLBCL. The GOYA trial compared R-CHOP with obinutuzumab-CHOP in over 1400 patients, and found no difference in response, PFS, or OS.[39] In the SWOG trial, patients with DLBCL were randomized to consolidative autologous stem cell transplant (ASCT) or observation after induction therapy with R-CHOP or CHOP. The benefit of ASCT was only seen among patients with high-risk disease.[40] In the PRELUDE trial, patients with an IPI of 3-5, who were in remission after R-CHOP, were randomized to 3 years of oral enzastaurin or placebo. No differences in PFS or OS were seen between the two arms of the study.[41] The conclusion drawn from the findings of the REMARC study was that 24 months' maintenance with lenalidomide after R-CHOP, in elderly patients with DLBCL, after complete remission (CR) or partial remission

(PR) significantly prolonged the PFS compared to R-CHOP followed by observation, but no trend towards improved OS was seen.[43] (see Table 4)

Treatment based on cell of origin

Assessment of the cell of origin (COO) has been incorporated into a number of trials, but is not yet standard in clinical practice. Patients with (GCB) DLBCL have shown better survival than those with (ABC) DLBCL phenotypes, based on GEP studies.[11] Apart from this, patients with the ABC or GCB subtype of DLBCL had similar prognosis with and without MYC and BCL2 coexpression. Coexpression of MYC and BCL2 occurs significantly more frequently in the ABC subtype, and contributes to the overall poorer prognosis for patients with (ABC) DLBCL.[17] A new treatment paradigm for the management of (ABC) DLBCL phenotypes is under intensive investigation. There is no consensus about the preferred treatment for these cases. A retrospective analysis of patients with relapsed DLBCL treated with lenalidomide alone, revealed an overall response rate (ORR) of 53% and CR of 29% in the ABC (DLBCL) subgroup, compared with a 9% response rate in the GCB (DLBCL) subgroup.[51] Several prospective trials have been performed in which lenalidomide was added to R-CHOP. The Mayo Clinic group found improved PFS and OS in (ABC) DLBCL patients treated with lenalidomide plus R-CHOP, compared to historical control (ABC) DLBCL patients receiving R-CHOP only.[52] Similarly, an Italian multicentre trial reported a better outcome in elderly non-GCB patients when lenalidomide was combined with R-CHOP 21.[53] An international phase III randomized trial is in progress comparing R-CHOP with lenalidomide+R-CHOP (ROBUST trial, NCT02285062). Ibrutinib as a single agent was found to be associated with an ORR of 37% in the (ABC) DLBCL subgroup, compared with only 5% ORR in those with the (GCB) DLBCL phenotype.[54] A trial is ongoing to compare R-CHOP with and without ibrutinib in patients with (ABC) DLBCL (PHOENIX trial, NCT01855750).

Double-hit lymphoma

DH lymphomas are relatively uncommon, accounting for 5-7% of DLBCLs. The dual rearrangement of *MYC* and *BCL2* leads to clinical resistance to therapy and poor long-term survival. The results obtained with R-CHOP are very poor.[55] Several retrospective studies have suggested that more intensive regimens, including DA-EPOCH-R and R-HyperCVAD are superior to R-CHOP.[56,57] In an

ongoing prospective trial, treatment with DA-EPOCH-R is being studied in patients with MYC-associated high-grade lymphoma (NCT01092182). Early results from this study are promising, showing an OS of approximately 75% at a median follow-up of 14 months.[58] Retrospective studies have provided no evidence of any significant benefit for consolidative ASCT in patients with DH lymphoma,[56,59] and most patients do not benefit from ASCT at relapse.[60,61]

Double-expressor lymphoma

DE lymphomas are more common. Up to 30% of DLBCL or high-grade lymphoma patients have protein overexpression of MYC and BCL2, according to results from gene amplification, transcriptional dysregulation, or both.[62-64] Double expression is recognized as a marker of poor prognosis. The 5-year OS has been reported to be 30% and 50% for DE and non-DE patients, respectively.[55,65] The optimal treatment of DE lymphoma is unknown. Trials are ongoing to test lenalidomide or venetoclax in addition to DA-EPOCH-R (NCT02213913 & NCT03036904).

The role of radiotherapy in DLBCL

Early stage disease

The prognosis is very favourable for early-stage disease (stages I-II) without risk factors. The benefit of radiotherapy after chemoimmunotherapy has been investigated in four randomized trials during the pre-rituximab era.[66-69] Taking these results into consideration, together with those of a phase II trial published in the rituximab era, the conclusion is that 3 cycles of chemoimmunotherapy plus involved field radiation therapy (IFRT) is at least as effective as a full course of the same chemoimmunotherapy in DLBCL patients with stage I disease (see Table 5).[70]

Table 5:
Studies on the efficacy of radiotherapy after chemo- and chemoimmunotherapy in early-stage DLBCL, published between 1998 and 2008

Study	Reference	Number of patients	Median age (y)	OS (%)		Comments
3 cycles CHOP + 40-55 Gy radiotherapy vs. 8 cycles CHOP	Miller et al.[66]1998 (SWOG)	401	50% of patients <60	CHOPx3+ 40-55 Gy	5-year 72	Randomized trial (66% stage I, 33% stage II) (no bulky tumours)
				CHOPx8	82	
3 cycles CHOP+40 Gy IFRT vs. ACVBP	Reyes et al.[67]2005 (GELA LNH 93-1)	647	61	CHOPx3+IFRT	5-year 81	Randomized study (66% stage I) (Stage II bulky disease needs more than 3 cycles CHOP)
				ACVBP	90	
8 cycles CHOP +/- 30 Gy IFRT	Horning et al.[68]2004 (ECOG)	172		CHOPx8	5-year 87	Randomized trial (stage I-II)
				CHOPx8+30Gy	73	
4 cycles CHOP vs. 4 cycles CHOP + 40 Gy radiotherapy	Bonnet et al.[69]2007 (GELA LNH 93-4)	574	>60	CHOPx4	7-year 72	Randomized trial (66% stage I)
				CHOPx4+40Gy	66	
3 R-CHOP cycles + IFRT	Persky et al.[70]2008 (SWOG)	71	66-69	4-year 92		Phase II study

Advanced stage disease

For patients with advanced stage DLBCL, treated with R-CHO(E)P regimens, consolidation radiotherapy to bulky sites may be beneficial. The MInT trial found bulky disease to be a strong prognostic factor. Further analyses of patients >70 years with bulky disease in the RICOVER-60 trial confirmed an improved outcome for patients treated with radiotherapy consolidation after chemoimmunotherapy.[71] The 3-year PFS was 75% versus 61%, and the 3-year OS was 90% versus 65%, for those treated with and without radiotherapy, respectively. The findings of another study suggested a beneficial effect of consolidating radiotherapy to sites of skeletal involvement.[72] The most sensitive method of assessment of response after chemoimmunotherapy is to use positron emission tomography (PET). For patients with negative findings at PET scan at the end of treatment, observation is an option, while consolidative radiotherapy should be considered in cases of initially bulky disease or skeletal involvement. However, in a recently published abstract from the German study group RT was reported to have no benefit in bulky disease where PET scans were negative after chemoimmunotherapy.[73]

Follicular lymphoma

Follicular lymphoma is the second most common type of lymphoma, and accounts for approximately 20% of all lymphomas in the Western world. The disease is associated with long-term survival, with a 10-year OS of approximately 70-80%, and a variety of initial treatment strategies are used.

In Western countries, the incidence of FL is approximately 2 to 3 per 100,000 person-years. The incidence of FL has not changed significantly overall during later years, although it increased among elderly patients (> 60 years) by 1.8% per year during the period 1992-2001. The median age at diagnosis is in the 60s. The incidence rate does not differ between men and women.

Aetiology

The results of epidemiological studies suggest that FL has a multifactorial aetiology. First-degree family history of NHL, higher BMI as a young adult, and work as a spray painter have been found to be associated with an increased risk of FL. In females, a history of cigarette smoking was also reported to be associated with a higher risk of FL.[6]

Histopathological features

Follicular lymphoma is derived from B-cells from the germinal centre. Genetically, the FL cells are characterized by the t(14;18)(q32;q21) translocation, which leads to overexpression of BCL2 in approximately 90% of cells. These proliferating neoplastic GCB-cells are both centrocytes and centroblasts which maintain a partial follicular pattern. The grading system for FL involves evaluation of the proportion of centrocytes to centroblasts (see Figure 3). Grade 1-2 FL is defined as ≤15 centroblasts per high-power field. Grade 3 FL has >15 centroblasts per high-power field. Grade 3 FL is further divided into 3A and 3B. Grade 3B FL has only centroblasts and is a biologically distinct entity frequently without

t(14;18) translocation and CD10 expression, and increased p53 and MUM1/IRF4 expression. A large retrospective study in Sweden demonstrated that the clinical course of grade 3A FL is similar to grade 1-2 FL, whereas 3B FL is more similar to DLBCL.[74]

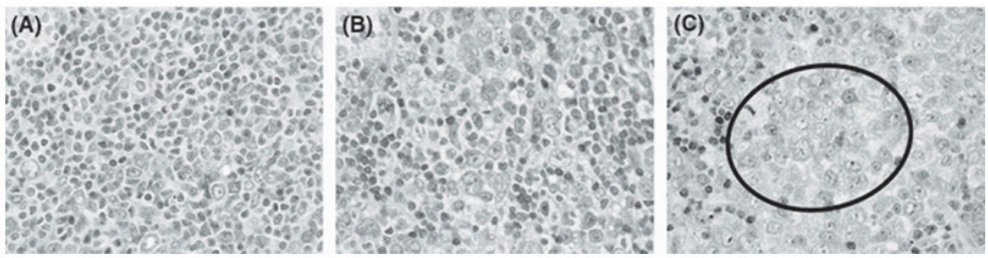

Figure 3:
Centroblasts in follicular lymphoma. (A) Follicular lymphoma grade 1–2. (B) Follicular lymphoma grade 3A. (C) Follicular lymphoma grade 3B, where a solid sheet of centroblasts is encircled. From[74](Reprinted by premission from John Wiley and Sons: British Journal of Haematology, copyright 2011)

Genetic features of FL

In addition to the *BCL2* translocation, inactivating mutations of *MLL2* are found in >80 % of cases of FL, which is an early event in FL. MLL2 activates gene transcription through H3K4 methylation. Mutations of other histone modifiers have also been identified (CREBBP, EZH2, MEF2B, EP300, TNFRSF14).[13] The FL microenvironment and the gene expression signature of the non-malignant stromal cells are prognostically more important than the neoplastic B cells.[75-80] The microenvironment is composed of tumour-infiltrating CD8+ T cells, follicular regulatory T cells, lymphoma-associated macrophages and mast cells, follicular helper T cells, follicular dendritic cells and follicular reticular cells. The complex relationship between the neoplastic cells and the microenvironment is still an active area of investigation.

Clinical characteristics and prognostic factors

The clinical presentation of follicular lymphoma is characterized by asymptomatic peripheral lymphadenopathy, and the growth and reduction of lymph node enlargement over several years is a common feature. The 10-year OS among patients with FL is approximately 70-80%.[81]

The prognosis for patients with FL is determined by several clinical and biological factors. The most commonly used tool for prognosis is the Follicular Lymphoma International Prognostic Index (FLIPI).[82] The FLIPI is based on a number of biological and clinical factors: age >60 years, Ann Arbor stage III-IV, serum haemoglobin level <120 g/L, >4 nodal involvement areas and elevated s-LDH. The FLIPI was developed and validated in the pre-rituximab era. The FLIPI 2 using data from the rituximab chemotherapy era and includes the following factors: age >60 years, elevated β-2 microglobulin, haemoglobin level <120 g/L, bone marrow involvement and lymph node diameter >6 cm.[83] More recently, a clinicogenetic risk model was established by a German research group, by integrating the mutational status of 7 genes with the FLIPI (m7-FLIPI) (see Table 6).[84]

Histological transformation (HT) is a well-known event in patients with FL, and is associated with an unfavourable prognosis. The incidence of HT ranges from 10-60% in different studies.[85-90] HT into an aggressive lymphoma has been reported to occur in 17%, 28% and 37% of FL patients after 5, 10 and 17 years, respectively, with an apparent plateau at 15 years.[86] Clinically, the transformation is characterized by a rapidly growing tumour mass, the presence of B symptoms, elevated s-LDH or hypercalcaemia. The acceleration of proliferation kinetics is correlated with an increased expression of Ki 67 within tumours, and a high maximum standardized uptake value (>12-14) on PET scanning.[91] Several retrospective studies have been carried out to investigate the overall incidence of clinical risk factors for transformation. Sarkozy et al. evaluated the incidence of HT at first recurrence in the PRIMA patient cohort, and studied the risk factors associated with this event. More than half of the HTs occurred during the first year after induction immunochemotherapy. Among the patients with HT, the median OS rate from the first relapse was 3.8 years, compared with 6 years for patients with FL histology without HT.[90] There is still disagreement over whether immediate treatment reduces the incidence of transformation, but the findings of three randomized studies suggest that observation alone has no negative effect on the rate of transformation.[92-94]

Based on analyses from the National LymphoCare Study (NLCS), it appears that one of the strongest predictors of long-term outcome is probably the length of the first remission after standard induction immunochemotherapy. Among patients who received R-CHOP as initial therapy, approximately 20% relapsed or progressed within 2 years of diagnosis.[95] The 5-year OS was 50% for this group, compared with 90% in patients without early progression. None of the prognostic tools currently available (FLIPI, FLIPI-2, or m7-FLIPI) is able to identify patients <60 years old with such a high risk of early death.

In a recently completed trial in France, on the cause of death due to FL in the current treatment era, it was demonstrated that lymphoma was the most common

cause of death in 49% of cases, followed by treatment-related complications in 15% and secondary cancer in 12%. HT had occurred in 85% of lymphoma-related deaths, while treatment-related mortality was also frequent (46%) among patients showing no HT treated with ASCT.[96]

Table 6:
Prognostic indices of FL

Prognostic tool	Biological and clinical factors	Comments
FLIPI	Age >60 y Ann Arbor stage III-IV s-haemoglobin level >120 g/L >4 nodal involvement areas Elevated s-LDH	Pre-rituximab era
FLIPI-2	Age >60 y Elevated β-2 microglobulin s-haemoglobin level >120 g/L Bone-marrow involvement Lymph node diameter >6 cm	Rituximab era
m7-FLIPI	Mutation status of seven genes (EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP, and CARD11) FLIPI WHO-PS	Highest accuracy in predicting progression of disease within 24 months

Treatment of follicular lymphoma

The watch-and-wait approach

Follicular lymphoma is usually considered to be an incurable chronic disease. Several investigations have been carried out to compare the outcomes of patients who were either treated immediately with immunotherapy or chemoimmunotherapy, or were carefully followed without initial systemic treatment. Two retrospective studies revealed no significant difference in OS between the groups managed with immediate treatment and watchful waiting.[97,98] Three prospective trials confirmed these findings. In the first of these, a study by the National Cancer Institute, patients with indolent lymphoma were randomized to watchful waiting or immediate intensive combined modality therapy with a multidrug regimen and total nodal irradiation. No significant difference was found in the OS of these two groups.[99] The second randomized trial, performed by the Group d´Etude des Lymphomes de Folliculaires (GELF), compared watchful waiting to less aggressive treatment, i.e., prednimustine or subcutaneous interferon-α. No difference in OS was seen between the two groups after 5 years of follow-up.[92] The third and largest randomized multicentre study, by Ardeshna et al., compared treatment with chlorambucil with watchful waiting in patients with indolent lymphoma, approximately 66% of which had FL. The 5-year OS for the watch-and-wait group was 58%, which can be compared with 78% in the GELF study. The lower OS was probably due to an older patient cohort with more bulky disease in the study by Ardeshna.[93] They also found that 19% of patients did not require treatment for lymphoma after 10 years' follow-up. Another prospective randomized study by Ardeshna et al., in which the watch-and-wait strategy was compared with rituximab monotherapy with or without rituximab maintenance, showed similar 3-year OS of 94% vs. 97% vs. 96%, respectively.[94] Both recent studies and those mentioned above provide support for watchful waiting as a primary strategy for patients with asymptomatic advanced-stage follicular lymphoma. The watch-and-wait strategy has also been found to be appropriate in advanced-stage FL in a population-based study using data from the Danish Lymphoma Registry. A favourable outcome was reported, with a 5-year PFS of 35% and 10-year OS of 65%. Abandoning this strategy could lead to overtreatment in some patients (see Table 7).[100]

Table 7:
Results of studies on watchful waiting in cases of FL

Study	Reference	Number of patients	OS(%)		Comments
Watch and wait vs. initial treatment	Portlock et al.[97] 1979	156	Watch and wait	4-year 77	Retrospective
			Initial treatment	83	
Untreated patients	Horning et al.[98] 1984	83	5-year OS 82% 10-year OS 73% 23% spontaneous regression		Retrospective (low grade NHL)
Watch and wait vs. aggressive combined modality treatment	Young et al.[99] 1988	104	No data on OS available		Prospective
Watch and wait vs. Prednimustine vs. Interferon-α	Brice et al.[92] 1997	193	Watch and wait	5-year 78	Prospective
			Prednimustine	70	
			Interferon-α	84	
Watch and wait vs. Chlorambucil	Ardeshna et al.[93] 2003	309	Watch and wait	5-year 57	Randomized prospective (66% of patients with FL) (19% of patients not needing chemotherapy after 10 years)
			Chlorambucil	58	
			Watch and wait	10-year 35	
			Chlorambucil	34	
			Watch and wait	15-year 21	
			Chlorambucil	20	
Watch and wait vs. Rituximabx4 vs. Rituximabx4+ rituximab maintenance	Ardeshna et al.[94] 2014	379	Watch and wait	3-year 94	Randomized prospective Low tumour burden Stage II-IV
			Rituximabx4	96	
			Rituximabx4+rituximab maintenance	97	
Watch and wait	El-Galaly et al.[101] 2015	286	10 year OS 95		Population-based, stage III-IVA

Rituximab monotherapy

In 1997, the anti-CD20 chimeric monoclonal antibody, rituximab, was the first antibody approved for use in the treatment of FL. The mechanism of action of rituximab is the induction of lymphoma cell lysis through complement-mediated cytolysis, antibody-dependent cell cytotoxicity and/or direct induction of apoptosis.[102-104] The efficacy of rituximab monotherapy was initially demonstrated in relapsed/refractory patients receiving 4 weekly doses of rituximab. The response rate was 48%, and the median PFS 13 months.[105] A phase II trial in previously untreated low-tumour-burden FL patients showed an ORR of 73%.[106] Only one prospective randomized phase III trial, by Ardeshna et al., has compared

rituximab monotherapy with watchful waiting in patients with advanced-stage, asymptomatic, non-bulky FL. [94]

Table 8:
Results of prospective studies on first-line rituximab monotherapy of patients with FL and in relapsed/refractory patients

Study	Reference	Number	Response rate			Comments
Rituximabx4	McLaughlin et al.[105]1998	166	ORR 48%			Prospective phase IIRelapsed/ refractory patients
Rituximabx4	Colombat et al.[106]2001	50	ORR 73%			Prospective phase II
Rituximabx4 + observation vs. Rituximabx4 + rituximab maintenance	Martinelli et al.[107]2010 (SAKK 35/98 trial)	202	Rituximabx4	+ observation	8-year PFS 5 %	Randomized prospective
				+ rituximab maintenance	27%	
Watch and wait vs. Rituximabx4 vs. Rituximabx4+ maintenance	Ardeshna et al.[94]2014	379	Watch and wait		3-year OS 94%	Randomized prospective Low tumour burden, stage II-IV
			Rituximabx4		96%	
			Rituximabx4+rituximab maintenance		97%	
Rituximab+ Rituximab retreatment vs. Rituximab+ rituximab maintenance	Kahl et al.[108]2014 (RESORT trial)	289	Rituximab + rituximab retreatment		5-year OS 94%	Randomized prospective Low tumour burden
			Rituximab + rituximab maintenance		94%	
Rituximab vs. Rituximab+ interferon	Kimby et al.[109]2015	318	Rituximab		8-year OS 87%	Randomized prospective Low and high-tumour burden included
			Rituximab+interferon		90%	
Rituximab vs. Rituximab-lenalidomide	Kimby et al.[110]2014 (SAKK 35/10 trial)	154	Rituximab		3-year OS 92%	Phase II study in cooperation with the Nordic Lymphoma Group
			Rituximab+lenalidomide		93%	

Chemoimmunotherapy

The addition of rituximab to chemotherapy has led to therapeutic advances in the treatment of FL, including, improved response rate, event-free survival (EFS), PFS and OS. Several large randomized trials on FL patients with advanced stage disease, previously untreated, in which combination chemotherapy was compared with rituximab + chemotherapy, confirmed a persistent improvement in the median OS of between 2 and 5 years.[111-114] Before the introduction of bendamustine, the most commonly used regimens were R-CHOP, rituximab plus cyclophosphamide, vincristine and prednisone (R-CVP), or R-fludarabine. A

randomized study in Italy, FOLL05, compared these regimens and found that R-CHOP had the best risk-benefit profile.[115]

The alkylating agent bendamustine was reintroduced for the treatment of several haematological malignancies in the late 1990s. The first alkylating agent was used at the beginning of the 20th century. So-called mustard gas, used during the First World War, was found to be extremely toxic to bone marrow.[116] Several decades later, in 1947, doctors at Yale University, observed that a patient who had been treated with nitrogen mustard, derived from mustard gas, showed a remarkable tumour response. The alkylating agent, bendamustine was first synthesized in Jena, in 1963, by Ozegowski and Krebs. Bendamustine was widely used clinically in East Germany to treat lymphomas and lung cancer, but was not studied systematically in patients until the 1990s. Bendamustine received its first marketing approval in Germany.[117]

One randomized multicentre phase III trial, carried out by the Study group indolent Lymphomas (StiL), compared the effects of rituximab-bendamustine (RB) to R-CHOP. The patients in the RB arm of the study showed a higher rate of CR and longer PFS. In addition, the frequency of haematological toxicity, alopecia, infections and peripheral neuropathy was lower in the RB arm.[118] Another randomized phase III trial in the US, the BRIGHT trial, compared RB to R-CHOP and R-CVP, and found that RB was not inferior to either of them, suggesting that RB is an attractive alternative to R-CHOP or R-CVP in the treatment of FL.[119] The occurrence of secondary malignancies was not increased in the bendamustine-treated patients.[118]

Table 9:
Results of randomized prospective trials of chemoimmunotherapy in the first-line setting

Study	Reference	Number of patients	Response rate		Comments
CHOP vs. R-CHOP	Hiddemann et al.[111] 2005	428	CHOP	ORR 90%	Randomized prospective
			R-CHOP	96%	
Mitoxantrone-chlorambucil-prednisone (MCP) vs. R-MCP	Herold et al.[112] 2007	358	MCP	4-year OS 74	Randomized prospective
			R-MCP	87	
CVP vs. R-CVP	Marcus et al.[113] 2008	321	CVP	4-year OS 77	Randomized prospective
			R-CVP	83	
Cyclophosphamide-adriamycin-etoposide-prednisone (CHVP)+interferon (I) vs. R-CHVP+I	Bachy et al.[114] 2013	358	CHVP+I	8-year OS 70	Randomized prospective
			R-CHVPI+I	78	
R-CVP vs. R-CHOP vs. R-fludarabine-mitoxantrone (FM)	Federico et al.[115] 2013 (FOOL05)	534	R-CVP	3-year PFS 52%	Randomized prospective
			R-CHOP	68%	
			R-FM	63%	
RB vs R-CHOP	Rummel et al.[118] 2013 (StiL trial)	549	Data on ORR, PFS or OS are not available		Randomized prospective (>50% FL)
RB vs. R-CHOP/R-CVPR-CHOP/R-CVP	Flinn et al.[119] 2014 (BRIGHT trial)	447	RB	ORR 97%	Randomized prospective (>70% FL)
			R-CHOP/R-CVP	91%	

Maintenance therapy

The first study comparing rituximab maintenance vs. retreatment at progression after rituximab induction showed that first-line treatment with scheduled maintenance after induction led to a higher ORR, CR and longer PFS (34 months) than reported with the standard four-week treatment.[120] In the results of studies by Swiss Group for Clinical Cancer Research (SAKK), 13% of the patients in the observation arm were event-free at the 5-year follow-up, and 5% at the 8-year follow-up. In the prolonged exposure arm 27% of the patients were event-free at 5 years and 27% remained event-free at 8 years.[107] In the previously mentioned study by Ardeshna et al., the patients were enrolled in three arms: watch and wait, rituximab weekly x 4 and rituximab weekly x 4 followed by maintenance therapy. They found no difference in 3-year OS between these three groups.[94] In the RESORT study, previously untreated, low-tumour-burden FL patients were randomized after 4 weekly doses of rituximab induction between rituximab maintenance or rituximab retreatment. No difference was found in the time to

treatment failure at the 3-year follow-up between the rituximab retreatment arm (61%) and the rituximab maintenance arm (64%). However, the rituximab retreatment strategy requires less rituximab. The conclusion drawn from this study was that rituximab retreatment is the preferred strategy when single-agent rituximab was the induction treatment.[108]
The efficacy of rituximab maintenance after induction with immunochemotherapy was investigated in the PRIMA trial, a phase III randomized trial. Different induction treatments R-CHOP, R-CVP or rituximab-fludarabine-cyclophosphamide-mitoxantrone (R-FCM) were used at different centres. Rituximab maintenance was administered every 2 months for 2 years. The 2-year PFS in the rituximab maintenance arm was 75%, vs. 58% in the observation arm, and the beneficial effect was not dependent on induction chemotherapy. No difference in OS was observed after 4 years (see Table 10).[121]

The results of these studies indicate that the watch-and-wait approach remains an appropriate choice for asymptomatic FL patients with low-tumour burden. However, treatment of low-tumour-burden FL patients with rituximab monotherapy was investigated in the RESORT trial, in which an extended rituximab schedule, including maintenance, was compared with retreatment at time of relapse. The rituximab retreatment strategy was associated with less use of rituximab, and provided disease control comparable to that achieved with the maintenance strategy. This study also showed modest reductions in immunoglobulin levels and more pronounced changes in IgA and IgM levels among patients treated with the extended schedule.[108] Furthermore, the results of studies by the Nordic Lymphoma Group and the SAKK suggest that rituximab monotherapy may also be an appropriate option for patients with symptomatic high-tumour-burden disease, sparing a large proportion of patients the side effects of chemotherapy.[107,109] The studies presented thus far provide evidence that rituximab should be considered as a treatment option for FL due to a number of benefits, such as improved PFS and longer time to the need of first chemotherapy.[94,108]

Table 10:
Results from prospective randomized trials on rituximab maintenance treatment of FL patients

Study	Reference	Number of patients	Response rate			Comments
Rituximabx4+ rituximab maintenance vs. Rituximab retreatment after progression	Hainsworth et al.[120]2002	114	Rituximabx4+rituximab maintenance		3-year OS 72%	Randomized phase II trial
			Rituximabx4+retreatment after progression		68%	
R-CHOP/R-CVP/R-FCM +/- rituximab maintenance	Salles et al.[121]2010 (PRIMA trial)	1217	Chemoimmunotherapy+rituximab maintenance	4-year OS 95%	2-year PFS 75%	Randomized prospective
			Chemoimmunotherapy	95%	58%	
Rituximabx4 +/- Rituximab maintenance	Martinelli et al.[107]2010 (SAKK 35/98 study)	202	Rituximabx4+rituximab maintenance		8-year PFS 27%	Randomized prospective
			Rituximabx4		5%	
Watch and wait vs. Rituximabx4 vs. Rituximab+rituximab maintenance	Ardeshna et al.[94]2014	379	Watch and wait		3-year OS 94%	Randomized prospective Stage II-IV Low tumour burden,
			Rituximabx4		96%	
			Rituximabx4+rituximab maintenance		97%	
Rituximab+rituximab retreatment vs. Rituximab+rituximab maintenance	Kahl et al.[108]2014 RESORT	289	Rituximab+rituximab retreatment		5-year OS 94%	Randomized prospective Low tumour burden
			Rituximab+rituximab maintenance		94%	

Radiotherapy

Radiotherapy plays an important role in the treatment of FL. In "limited-stage" disease, which usually means stage I and contiguous stage II, radiotherapy has curative potential. Approximately 25-30% of patients with FL present with limited-stage disease. Goffinet et al. presented a series on 206 patients with nodal lymphoma in which 31% had stage I-II disease, based on imaging and physical examination. After laparotomy/splenectomy and bone marrow biopsy, only 12% remained in stage I-II.[122] The accurate staging of limited-stage FL is crucial for the decision to use IFRT. The role of PET in the staging of FL has been documented in a retrospective analysis, where it was found to be able to detect more nodal and extranodal lesions than CT.[123] A study by Wirth et al. showed that PET findings suggested a change in stage or management in 31% of patients, who were upstaged to stage III-IV, leading to a change in the curative therapy strategy from IFRT to watch and wait or systemic therapy.[124] The role of PET/CT in the initial staging of FL was investigated in another retrospective analysis (FOLL05 phase III trial). The results of this study showed that the impact of PET on the classification of stage was highest in patients with lower stages of disease.[125] The greatest contribution of PET was the detection of extranodal involvement in bone, spleen, gastrointestinal tract and skin. PET appeared not to be sufficiently sensitive to detect bone marrow infiltration and can, therefore, not replace bone marrow biopsy in FL patients.[123,125]

IFRT with a radiation dose of 24-30 Gy delivered in 12-15 fractions is a curative option for stage I-II disease.[126] Radiotherapy has been reported to be associated with a 14% absolute improvement in 10-year OS of patients with early-stage low-grade FL, compared to patients who did not receive any initial therapy or were treated with chemotherapy.[127,128] Preliminary results have been presented by Brady et al. from a study in which 310 patients were treated from 2000-2016 at 11 centres. These patients, with stage I-II FL, were staged using PET-CT and received radiotherapy. The results showed 70% PFS and 96% OS after 5-years. The PFS was 74% for patients with stage I disease, and 48% for those with stage II, after 5 years.[129]

Almost 50% of patients with limited stage disease will relapse within 10 years, usually at sites outside the primary irradiated field.[130]Combined modality therapy involving rituximab monotherapy and (sequential and/or concomitant) IFRT has been suggested as a means of improving disease control. The MIR study is a phase II study assessing the combination of rituximab and radiotherapy.[131] The

results of this study were presented at the American Society of Hematology Meeting in 2012, confirming a 2-year PFS of 90%.[132]

Very-low-dose radiotherapy (4 Gy) is frequently administered for local palliation, especially in patients with poor PS. This treatment provides effective symptomatic relief for bulk tumours of all sizes, with an overall response rate of 81%.[133] Local treatment can be repeated in cases of local progression.

The role of stem cell transplant in follicular lymphoma

The role of high dose treatment (HDT) and autologous or allogeneic stem cell transplant in FL has not yet been fully elucidated. Stem cell transplant should be considered when the disease is resistant to standard treatment, or it is feared that young patients with FL will die of their disease. A number of randomized trials have been carried out on the use of HDT with ASCT. (see Table 11). The only prospective randomized trial on patients with relapsed FL published to date is the CUP trial.[134] The results of this trial suggested that ASCT gave benefits in terms of PFS and OS, but the trial was closed early due to slow enrolment of patients. In the GLSG trial, previously untreated patients were randomized to ASCT or interferon maintenance after induction therapy.[135] The 5-year PFS was found to be longer in the ASCT arm. This study had a short follow-up, and higher toxicity was reported in the ASCT arm. In the GOELAMS study advanced-stage FL patients were randomized to either cyclophosphamide, doxorubicin, teniposide, prednisone and interferon or to HDT followed by ASCT.[136] The patients treated with HDT showed a higher response rate, but the OS was lower due to an excess of secondary malignancies in the ASCT arm. Furthermore, the French GELF-94 trial was not able to confirm any improvement OS following ASCT compared to chemotherapy in previously untreated advanced-stage FL patients.[137] From a meta-analysis of the results presented above it was concluded that HDT with ASCT does not improve OS in FL patients. [138]

Table 11:
Results of randomized studies on the role of ASCT in FL

Study	Reference	Number of patients	Response rate		Comments
Chemotherapy vs. ASCT	Schouten et al.[134]2003 (CUP trial)	140	Chemotherapy	4-year OS 46%	Prospective randomized Relapsed patients
			ASCT	71%	
CHOP/MCP+ ASCT vs. Interferon maintenance	Lenz et al.[135]2004 (GLSG trial)	307	CHOP/MCP+ASCT	5-year PFS 65%	Prospective randomized Previously untreated patients
			CHOP/MCP+ interferon maintenance	33%	
CHVP+IFN vs. HDT +ASCT	Deconinck et al.[136] (GOELAMS trial)	172	CHVP+IFN	5-year OS 82%	Prospective randomized Newly diagnosed patients
			HDT+ASCT	73%	
CHVP+IFN vs. CHOPx4+HDT+TBI+ASCT	Sebban et al.[137]2006 GELF-94	401	CHVP+IFN	Estimated 7-year OS 71%	Prospective randomized Previously untreated patients
			CHOPx4+HDT+TBI+ASCT	76%	

In a retrospective analysis of data from the International Bone Marrow Transplant Registry, van Besien et al. summarized the results after stem cell transplant in 904 patients with FL. Among these patients, 176 (19%) received alloSCT, 131 (14%) underwent purged ASCT and 597 (67%) were treated with unpurged ASCT. The conclusion of this analysis was that both alloSCT and ASCT can induce durable remission, with a 5-year probability of survival of 51%, 62% and 55%, after alloSCT, purged ASCT and unpurged ASCT, respectively. The 5-year treatment-related mortality rates were 30%, 14% and 8% after alloSCT, purged ASCT and unpurged ASCT, respectively. Long-term PFS has been reported after alloSCT in 29 FL patients with advanced FL, 11 of which had refractory disease.[139] The disease-free 5-year survival rate was approximately 50% for this cohort.

The histological transformation of FL to high-grade lymphoma is associated with poor outcome, and the role of ASCT has been established in this situation.[140] The Canadian Blood and Marrow Transplant Group studied 172 patients with biopsy-verified HT of FL. In these patients, 13% underwent alloSCT, 56% underwent ASCT and 31% were treated with rituximab-containing chemotherapy. The 5-year OS after HT was 46%, 65% and 61% after alloSCT, ASCT and R-chemotherapy, respectively. In a multivariate analysis, patients treated with ASCT showed improved OS compared with patients who received rituximab-containing chemo-therapy. The 5-year treatment-related mortality was reported to be 23% among the patients treated with alloSCT and 5% for those who underwent ASCT. In a prospective phase II study in Norway, Eide et al. reported that the majority of

patients with HT of FL (60%) who underwent ASCT achieved CR and had prolonged OS.[141]

In summary, these studies indicate that consolidation with ASCT should be considered for patients with HT FL.

Novel agents in FL

The identification of new therapeutic pathways and targets has improved the treatment of FL. These novel strategies include targeting the BCR, CD20, the tumour microenvironment, epigenetic modifiers and checkpoint inhibitors.

Obinutuzumab (GA-101) is a third-generation, fully humanized, type II anti-CD20 monoclonal antibody. According to the GAUGUIN study, obinutuzumab, as a single agent, has shown promising activity in patients with relapsed/refractory FL, with an ORR of 50%.[142] In a study on patients with rituximab-refractory FL (the GADOLIN, phase III study), patients were randomized to receive either bendamustine or bendamustine+obinutuzumab. Maintenance therapy with obinutuzumab was given to responders in the bendamustine+obinutuzumab arm. Obinutuzumab increased the PFS, but no difference was seen in OS.[143] The GALLIUM trial is a phase III trial, in which previously untreated advanced-stage FL patients were randomized to rituximab- or obinutuzumab-based chemotherapy. Obinutuzumab-based chemotherapy resulted in a significantly lower risk of progression, relapse, or death than rituximab-based chemotherapy (3-year PFS 80% vs. 73%). However, obinutuzumab was associated with increased infusion-related toxicity and neutropenia compared with rituximab, while the OS was similar in the two groups.[144]

Ibrutinib evaluated in the relapsed setting, has been found to have moderate activity as a single agent in FL, with an ORR of 37.5% and a 2-year PFS of 20%. *CARD11* mutations were present in 16% of the patients, who were thus predicted to exhibit resistance to ibrutinib.[145] In the first-line setting, ibrutinib has been combined with rituximab in a study comparing two different schedules. In arm 1, rituximabx4 weekly was combined with 560 mg ibrutinib daily until progression or toxicity. The treatment in arm 2 was 560 mg ibrutinib daily for 8 weeks, followed by the original schedule of arm 1. A higher ORR was found in arm 1 (85% vs. 75%), while a higher grade of toxicity, with rash and diarrhoea, was found in arm 2.[146]

Idelalisib is an oral inhibitor of PI3K delta which is a regulator of B-cell function downstream of the BCR. In relapsed/refractory FL, idelalisib was found to give an ORR of 57%, and has been approved for this indication.[147] However, several

studies on combination therapies involving idelalisib were closed early due to severe toxicity, such as cytomegalovirus reactivation, the occurrence of opportunistic infections and pneumonitis. However, single-agent idelalisib has a clear role in rituximab-refractory FL. The second-generation inhibitors of PI3K, duvelisib and TGR1202, are currently being studied, and show an encouraging ORR (87-91%) with toxicity profiles similar to that seen for idelalisib.[148]

Lenalidomide is an oral immunomodulatory agent that is effective against malignant B-cells and their microenvironment through anti-proliferative and anti-angiogenic action. Lenalidomide as a single agent has been found to give only modest response rates, but the response is markedly improved when rituximab is added (ORR 63-77%). A randomized phase II trial on patients with recurrent FL (ALLIANCE) reported an ORR of 95% and 5-year OS of 100% in the lenalidomide plus rituximab combination arm.[149] In another phase II-trial on advanced-stage, untreated FL, the combination of rituximab and lenalidomide showed an ORR of 98% and CR after 36 months was seen in 79%.[150] Preliminary results from the SAKK phase II study in cooperation with the Nordic Lymphoma Group, comparing rituximab monotherapy with rituximab-lenalidomide, showed that combination therapy led to a significantly higher rate of CR and an improvement in CR after 30 months, but no difference was seen in the OS.[110] The RELEVANCE trial comparing lenalidomide and rituximab vs. rituximab-chemotherapy is undergoing evaluation. This is an important study as a chemotherapy-free approach is being directly compared with standard chemoimmunotherapy in patients with high-tumour-burden FL (NCT 01650701). According to a press release from December 2017, no difference had been found in the primary endpoints, CR or PFS between lenalidomide-rituximab and the rituximab-chemotherapy arm.[151]

Targeting apoptosis involves both pro- and antiapoptotic effectors. Venetoclax is an inhibitor of the antiapoptotic BCL2 protein. The response rate of FL patients to venetoclax was found to be 38%, which is lower than could have been expected.[152] Several ongoing studies are investigating the combination of venetoclax with chemotherapy and other novel agents.

The programmed death receptor and its ligand (PD-L1) inhibitors have demonstrated very encouraging efficacy in the treatment of lymphoma, particularly in cases of relapsed Hodgkin's lymphoma. The PD-1 inhibitor, pembrolizumab is being studied in combination with rituximab, in cases of relapsed FL. At the pre-planned interim analysis, the ORR was found to be 80% and CR 60%.[153] Another PD-1 inhibitor, nivolumab, studied in 10 patients with FL, showed an ORR of 40%.[154] Other checkpoint inhibitors, against PD-L1, durvalumab and atezolizumab, are currently being investigated.

Vitamin D

Only limited data are available on the variation in prognosis in cases of DLBCL with season of diagnosis. One of the most potent effects of sunlight is the cutaneous synthesis of vitamin D. Calcitriol (1,25-dihydroxyvitamin D3) is the biologically most active form of vitamin D, and exerts its effects through the vitamin D receptor (VDR). The VDR is not only present in cells involved in calcium regulation, but also in malignant cells.[155] Calcitriol exerts antiproliferative and prodifferentiating effects, and inhibits the proliferation of malignant cells by inducing cell cycle arrest, causing cells to accumulate in the G0/G1 phase.[156-158] Calcitriol also induces apoptosis, causing the disruption of mitochondrial function, cytochrome release, and the production of reactive oxygens species. Calcitriol also inhibits angiogenesis, reducing the metastatic and invasive potential of the malignant cell.[159-161] Calcitriol also exerts an anti-inflammatory effect by suppressing the activation and signalling of NF-κB, which regulates the genes involved in the inflammatory and immune responses and cellular proliferation. The VDR is expressed in activated CD4+ and CD8+ T lymphocytes and antigen-presenting cells such as macrophages and dendritic cells.[162,163] These results illustrate the important immunomodulatory effects of vitamin D (see Figure 4).

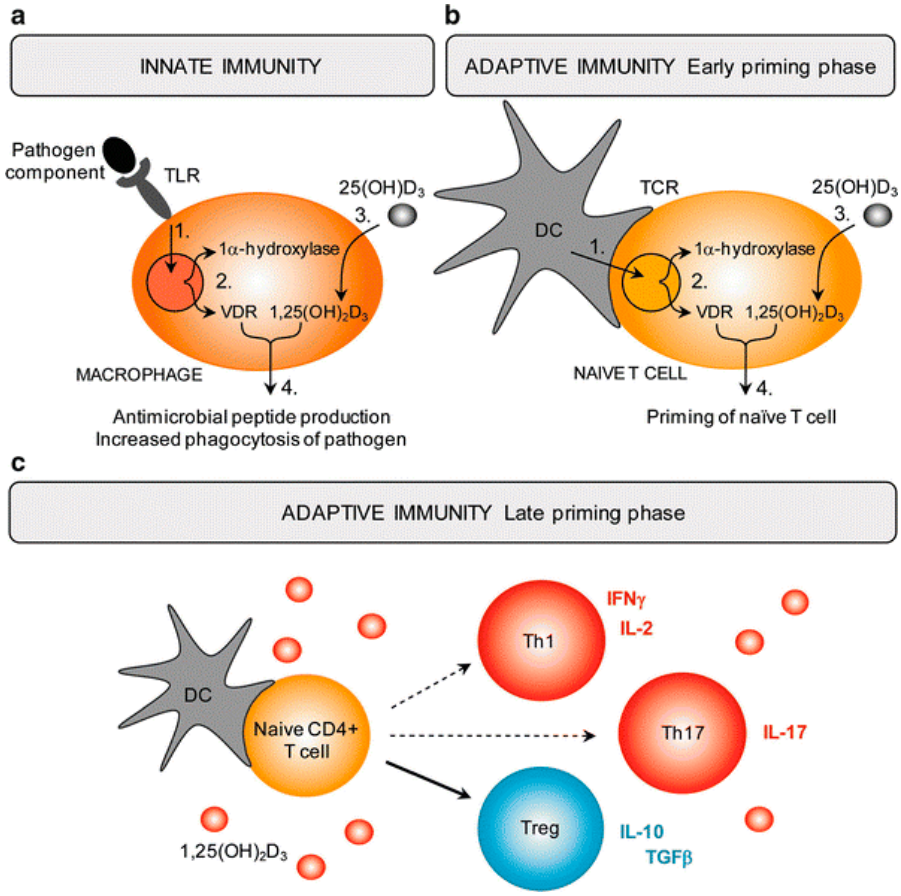

Figure 4:
Proposed model for the immunomodulatory effects of 1,25-dihydroxyvitamin D3-VDR. (a) Innate immunity: (1) In response to TLR stimulation, (2) macrophages induce expression of 1α-hydroxylase and VDR, (3) leading to the production of 1,25-dihydroxivitamin D3 and the activation of VDR, (4) promoting the production of antimicrobial peptides and increased phagocytosis of the pathogen. (b) Adaptive immunity: (1) In response to DC-induced TCR stimulation, (2) naive T cells induce the expression of 1α-hydroxylase and VDR, (3) leading to the production of 1,25-dihydroxyvitamin D3 and the activation of VDR, (4) encouraging priming of the naïve T cells. (c) In the secondary lymphoid organs, where T-cell priming takes place, an increase in 1,25-dihydroxyvitamin D3 is expected. 1,25-dihydroxyvitamin D3 influences the DCs to favour differentiation of Treg cells as opposed to Th1 and Th17 effector T cells. 1,25-dihydroxyvitamin D3 also enhances the function of the Treg cells and inhibits the function of Th1 and Th17 cells. Dotted lines and text rendered in red indicate inhibitory actions of 1,25-dihydroxyvitamin D3 and black lines and text rendered in blue increased actions. From[164] (Reprinted by permission from Springer Nature: Springer eBook, copyright 2018)

Several reports suggest that low serum 1,25-hydroxyvitamin D3 levels may be associated with increased cancer incidence and mortality. The most investigated cancers in this respect are colorectal and breast cancer.[165,166] Although a recently presented meta-analysis found no evidence of a protective role of vitamin D in NHL[167], there is evidence that vitamin D may have an effect on NHL survival. Drake et al. found that vitamin D deficiency was associated with poorer event-free

and overall survival in DLBCL and T-cell lymphoma patients.[168] It has also been found in a Norwegian study on Hodgkin's lymphoma that the season of diagnosis was a strong prognostic factor, particularly for patients younger than 30 years.[169] Vitamin D deficiency has also been found to impair rituximab-mediated cellular cytotoxicity, and was associated with inferior outcome in elderly DLBCL patients treated with rituximab.[170] These previous studies provided the rationale for initiating a phase III trial to test whether vitamin D replacement affected tumour response and prognosis in NHL (NCT01787409).

Other sunlight-potentiated and vitamin-D-independent pathways may play a role in reducing cancer risk, particularly prostate cancer and NHL. Exposure to sunlight has been found to modulate subclinical immunosuppression[171] and the circadian rhythm,[172] and to be associated with increased degradation of folic acid[173]. Previous studies have shown that patients with solid tumours and lymphomas with high inflammatory activity have inferior overall survival. Thus, immunomodulation induced by sunlight may have a favourable effect in this respect.[174]

Patients

The study populations in these studies were identified through the Swedish Lymphoma Registry (SLR). The SLR was established in 2000 by the Swedish Lymphoma Group in order to expand the data available in the Swedish Cancer Registry, which was set up in 1958. Reports to the Swedish Cancer Registry are made primarily by the pathologist, and do not include clinical data on prognostic classification and treatment. The SLR is administered by the Regional Cancer Centre, which is notified upon the registration of a lymphoma diagnosis in the Swedish Cancer Registry. The Regional Cancer Centre initiates a case file, and the local health care unit responsible for the patient completes the case file. Between 2000 and 2007, cases were reported on paper, and the SLR contained basic clinical characteristics. Detailed data on treatment were added to the SLR from 1 January 2007, and since then registration has been managed in a web-based reporting system. The coverage of the SLR has been validated, showing that the data cover approximately 95-95% of all lymphoma cases diagnosed in Sweden.

Paper I.

The study population included all patients diagnosed with DLBCL in Sweden from 1 January 2000 to 31 December 2010, who were included in the SLR. Patients with primary CNS lymphoma or HIV-related lymphoma were excluded. In total, 5349 patients diagnosed with DLBCL were identified in the SLR during this period.

Paper II

The study population consisted of all patients diagnosed with DLBCL in Sweden from 1 January 2007 to 31 December 2012. This gave a total of 3443 patients after patients with CNS involvement had been excluded.

Paper III.

In total, 5875 cases of DLBCL and 1693 cases of HL were identified in the SLR from 1 January 2000 to 31 December 2011. Of these, 4812 (82%) of patients with DLBCL and 1510 (90%) of the patients with HL received treatment with curative

intent. Patients with primary CNS lymphoma or HIV-related lymphoma were excluded.

Paper IV.

The study population included all patients diagnosed with FL grade I-IIIA, from 1 January 2000 to 2 December 2015. Out of a total number of 4679 registered patients with FL, information on initial treatment was available in 2251 cases. Patients with FL grade IIIB or confirmed histological transformation at the time of diagnosis were excluded. Detailed information on treatment was included in the SLR from 1 January 2007. In the case of patients registered before 2007, information on treatment was collected from patient records.

Statistical methods

In all the studies, OS was defined as the time from the diagnosis to the time of death from any cause, or the latest follow-up. The Kaplan-Meier method was used to estimate OS. The log-rank test was used to compare survival curves, and the Cox regression model was used for uni- and multivariable analyses.

Paper I

A Cox model with one term for sex dependence and two terms, one linear and one quadratic, accounting for the age dependence, was used to evaluate the prognostic impact of clinical risk factors and disease presentations. The χ^2 test was used for relationships between prognostic factors. The χ^2 test and linear by linear association were used for comparisons of time periods. Age-standardized incidence was calculated according to the direct method (general population Sweden 2000). Linear regression was used for the estimation of differences in age-standardized incidence. To understand the interdependence of age and gender, the following Cox model was used: RR=exp[-0.11x(female gender)+0.057x(age at diagnosis-70)+(age at diagnosis-70)^2]. Statistical analyses were performed using SPSS version 18, and R version 2.12.0.

Paper II

In multivariable analyses, the effect of chemotherapy was adjusted for WHO-PS (linear), s-LDH, gender, bulky disease, stage (as a factor on four levels) and age. Age was modelled as a restricted cubic spline with five knots, to more truthfully allow the effect of increased age on survival to vary in impact among different ages. To test the stability of the results and further reduce the risk of bias due to differences in age and prognostic factors between patients receiving etoposide vs. those not receiving etoposide, stratified Cox regression was performed, thus allowing for different baseline hazards across strata. The strata were defined by age in eight groups, including patients up to 65 years, adjusted for s-LDH, WHO-PS, stage, gender, bulky disease, as above, as well as age in eight groups separated by age-adjusted IPI (analyses adjusted for gender and bulky disease). Data were analysed using STATA version 13 and SPSS version 22.

Paper III

The impact of light exposure was defined as a periodical, continuous variable: cos[(month of diagnosis-φ)/12x2π], where φ is the phase. For example, a value of 6 implies that the function varies between -1 in December and +1 in June. The phase was estimated over the integers assigned to the 12 months. In addition, age, gender, disease stage, year of diagnosis, WHO-PS, number of extranodal sites and s-LDH were included as cofactors. Potential time trends were investigated by introducing the interaction between the terms for light exposure and the time periods 2000-2004, 2005-2007 and 2008-2011. Data from patients with the diagnoses DLBCL and HL were analysed separately. Analysis of the distribution of time from diagnosis until the start of treatment was performed with the Kruskal-Wallis test. Data were analysed in STATA.

Paper IV

Multivariable Cox regression was employed for the analysis of OS using 95% confidence intervals (CIs). The analyses were adjusted for the prognostic factors gender, FLIPI, bulky disease and date of diagnosis. Survival curves were estimated according to the Kaplan-Meier method, and compared with the log-rank test. Statistical analyses were performed using SPSS version 22. The χ^2 test was used to test for relationships between prognostic factors. To minimize immortality bias in the analysis of maintenance therapy, data were conditioned to reach 200 days overall survival.

Results

Improvement in survival of patients with diffuse large B-cell lymphoma

This study addresses incidence patterns and temporal changes in survival, within age categories, gender and prognostic groups, according to disease presentation.

The median age at diagnosis in the cohort was 70 years, with a male predominance (55% of patients). The median age at diagnosis was lower for males, 69 years, than for females, 72 years. The relative risk of OS according to multivariable analyses was higher for men (p=0.001), and this difference was constant during the observation period.

As expected, a pronounced association was found between age at diagnosis and OS. During the whole period, the incidence of DLBCL was higher for men than for females. There was an estimated yearly increase in incidence for men of 0.019 per 10,000 (see Figure 5).

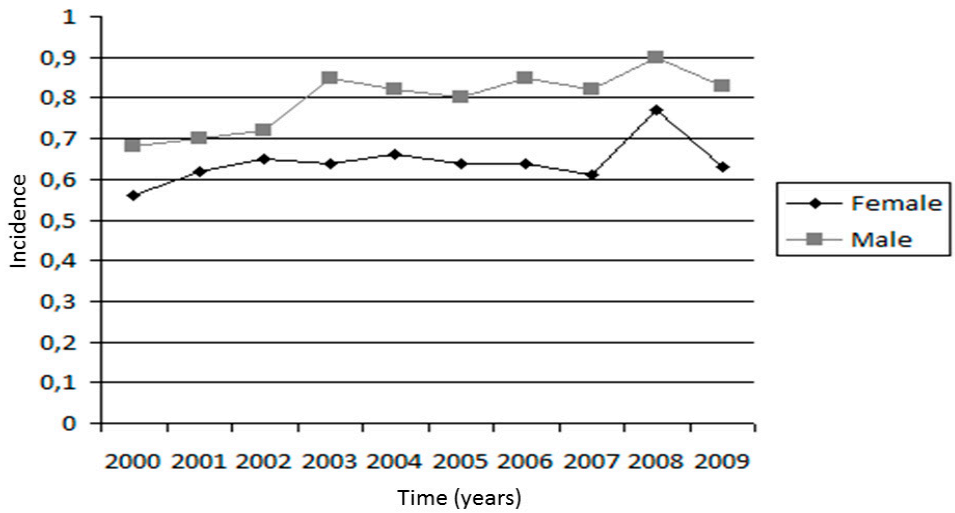

Figure 5:
Age-standardized incidence of DLBCL Sweden by year of diagnosis and gender.

Extranodal involvement of CNS, bone, bone marrow and urinary bladder were associated with adverse survival both in uni- and multivariable analyses (see Table12).

Table 12:
Hazard ratios (HR) for OS, unadjusted and adjusted for age, gender, stage, s-LDH, PS and number of extranodal sites

	Univariable			Multivariable		
	HR	95% CI	p-value	HR	95% CI	p-value
CNS, n=51	2.49	(1.84-3.03)	p<0.001	2.18	(2.70-3.73)	p<0.001
Bone, n=407	1.15	(0.99-1.32)	p=0.062	1.70	(1.43-2.03)	p<0.001
Bone marrow, n=634	1.61	(1.47-1.79)	p<0.001	1.29	(1.13-1.46)	p<0.001
Urinary bladder, n=33	1.96	(1.23-2.93)	p=0.001	1.60	(1.00-2.57)	p=0.049

The OS of DLBCL patients has improved markedly during the past decade. The improvement was most prominent in the age group 60-78 years, and in patients presenting with favourable WHO-PS, and was significant in all IPI groups, except for the low-risk category (see Figures 6-7).

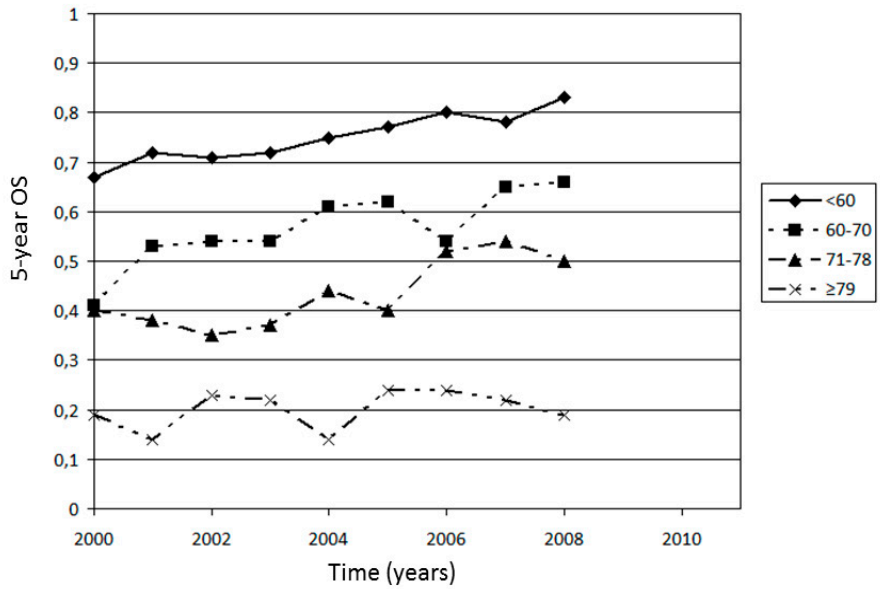

Figure 6:
5-year OS of DLBCL patients in Sweden during the period 2000-2010, according to age quartile.

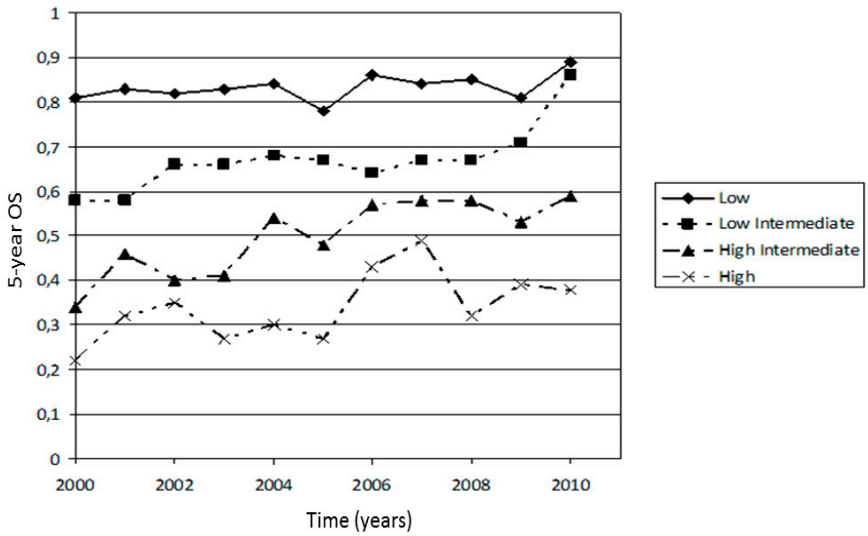

Figure 7:
5-year OS of DLBCL patients in Sweden during 2000-2010 according to IPI.

Patients aged 60-78 years have been treated to a higher degree with curative intent. The fraction without curative treatment decreased during this period in all age groups, except for the oldest (≥79 years), being 37% in 2000-2005, and 32% in the later half of the decade (p=0.14). In the youngest quartile, this fraction decreased from 4.2 in 2000-2005 to 1.4% in 2006-2010 (p=0.007), and the change in treatment intent was most pronounced among patients aged 70-78 years: 13 to 5.4% (p<0.001).

Impact of the addition of etoposide to chemotherapy in DLBCL patients

This study compared the OS for DLBCL patients treated with different chemotherapeutic regimens. The median age at diagnosis was 70 years, and there was a slight male predominance of 55%.

Data on treatment were available for 2838 (82%) patients. R-CHOP-14 was the most commonly used regimen (42%). As expected, the most intensive regimen, R-CHOEP-14, was more frequently administered to younger patients, with a poor prognosis. Patients receiving R-CHOP-21 had a higher median age. A lower proportion of patients with elevated s-LDH, Ann Arbor stage III-IV and the presence of bulky disease were treated with R-CHOP-21.

According to univariable analysis, patients receiving R-CHOEP-14 had a superior 5-year OS rate of 84%, compared to 70% for-CHOP-14 and 56% for R-CHOP-21 (see Figure 8). In the univariable Cox regression analyses, a strong association was found between chemotherapy regimen and survival, but after adjustment for prognostic factors, no evidence of an overall difference between the chemotherapy regimens remained.

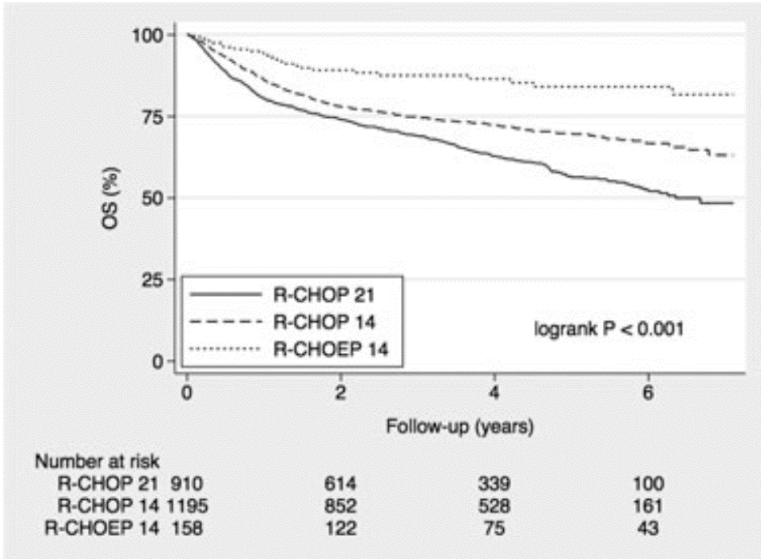

Figure 8:
OS of DLBCL patients in Sweden during 2007-2013, according to chemotherapy regimen.

A cohort of 1304 patients aged up to 65 years, consisting only of patients eligible to receive etoposide, in terms of toxicity tolerance, was studied. Of these, 201 patients received R-CHOP-21, 657 R-CHOP-14 and 155 R-CHOEP-14. Patients in this subgroup given R-CHOP-21 also showed more favourable prognostic features, apart from a higher median age. Five-year OS rates were 85%, 78% and 84% for the patients who received R-CHOP-21, R-CHOP-14 and R-CHOEP-14, respectively. After adjusting for the prognostic factors, R-CHOEP-14 was found to be associated with superior outcome compared with R-CHOP-14 (HR: 0.64 95% CI: 0.4-1.0 p=0.06) and R-CHOP-21 (HR: 0.49 95% CI: 0.3-0.9 p=0.028).

Impact on survival of season of diagnosis in DLBCL and in HL

In this study, the hypothesis that patients with DLBCL and HL diagnosed and treated during the summer season may have a better outcome was investigated. The study was restricted to patients receiving treatment with curative intent, thus including 82% of patients with DLBCL and 90% of patients with HL. The median age of the patients with DLBCL was 67 years, and that of the HL patients, 38 years. Data on the start of treatment were available for patients diagnosed from 2007 and onwards. The median time to start of treatment was 20 days for patients with DLBCL and 18 days for those with HL.

The season variable was analysed with multivariable Cox regression, adjusted for age, gender, stage, WHO-PS, s-LDH, number of extranodal sites and year of diagnosis. According to this analysis, diagnosis during the summer was associated with a significantly better OS in patients with curatively treated DLBCL (HR: 1.08 95% CI: 1.02-1.14 p=0.006) (see Figure 9). Further multivariable analysis, according to gender, showed improved survival to be restricted to males (HR: 1.09 95%CI: 1.01-1.17 p=0.026). However, no statistically significant association was found for patients with HL.

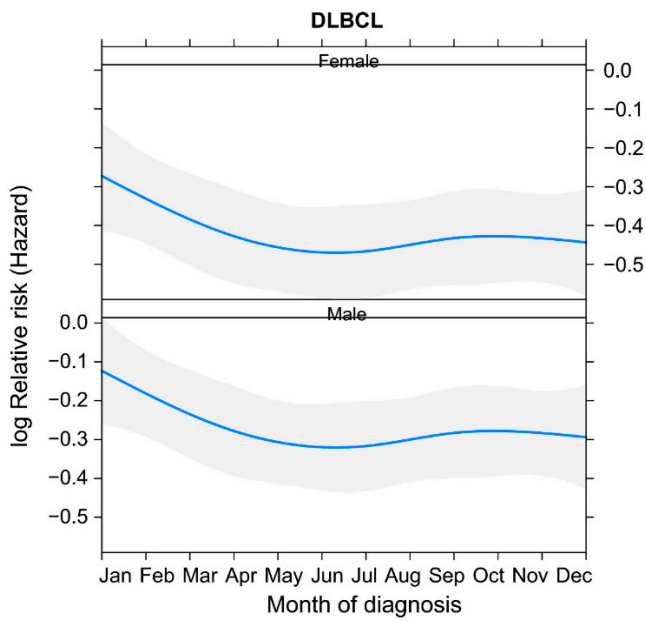

Figure 9:
The relative risk of OS according to month of diagnosis of DLBCL, with January as the reference category

First-line treatment of follicular lymphoma

The aims of this study were to determinate the initial management and efficacy of different treatment options for FL patients in Sweden, including watchful waiting, rituximab monotherapy, chemoimmunotherapy and radiation therapy.

Information on initial treatment was available in 2251 (48%). The median follow-up time for surviving patients with treatment data was 48 months, the median age at the time of diagnosis was 65 years (range: 18-100 years), and a slight majority were female (1177, 52%). In the group lacking treatment data, the incidence of FLIPI=2 was statistically significantly lower ($p=0.018$), and the incidence of bone marrow involvement was higher ($p<0.001$). Otherwise, there were no differences between the two groups. The 5-year OS for patients with available treatment data was 75%, vs. 72% for patients for whom no treatment data were available.

In 617 (28%) cases of newly diagnosed FL, watchful waiting was the primary option. No significant difference was found between female and male patients in the choice of immediate treatment or no treatment. However, significantly more patients <70 years ($p<0.001$), with bulky disease ($p<0.001$) and with verified bone marrow involvement ($p<0.001$) were given immediate treatment. The 5-year OS for the immediate treatment group was 77%, vs. 75% for the watchful waiting group. As can be seen in Figure 10, according to univariate analysis, there was no difference in survival between patients receiving immediate treatment and those in whom watch and wait was applied. Neither was there any significant difference in survival after adjustment for prognostic factors (HR, 0.909; 95% CI 0.691-1.195, $p=0.492$).

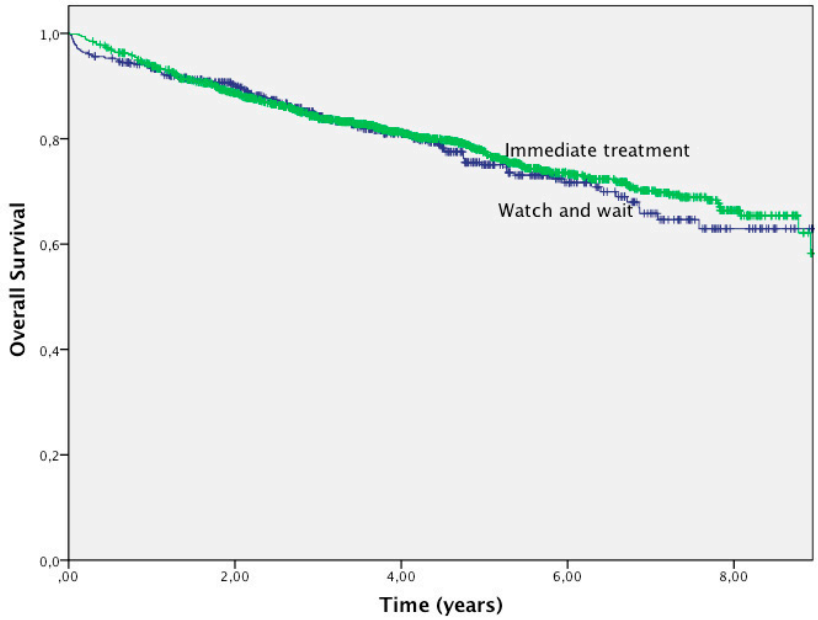

Figure 10:
Survival curves estimated using the Kaplan-Meier method (unadjusted analyses, all patients), for immediate treatment and watch and wait.

Data on the type of immediate treatment were available for 1603 patients. In 12 cases the patients were treated by surgery, steroids, etc., and in 19 cases, data on immediate treatment was not available. The 5-year OS for patients treated with immunotherapy was 84%, vs. 75% and 89% for those treated with chemoimmunotherapy or radiotherapy, respectively. The patient characteristics according to treatment modality are given in Table 13. Figure 11 shows the OS of patients treated with each modality. According to the multivariable analyses, the only significant finding was that chemotherapy alone was inferior to immunotherapy (p=0.009). Otherwise, no differences were seen in the overall survival of patients treated with immunotherapy vs. chemoimmunotherapy (p=0.283) or immunetherapy vs. radiotherapy (p=0.737).

The majority of the patients treated with radiotherapy were stage I (N=218, 74%) or II (N=45, 15%). The radiation dose was 30 Gy in 168 patients (57%), 24 Gy in 84 patients (29%) and 4 Gy in 41 patients (14%).

Table 13:
Patient characteristics according to therapy modality

Variable	Immunotherapy	Chemoimmuno-Therapy	Radiotherapy	Combined modality therapy	Chemotherapy	All
N	347	736	342	30	148	1603
Gender						
Female	195 (56%)	341 (46%)	192 (56%)	16 (53%)	72 (49%)	816 (51%)
Male	152 (44%)	395 (54%)	150 (44%)	14 (47%)	76 (51%)	787 (49%)
Age group						
<70	263 (75%)	489 (67%)	239 (70%)	26 (86%)	58 (39%)	1075 (67%)
70-80	61 (18%)	193 (26%)	69 (20%)	2 (7%)	46 (32%)	371 (23%)
=>80	23 (7%)	54 (7%)	34 (10%)	2 (7%)	44 (29%)	157 (10%)
Bulky disease						
Yes	29 (8%)	239 (33%)	11 (3%)	7 (23%)	21 (14%)	307 (19%)
No	302 (89%)	487 (66%)	328 (96%)	23 (77%)	117 (79%)	1264 (79%)
Unverified	9 (3%)	10 (1%)	3 (1%)	0	10 7%)	32 (2%)
Date of diagnosis						
<2010	176 (51%)	373 (51%)	138(40%)	16 (53%)	93 (62%)	796 (50%)
>=2010	171 (50%)	363 (49%)	204 (60%)	14 (47%)	55 (38%)	807 (50%)
FLIPI						
0	25 (7%)	24 (3%)	68 (20%)	6 (20%)	2 (1%)	125 (8%)
1	94 (27%)	135 (19%)	147 (43%)	7 (20%)	27 (18%)	410 (26%)
2	98 (28%)	202 (27%)	30 (9%)	4 (14%)	39 (27%)	373 (23%)
3	32 (9%)	127 (17%)	3 (0,5%)	3 (11%)	35 (23%)	200 (12%)
4	6 (2%)	38 (5%)	2 (0,5%)	1 (4%)	7 (5%)	54 (3%)
Missing	92 (27%)	210 (29%)	92 (27%)	9 (31%)	38 (26%)	441 (28%)
Bone marrow involvement						
Yes	96 (28%)	260 (35%)	7 (2%)	5 (17%)	52 (35%)	420 (26%)
Missing	251 (72%)	476 (65%)	335 (98%)	25 (83%)	96 (65%)	1183 (74%)
5-year OS	84%	75%	89%	78%	53%	

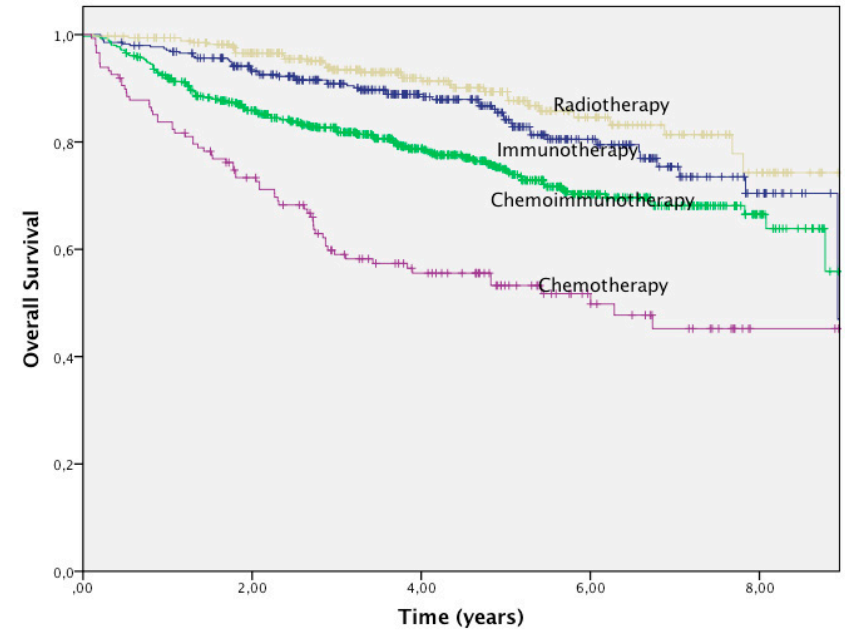

Figure 11:
Survival curves estimated using the Kaplan-Meier method (unadjusted analyses) for radiotherapy, immunotherapy, chemoimmunotherapy and chemotherapy.

A total of 1146 patients with detailed medical treatment data were identified. Table 14 summarizes the patient characteristics and response assessment according to the choice of induction therapy. In 342 cases, the patients were treated with radiotherapy, and in 30 cases with combined modality therapy.

The 5-year OS for the R-CHOP group was 80%, vs. 84% and 82% for single-agent rituximab and RB, respectively.

Overall survival is shown in Figure 12. Multivariate analysis revealed no difference in efficacy between rituximab monotherapy and R-CHOP (p=0.891), or between rituximab monotherapy and RB. (p=0.501)

Table 14: Patient characteristics and response assessment according to the choice of induction therapy

Variable	R-CVP	R-CHOP	Rituximab monotherapy	RB	R-chlorambucil	Chlorambucil	All
N	36	486	347	144	31	102	1146
Gender							
Female	16 (46%)	225 (46%)	295 (85%)	68 (47%)	16 (51%)	49 (48%)	568 (50%)
Male	20 (54%)	261 (54%)	152 (15%)	76 (53%)	15 (49%)	53 (52%)	578 (50%)
Age groups							
<70	9 (25%)	353 (73%)	263 (76%)	97 (67%)	8 (26%)	30 (29%)	760 (66%)
70-80	17 (47%)	111 (23%)	61 (18%)	40 (28%)	9 (29%)	39 (38%)	277 (24%)
=>80	10 (28%)	22 (5%)	23 (6%)	7 (5%)	14 (45%)	33 (33%)	109 (10%)
Bulky disease							
Yes	4 (11%)	178 (37%)	29 (8%)	41 (29%)	7 (23%)	9 (9%)	268 (23%)
No	32 (89%)	301 (62%)	309 (89%)	102 (71%)	22 (71%)	86 (84%)	852 (75%)
Unverified	0	7 (1%)	9 (3%)	1	2 (6%)	7 (7%)	26 (2%)
Rituximab maintenance							
Yes	1 (2%)	210(43%)	26(7%)	55(38%)	6(19%)	0	298(26%)
No	35(98%)	253(52%)	310(90%)	81(56%)	25(81%)	94(92%)	798(70%)
Unverified	0	23(5%)	11(3%)	8(6%)	0	8(8%)	50(4%)
Date of diagnosis							
<2010	26 (72%)	294 (60%)	176 (51%)	14 (10%)	16 (51%)	61 (60%)	587 (51%)
=>2010	10 (28%)	192 (40%)	171 (49%)	130 (90%)	15 (49%)	41 (40%)	559 (49%)
FLIPI							
0	1 (3%)	13 (3%)	25 (7%)	5 (3%)	2 (6%)	2 (2%)	48 (4%)
1	2 (6%)	100 (21%)	94 (27%)	29 (20%)	1 (3%)	21 (21%)	247 (22%)
2	6 (17%)	124 (26%)	98 (28%)	53 (37%)	8 (26%)	22 (21%)	311 (27%)
3	4 (11%)	85 (17%)	32 (9%)	25 (17%)	5 (16%)	28 (27%)	179 (15%)
4	7 (19%)	17 (3%)	6 (2%)	8 (6%)	2 (6%)	3 (3%)	43 (4%)
Missing	16 (44%)	147 (30%)	92 (27%)	24 (17%)	13 (43%)	26 (26%)	318 (28%)
Bone marrow involvement							
Yes	15 (42%)	170 (35%)	96 (28%)	50 (35%)	11 (35%)	34 (33%)	376 (33%)
Missing	21 (58%)	316 (65%)	251 (72%)	94 (65%)	20 (65%)	68 (67%)	770 (67%)
Response evaluation			255(73%)				
ORR (CR/PR)	22(61%)	445(92%)	103(30%)	129(90%)	18(58%)	71(70%)	940(82%)
CR	9(26%)	232(48%)	152(44%)	72(50%)	7(23%)	14(14%)	437(38%)
PR	13(36%)	213(44%)	34(10%)	57(40%)	11(36%)	57(56%)	503(44%)
SD	2(5%)	7(1%)	29(8%)	2(1%)	1(3%)	10(10%)	56(5%)
PD	2(5%)	19(4%)	29(8%)	7(5%)	2(6%)	7(6%)	66(6%)
Missing	10(28%)	15(3%)		6(4%)	10(32%)	14(14%)	84(7%)
5-year OS	18%	80%	84%	82%	53%	53%	

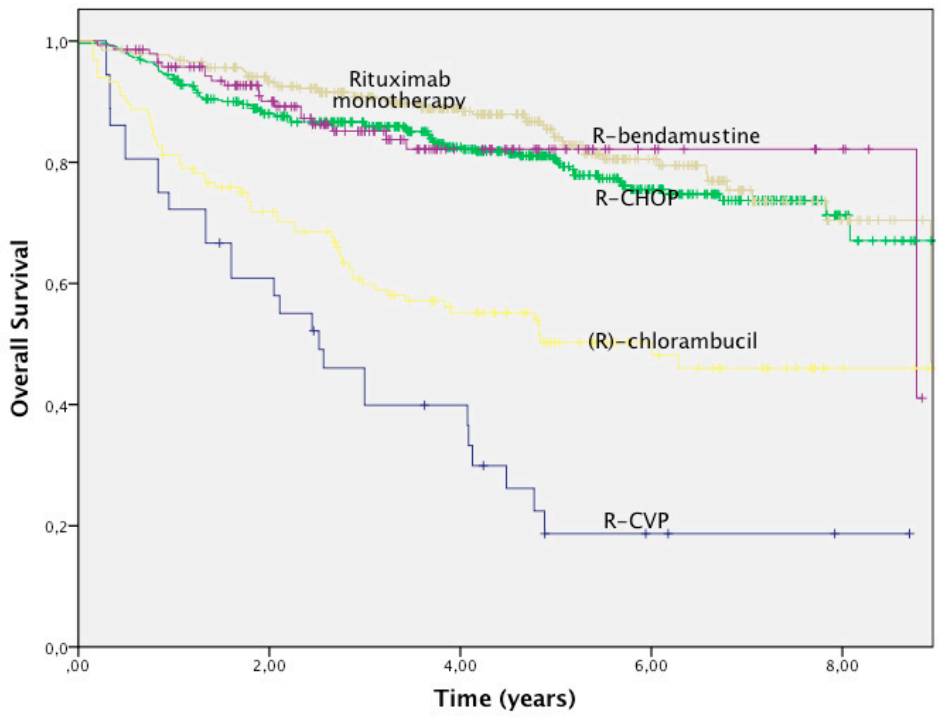

Figure 12:
Survival curves estimated using the Kaplan-Meier method (unadjusted analyses) for single-agent rituximab, R-CHOP, R-bendamustine, chlorambucil, R-chlorambucil and R-CVP.

A minority of the patients, 298 (26%), receiving different chemoimmunotherapy regimens, also received rituximab maintenance therapy. The 5-year OS for the patients treated with rituximab maintenance was 83%, vs. 75% for the group receiving no further treatment. According to multivariate analysis, corrected for immortal time bias, and adjusted for gender, FLIPI, bulky disease, quality of initial response, and date of diagnosis, rituximab maintenance treatment was associated with superior overall survival (HR 0.56; 95% CI 0.37-0.87, p=0.010). To minimize immortality bias, in the analyses of maintenance therapy, data were conditioned to reach 200 days overall survival.

Discussion and future perspectives

The cohorts of patients in all four studies described in this thesis were identified through a population-based registry. Population-based studies have both strengths and limitations, and can serve as complements to randomized controlled trials. Booth et al. have compared and summarized the strengths and limitations of randomized and population-based studies.[175] Population-based observational trials can offer good external validity, which provides an excellent opportunity to evaluate the effect of new treatments and their outcomes in routine practice. This is in contrast to randomized trials, which have good internal validity through randomization, minimizing the risk of bias by confounding. Randomization ensures that the only difference between the treatment arms is their exposure to the treatment of interest. Patients are highly selected for participation in randomized trials, which is a major limiting factor in interpreting the results, especially if the patients in routine practice are very different from those included in randomized trials. Patients with advanced age and greater comorbidity,[176,177] and those with lower socioeconomic background[178] are under-represented in randomized trials. In contrast to randomized trials, population-based studies provide insight into the delivery of care in routine practice to all patients, including the elderly and those with comorbidity. Through large samples, population-based studies also provide the opportunity to study rare diseases, which is not possible in randomized studies. Another strength of population-based studies is the insight gained into short- and long-term toxicity in routine practice, and the study of other questions that have not, and will not, be evaluated in randomized trials. In addition, a population-based study can be used to formulate a hypothesis for a randomized trial and help establish the appropriate sample size[179].

Population-based studies also have several important limitations. Limited internal validity means difficulties in separating the effects of new treatment from other factors. The absence of detailed data regarding comorbidity and factors that may not be identified or measurable using observational data include stage migration, changes in the disease biology, confounding by indication and treatment decisions based on partly undocumented factors.

One of the major limitations of the present studies is missing data. The data are collected retrospectively, and in general, only standard parameters are recorded, which is also a limitation in the investigation of novel prognostic factors.

The lack of a central pathology review, which is not feasible in large cohorts, and the lack of central evaluation of imaging, are limitations of the studies presented here. In the study described in Paper IV, the median follow-up time was relatively short. The lack of data on PFS, duration of response, further lines of treatment and comorbidity are also limitations that will affect the OS, especially in indolent diseases, such as follicular lymphoma.

Incidence and risk factors in DLBCL

It has previously been shown that the incidence of DLBCL among men increased up to the year 2000.[5] In the United States, the incidence shows a plateau during the 1990s.[4] An increase in incidence was also seen within the past decade among men, but not among women, in the present work (Paper I). The reason why this increase is restricted to males is not clear, but may be due to gender differences in exposure to environmental toxic or infectious agents. DLBCL has a complex multifactorial aetiology. B-cell-activating autoimmune disease, hepatitis C virus seropositivity, a family history of NHL, lower socioeconomic status, lower recreational sun exposure and higher young adult BMI have previously been associated with increased risk of DLBCL.[180] The most important risk factors are probably those associated with immune function. B-cell-activating autoimmune disease and hepatitis C virus seropositivity are associated with chronic immune stimulation. Further work is required to establish the relation between the molecular characterstics of distinct subtypes of DLBCL (GCB vs. ABC) and relation to immune stimulation due to different risk factors.

Prognostic factors in DLBCL

The assessment of prognosis in DLBCL is based on the clinical prognostic factors summarized in the IPI. Clinical trials have shown a gradual improvement in outcome for patients with DLBCL, but less is known about the subgroups with a dismal prognosis. Specific involvement of bone marrow, CNS, bone and urinary bladder was found to be associated with inferior outcome in multivariable analyses in the present work (Paper I). Bone marrow involvement is the most commonly involved extra nodal site with an inferior prognosis.[101] There is still no consensus concerning the need for bone marrow biopsy or PET/CT for the evaluation of bone marrow involvement in DLBCL.[181-183] However, based on a retrospective study, Goldschmidt et al. suggested that the finding of monoclonal *IGHV* gene rearrangement in bone marrow for a subset of DLBCL patients with high IPI

played a prognostic role.[184] CNS involvement confers a dismal prognosis, which requires specific CNS-directed treatment.[185] Bone involvement has also previously been found to be associated with inferior survival.[186] Involvement of urinary bladder is a relatively uncommon presentation in DLBCL. After review of the literature, no data was found on the prognosis of DLBCL with urinary bladder involvement. Further studies, which take this extranodal presentation into account, will need to be undertaken.

Male sex has been found to be associated with inferior OS, both in the present work and in other studies. One possible explanation has been provided by the German Study Group, who found that the elimination half-life of rituximab was significantly longer in women than in men. A subsequent Swedish population-based study confirmed that male gender is a risk factor in young patients with DLBCL.[187] It is possible that hormonal factors are responsible for these findings.

Age is a strong prognostic factor in DLBCL. Approximately half of all lymphoma cases occur in patients older than 65 years, and one-third of reported cases are aged over 75 years. The incidence of DLBCL in older patients has increased during recent decades due to increased longevity. A geriatric assessment, including the evaluation of comorbidity, organ function, list of medications, evaluation of geriatric factors and geriatric syndromes is necessary to tailor individual treatment. A pronounced association was found between age at diagnosis and OS in the present work (Paper I), probably because elderly patients were systematically considered too frail to receive full treatment, and were thus treated with half-dose regimens. When the population was divided into quartiles according to age, the estimated 5-year survival for the youngest quartile was 74%, compared to 22% for patients in the oldest quartile (≥79 years).

Considerable effort has been devoted to improving prognostic indices for DLBCL. The metabolic tumour volume assessed by PET/CT may have a prognostic value. In several recently published studies,[188-190] this was confirmed as a possible prognostic marker with more potential predictive power than Ann Arbor stage.[191]

The SLR database does not contain information on subtypes of DLBCL, which is also a limitation. Such data should be included in the registry, possibly through linkage to pathology databases.

Treatment of DLBCL

DLBCL is considered a highly curable disease with conventional anthracycline-based chemotherapy, such as CHOP. The introduction of monoclonal antibodies over recent decades has led to an increase in survival of approximately 15% to

20% across age groups, and established R-CHOP as the standard treatment option. Despite the successful results achieved with R-CHOP, a subset of patients is not cured. In the perspective of clinical prognostic factors, elderly patients and patients with a high IPI have cure rates of approximately 50%, indicating that efforts to improve outcome should be focused on these groups. Methods to improve the efficacy of R-CHOP have included shortening the cycle length, administering more cycles, adding more cytotoxic agents, or delivering chemotherapy as a long-term infusion. Patients who relapse early after first-line chemoimmunotherapy have an inferior prognosis. High-dose chemotherapy with ASCT can cure patients with relapsed DLBCL.[192] Patients <60 years previously showing CR, no CNS or bone marrow involvement, and previous response to a conventional rescue protocol have benefited most from this treatment. Thus, this subset of patients, which requires more aggressive initial treatment, and is in need of novel, targeted therapy, could be identified already at the time of diagnosis. Patients with a high IPI should be considered for high-dose chemotherapy, methotrexate and cytarabine, in addition to R-CHO(E)P to avoid disease recurrence in the CNS.[193]

Elderly patients with DLBCL exhibit inferior survival, especially when comorbidities are present.[194] R-mini-CHOP is a well-tolerated therapy, and is currently considered to be the standard treatment for elderly patients.[50] Achieving CR is of critical importance for disease-free survival.[195,196] This group of patients is in need of novel strategies, new chemoimmunotherapy agents and tailored treatment to increase the rate of complete remission.

The role of etoposide in DLBCL treatment

The aim of the study presented in Paper II was to compare the outcome using three chemoimmunotherapy regimens (R-CHOP-21, R-CHOP-14 and R-CHOEP-14) in a population-based cohort, in terms of OS, adjusted for clinical prognostic factors. No difference in efficacy was seen between R-CHOP-21, R-CHOP-14 and R-CHOEP-14 in the whole population group, after adjustment for prognostic factors. However, when restricting the analysis to patients aged ≤65, the data indicated that R-CHOEP-14 was associated with a superior OS. No randomized trial has been performed to evaluate the addition of etoposide to the treatment of DLBCL in the rituximab era. However, the present results are in line with those from a Danish population-based study.[197,198] It has also been reported that R-CHOEP-14 is well tolerated by patients younger than 65 years, with acceptable toxicity.[44,199] A recently published Swedish population-based study, based on SLR data, indicates that patients <60 years with age-adjusted IPI≥2 appear to show superior OS after more intensive treatment with the addition of etoposide and high-dose cytarabine or methotrexate.[200]

Despite the absence of randomized studies,R-CHOEP-14 should be considered as a possible treatment alternative for high-risk young patients with DLBCL.

Initial treatment of follicular lymphoma

There is still no consensus regarding the optimal initial treatment of FL. The aim of the study described in Paper IV was to shed additional light on this matter. The overall survival of patients treated with rituximab versus chemoimmunotherapy (R-CHOP and RB) was compared. The results indicated that initial treatment with rituximab as a single-agent does not seem to impair long-term outcome. In addition, long-term follow-up of patients treated with rituximab only, suggests that a considerable proportion of patients, about 30%, will not need additional chemotherapy.[94,107] To the best of our knowledge, no randomized trials have been performed, or are planned, to compare the efficacy of rituximab to that of chemoimmunotherapy. However, without a randomized trial, we cannot exclude a bias, that the patients receiving rituximab monotherapy in this study, may have had more favourable characteristics.

Several studies support a chemotherapy-free approach in newly diagnosed cases of FL. The SAKK 35/98 trial investigated the potential benefits of extended rituximab treatment compared to a standard schedule of rituximab induction. Approximately 85% of the patients in that study presented with advanced stage disease, and more than 50% had bulky lesions. PFS was longer in the maintenance arm but the OS was not significantly different.[107] Increased toxicity was also seen in the maintenance arm. The RESORT study, including patients with low-tumour-burden FL, confirmed that rituximab retreatment is preferable to maintenance treatment.[108] These results confirm that rituximab monotherapy is a valid first-line treatment option, associated with a similar OS to that achieved with chemoimmunotherapy.

When comparing different chemoimmunotherapy regimens in the present work, it was found that RB and R-CHOP had similar efficacy in terms of OS. These findings are in line with those from randomized studies (StiL and BRIGHT).[118,119] RB is a well-tolerated and less toxic regimen than R-CHOP. Thus, based on the results of randomized trials, the recommended treatment for high-tumour-burden symptomatic patients with FL, requiring chemoimmunotherapy for rapid tumour reduction, is RB.

The addition of rituximab maintenance after RB induction, which could further improve the efficacy of the RB induction regimen, is being assessed in an ongoing StiL study, MAINTA

IN (NCT00877214). The role of rituximab maintenance therapy after chemoimmunotherapy was investigated in the PRIMA trial, which showed a significant improvement in PFS with rituximab maintenance therapy, but no effect on OS. Contrary to expectations, a significant difference in OS was found in the present work between patients treated with induction and rituximab maintenance, compared with patients receiving induction therapy and no further treatment. These results should be interpreted with caution as no information was available on the schedule or duration of treatment. However, rituximab maintenance therapy is recommended after induction with R-CHOP in the Swedish national guidelines, based on the improved PFS in the PRIMA trial.

Generally, the overall survival of patients with FL has improved, and the median OS now exceeds 12 years. This indolent, chronic disease is characterized by a heterogeneous clinical evolution. Some FL patients never require therapy, or require only one line of therapy, and the latter may be considered cured.[201] Other subsets of FL patients have a high risk of death due to their disease. Histological transformation or recurrence within 2 years of chemoimmunotherapy should be considered as high-risk factors. Future research should focus on the search for prognostic biomarkers that can be used to identify these high-risk patients at diagnosis. The development of predictive biomarkers for targeting agents and strategies to reduce the risk of HT needs to be investigated.

Season of diagnosis in DLBCL

Several studies have been carried out on the effect of sunlight on the development of lymphoma. In the InterLymph NHL Subtype Project, increased sunlight exposure was found to be associated with a decreased risk of DLBCL.[180] This suggests that serum vitamin-D level or sunlight-mediated immune modulation may play a role in the lymphomagenesis of DLBCL. However, the meta-analysis of large amounts of data was unable to confirm the protective role of vitamin D in DLBCL.[167] This may indicate that other effects of sunlight could have a protective role. Immune modulation may play a crucial role in lymphomagenesis, but other effects, such as circadian rhythm or folate degradation may also be important.

The aim of the study presented in Paper III was to investigate whether the season of diagnosis had any effect on the survival of patients with DLBCL and HL. In a previous Norwegian study on HL,[202] the authors found that the season of diagnosis was a strong prognostic factor. Drake et al. found that vitamin D deficiency was associated with inferior prognosis in DLBCL and T-cell lymphoma patients.[168] A recently presented prospective study on DLBCL patients showed that achievement of a normal serum vitamin D level after vitamin D3 supplementation was

associated with improved outcome.[203] Vitamin D deficiency has been defined as a vitamin D level below 10 ng/mL, and is very common among elderly patients.[202] Other characteristics associated with low vitamin D level are poor WHO-PS, overweight, B symptoms, elevated LDH, and lower albumin and haemoglobin levels. These associations are probably not unexpected and explainable. After oral supplementation of vitamin D, with a loading dose and a maintenance phase, the serum vitamin D level increased significantly in about 56% of patients. All the patients were treated with R-CHOP. The conclusions drawn from the results of this study were that patients with a vitamin D level below 20 ng/mL at diagnosis had an inferior EFS, and that patients with normalized vitamin D levels following supplementation showed better EFS than patients with persistently low vitamin D levels. One explanation of the effect of vitamin D supplementation on patients with DLBCL receiving R-CHOP has been suggested to be the major mechanism of action of rituximab, antibody-dependent cell mediated cytotoxicity.[170]

The findings discussed above indicate that vitamin D levels should be monitored in newly diagnosed DLBCL patients, and supplements given if deemed necessary. This will probably most often be the case in the older patient group, based on their lower serum vitamin D-level and inferior outcome. Further studies are needed to elucidate the role of vitamin D in DLBCL, and explain the genetic polymorphism in the genes coding for proteins involved in the vitamin D pathway.

Conclusions

Paper I

The findings of this study indicate that attention should be focused on DLBCL patients with high-risk factors and on elderly patients who may require specially tailored treatment.

Paper II

R-CHOEP-14 was associated with superior overall survival in patients with DLBCL aged up to 65 years, indicating that this may be a valid treatment option for this patient population.

Paper III

The season of diagnosis was shown to have an impact on overall survival in male patients with DLBCL. It can be speculated that this seasonal effect is due to vitamin D synthesis in the skin by sun exposure, but other explanations are possible. Further investigations are required to investigate the effect of vitamin D status during the treatment of DLBCL.

Paper IV

For patients with FL, an initial watch-and-wait strategy led to meaningful OS and remains a viable option in the modern era. For patients with symptoms requiring early treatment, rituximab monotherapy should be considered as one of the first-line treatment options.

Concluding remarks

DLBCL is a curable disease in 60-70% of cases, and the prognosis for patients with FL is very good, with a 12-year median OS. However, some subgroups of patients with both diagnoses have an inferior outcome. Identifying these subgroups using current molecular techniques and customizing treatment with existing and novel agents is the challenge facing us at present. The findings presented in this thesis show that clinical prognostic factors and the season of diagnosis affect the survival of patients with DLBCL, and underline the need for further research to find biological prognostic markers.

Future management of DLBCL

The treatment of DLBCL patients has improved over recent decades, and the majority of patients are cured with standard R-CHOP. The evaluation of clinical prognostic factors through better staging with the help of PET/CT and increased knowledge of the epidemiology of DLBCL has probably contributed to this improvement in survival. The administration of granulocyte-colony stimulating factor during R-CHOP treatment, to minimize the risk of neutropenic infections and provide an adequate cycle length, may also have contributed. The assessment of COO has recently become part of the clinical routine, allowing treatment to be tailored to specific groups of patients with DLBCL. The adaptation of treatment based on COO is not yet standard in clinical care, although we await the results of ongoing trials. Both the GCB and ABC subtypes of DLBCL, as defined by GEP, are heterogeneous and contain biological subgroups that have different prognoses and may require different therapy.

There are two ongoing phase III trials focusing on COO in which R-CHOP is being compared with R-CHOP+targeted agent in ABC (DLBCL). In the ROBUST trial, R-CHOP is being compared with lenalidomide plus R-CHOP (NCT02285062) and in the PHOENIX trial (NCT 01855750) R-CHOP is being compared with ibrutinib plus R-CHOP.

DH lymphoma has an extremely aggressive clinical course, and the results with R-CHOP are very poor.[55] DH lymphoma occurs in 10% of DLBCLs and almost 91% belong to the (GCB) DLBCL phenotype. According to the results of retrospective studies, more intensive regimens, DA-EPOCH-R and R-HyperCVAD, are the preferred treatments.[57,205] In the prospective phase II study CALGB 50303, DA-EPOCH-R is being tested in *MYC*-associated high-grade lymphoma. The preliminary results after a median follow-up time of 14 months show 77% OS.[58] This preliminary finding also supports the choice of more aggressive regimens. The role of consolidative stem cell transplant is unclear.

DE lymphoma patients with coexpression of MYC and BCL2 proteins have a poor prognosis. DE lymphoma is more common in the ABC subtype and it has been suggested that it contributes to the overall inferior prognosis of patients with this subtype.[17] The results of previous trials and the preliminary results of ongoing trials suggest that R-CHOP or DA-EPOCH-R+targeted agent (lenalidomide or ibrutinib), could be considered as a treatment alternative. In general, the optimal treatment of DE lymphoma is still unknown, and clinical trials are recommended.

The treatment of DLBCL is still evolving, based on the recognition of major genetic and biologic subtypes. Mutation of the *EZH2* gene, altered germinal centre B-cell migration based on the mutation of different genes (*S1PR2, GNA13, ARHGEF1* and *P2RY8* inactivating the Gα13-dependent pathway) and mutations altering the tumour microenvironment (HVEM receptor *TNFRSF14* gene) are the subject of ongoing trials regarding (GCB) DLBCL. In the case of (ABC) DLBCL, the BCR signalling NF-κB pathway and mutations of *CD79A/B*, *MYD88* and *CARD11*, which affect this pathway, have a crucial role.

BCL2 expression is common in aggressive B-cell lymphoma. Venetoclax is a selective BCL2 inhibitor, and the addition of venetoclax to DA-EPOCH-R in DH and TH lymphoma patients is being investigated in an ongoing phase I trial (NCT03036904). A CD79B immunotoxin, polatuzumab vedotin, is also under investigation in clinical trials. In a phase III trial, POLARIX, the addition of polatuzumab vedotin to rituximab-cyclophosphamide-doxorubicin-prednisone is being compared with R-CHOP (NCT03274492).

The immune checkpoint inhibitors have resulted in therapeutic benefits, particularly in patients with Hodgkin lymphoma. The general response rates of patients with DLBCL and FL are lower, about 20-40%, and response is not sustained.[154,206] Combinations of these agents are under investigation. Another approach to the treatment of lymphoid malignancies is the development of genetically modified chimeric antigen receptor T cells.

A considerable amount of research is ongoing on the role of vitamin D deficiency in the outcome of lymphoma. The findings of the study included in this thesis

support those from other research, that the season of diagnosis may affect the outcome of patients with DLBCL. Taken together, these findings suggest that the sunlight-activated vitamin D could play an important role in immunomodulation and in the enhancement of the effect of chemoimmunotherapy. Analyses of serum vitamin D levels and replacement therapy are simple and feasible, and their introduction into clinical practice and in national guidelines should be considered.

Future management of FL

The results of the population-based study on FL included in this thesis, support the results obtained in previous randomized studies. Initial management with watchful waiting is recommended for patients without symptoms related to the disease, in order to prolong the time to first treatment.

If the patients are in need of therapy, monotherapy with rituximab without chemotherapy is effective, with a high ORR and prolonged PFS. The efficacy of rituximab monotherapy, in terms of OS, was equal to that of R-CHOP and RB, according to the present work. No randomized trials have been performed, or are planned, to compare the efficacy of rituximab to that of chemoimmunotherapy.

Comparison of the different chemoimmunotherapy regimens confirmed that RB is a suitable treatment for patients in need of rapid tumour reduction. The most important goals in the management of FL are to avoid overtreatment of asymptomatic patients with indolent disease, prolong the PFS for patients with high-tumour-burden disease receiving therapy, and identify patients at high risk of HT and refractory/recurrent disease. Another important goal is to prevent toxicity and the late effects of standard chemoimmunotherapy due to the long natural history of the disease and the repeated need for therapy during the patient's lifetime. Thus, several trials are ongoing to identify strategies including targeting the BCR, CD20, the tumour microenvironment, epigenetic modifiers and checkpoint inhibition. Promising preliminary results from the RELEVANCE study, comparing R-lenalidomide to R-chemotherapy, indicate that there was no difference in the primary endpoints, CR and PFS, between R-lenalidomide and R-chemotherapy.[151] This is an important study as it compares a chemotherapy-free approach to chemoimmunotherapy in high-tumour-burden FL. The effect of venetoclax in patients with FL has been investigated by Davids et al., who observed a 38% response rate, with 14% achieving CR.[152] Several trials are in progress testing venetoclax in combination with other novel agents and with chemotherapy. A number of ongoing trials are investigating obinutuzumab in combination with lenalidomide or venetoclax or polatuzumab vedotin. The results

from the GALLIUM trial, confirming a significantly prolonged PFS with obinutuzumab combined chemotherapy, suggest that obinutuzumab could be included in future therapy combinations instead of rituximab. However, no improvement in response rate, according to CT-based assessment, was demonstrated in this study, and the overall survival was similar in the two groups. The role of maintenance therapy after induction in patients in CR is also debatable due to lack of evidence in terms of improving overall survival.

In the case of rituximab-refractory disease, the GADOLIN study showed that obinutuzumab combined with bendamustine may be the treatment of choice. Checkpoint inhibitors, such as the PDL1 inhibitors durvalumab and atezolizumab are being investigated both in combination with chemotherapy, and with other targeted agents.

With the development of DNA- and RNA-sequencing techniques, many genetic alterations will be detected in different NHL subtypes, allowing tailoring of treatments with novel strategies. There is thus a need to adopt new and more efficient clinical trial designs. Dividing the patients according to genetic alterations will lead to smaller subsets of patients, necessitating multicentre collaboration.

Together, population-based studies and randomized clinical trials will provide complementary information to facilitate tailored treatment with novel agents to the individual patient with DLBCL and FL.

Populärvetenskaplig sammanfattning (Summary in Swedish)

Maligna lymfom är tumörer utgående från immunsystemets celler, så kallade lymfocyter. I Sverige drabbas ca 2000 patienter per år. Dessa utgörs av ca 50 olika sjukdomar med mycket varierande symptombild och biologi. Den stora majoriteten maligna lymfom, ca 90 %, utgår från B-lymfocyter, så kallade B-cellslymfom. Resterande lymfom ca 10 %, utgår från T-lymfocyter och NK (natural killer) celler. Vissa av B-cellslymfomen uppvisar ett snabbt och aggressivt förlopp, så kallade aggressiva lymfom, medan andra är lågproliferativa, kroniska sjukdomar, så kallade indolenta lymfom.

Den vanligaste subtypen (ca 500 fall/år, 25%) är diffust storcelligt B-cellslymfom (DLBCL) som är ett aggressivt lymfom. Med modern cytostatika- och antikropps-behandling botas uppskattningsvis 50 % av patienterna med denna typ av lymfom. Prognosen är i hög grad beroende av kliniska riskfaktorer, vilka sammanfattas i ett prognostiskt index, IPI.

Standardbehandlingen av denna sjukdom är idag cytostatika (CHOP) i kombination med en antikropp (rituximab), så kallad R-CHOP-behandling. Anti-kroppen rituximab är riktad mot ett ytprotein, CD20, som finns uttryckt på majoriteten av B-cellslymfom.

R-CHOP kan ges med tre respektive två veckors intervall (R-CHOP-21 resp R-CHOP-14). Tillägg av etoposid till R-CHOP har testats i studier och det föreligger stöd för användning av R-CHOEP för yngre patienter med avancerad sjukdom. Pga toxiciteten avråds patienter över 70 år från denna behandling.

Follikulärt lymfom (FL) är den näst vanligaste lymfomtypen. De flesta av dessa tumörer är långsamväxande sjukdomar med indolent förlopp, och anses f n ej botbara med konventionell terapi. För denna sjukdom finns ett stort antal behandlingsalternativ. Initialt kan man ofta avstå från behandling, men ca 80% av patienterna kommer att bli i behov av aktiv behandling. Detta kan innefatta antikroppsbehandling (rituximab), antikroppsbehandling kombinerad med cyto-statika (bendamustin, CHOP eller CVP) eller lokal strålbehandling. Också för denna sjukdomsgrupp finns ett prognostiskt index, FLIPI, vilket väl kan förutsäga

prognosen för sjukdomen. Jämförande randomiserade kliniska prövningar av de ovan nämnda behandlingsalternativen saknas i de flesta fall.

Den Svenska Lymfomregistret (SLR) innehåller insamlade kliniska uppgifter på 95% av de lymfompatienter som insjuknade i Sverige sedan år 2000. Sedan 2007 samlas även behandlingsdata in via elektronisk plattform. De fyra studierna inkluderade i den här avhandlingen baseras på SLR. Retrospektiva populations-baserade studier är ett bra komplement till randomiserade kliniska studier som bara innefattar utvalda patienter.

I den första studien beskrevs de kliniskt relevanta prognostiska faktorerna och incidensutvecklingen av patienter med DLBCL, insjuknade i Sverige mellan 2000-2010. Enligt förväntningarna påverkades prognosen negativt av hög ålder och extranodala manifestationer i form av benmärg, skelett och CNS. Manligt kön påverkade också prognosen negativt. Incidensen av sjukdomen ökade under den studerade tidsperioden, ffa hos män. Generellt har överlevnaden av patienter med DLBCL förbättrats under decenniet, förutom för patienter över 78 år och patienter med dåligt allmäntillstånd. Framtidens behandlingsfokus bör därför läggas på dessa två patientgrupper för att även där förbättra överlevnaden.

I den andra studien jämfördes de olika behandlingsregimer som används vid behandling av DLBCL. Resultaten visade ingen skillnad i överlevnad om patienter behandlades med 2 veckors eller 3 veckors intervall. Etoposid tillägget till R-CHOP för patienter under 65 år, visade bättre överlevnad jämfört med patienter som behandlades bara med R-CHOP.

I den tredje studien analyserades överlevnaden av patienter med DLBCL och Hodgkin-lymfom, beroende på när de insjuknade under olika årstider. De patienter som fick sina diagnoser under sommarmånaderna uppvisade bättre överlevnad än de patienter som blev sjuka under vinterperioden. Detta gällde enbart DLBCL patienter och var mer uttalad hos män. Resultaten tolkas i första hand med olika D-vitamin nivåer under årstiderna, som har en immunomodulerande effekt.

I den fjärde studien undersöktes överlevnadsskillnader mellan patienter med follikulärt lymfom. Syftet med studien var att jämföra total överlevnad beroende på om patienten aktivt observeras eller omedelbart behandlas. I studien jämfördes även överlevnaden av patienter som omedelbart behandlas med singel rituximab jämfört med R-CHOP och R-bendamustin. Överlevnadsanalyserna visade ingen skillnad mellan aktiv uppföljning utan behandling jämfört med omedelbar behandling och behandlingen med singel rituximab verkar vara lika effektiv som rituximab kombinerad med cytostatika.

Sammanfattningsvis har de här fyra studierna bidragit till en samlad beskrivning av patienter med DLBCL och FL, avseende riskfaktorer, incidens och behandlingsval. Resultaten från den andra och den fjärde studien kan bidra till

ökad kunskap om effekten av de olika behandlingar som används för DLBCL och FL patienter i Sverige. Dessa retrospektiva studier är värdefulla eftersom randomiserade studier inte har gjorts eller planeras för dessa behandlingsregimer. Resultaten från den tredje studien ger stöd för att vidare undersöka D-vitaminets verkningsmekanismer även på genetisk nivå.

Populationsbaserade analyser av behandlingsresultat utgör ett värdefullt komplement och det Svenska Lymfomregistret har genom hög täckningsgrad en unik möjlighet att bidra med data som kan utgöra bakgrund för fortsatt utveckling av riktlinjer för lymfombehandling och i sin tur för det dagliga beslutfattandet inom sjukvården.

Ismeretterjesztő összefoglalás (Summary in Hungarian)

A malignus limfómák az immunrendszer limfocitáiból erednek. Svédországban kb 2000-en betegednek meg limfómában évente. A limfóma diagnózis magába foglal kb 50 féle különböző altípust eltérő biológiai háttérrel és tünetegyüttessel. A limfómák kb 90%-a a B-limfocitákból ered, a maradék 10%-a a T-limfocitákból és NK-sejtekböl indul ki. A B-sejtes limfómák egy csoportja magas malignitású, ezek az aggresszív limfomák. A kevésbé malignus és alacsony proliferációs B-limfómák krónikus, hosszú lefolyású megbetegedések, úgynevezett indolens limfómák.

A legismertebb aggresszív limfóma a diffúz nagy B-sejtes limfóma (DLBCL) amely mintegy 25%-át teszi ki a limfómáknak. Ez a megbetegedés kb 50%-ban gyógyítható modern citosztatikum és antitest kombinációjával. A prognózis azonban nagy mértékben függ a klinikai prognosztikai faktoroktól, amelyeket egy nemzetközi prognosztikai index, az IPI foglal magába. A standard kezelés egy citosztatikum kombináció (CHOP) amelyet egy antitesttel, rituximabbal együtt alkalmaznak.(R-CHOP). A rituximab egy olyan antitest, amely a CD20 antigénre fejti ki a hatását. Ez az antigén a B-limfociták több mint 90-án megtalálható. Az R-CHOP kezelés 2 vagy 3 hetes intervallummal adható. Az R-CHOP kezelés effektivitását az etoposid hozzáadásával probálták növelni és tudományosan igazolt hatása van a 70 év alatti, előrehaladottabb megbetegedések esetén. 70 év feletti betegek esetén túl toxikusnak bizonyult.

A follikuláris limfóma a második leggyakoribb altípus, amely alacsony malignitású, indolens, krónikus lefolyasú betegség. Jelenlegi terápiás módszerekkel nem gyógyítható. Széles kezelési arzenál áll rendelkezésre ennek a megbetegedésnek a megfékezésére és az életkor meghosszabbítására. Az egyik terápiás stratégia az aktív ”várakozás”, kezelés nélkül, azokban az esetekben amikor a betegség nem jár tünetekkel. Azonban a betegek kb. 80%-a előbb-utóbb kezelésre szorul. Egy másik kezelési lehetőség a rituximab monoterápia. Előrehaladottabb megbetegedés esetén a rituximab kombinációja citosztikummal (CHOP, bendamusztin, CVP) is egy lehetséges kezelési alternatíva. Lokális sugárterápia is alkalmazható, jó hatással. A follikuláris limfómának is van egy

prognosztikai indexe, a FLIPI. Összehasonlító randomizált tanulmányok, amelyek a fenntebb említett kezelési stratégiákat vizsgálnák nincsenek.

Ebben a disszertációban szereplő tanulmányok alapját a svéd limfómaregiszter szolgálja, amely a limfóma megbetegségek klinikai paramétereit tartalmazza 2000 óta. A regiszter fedettsége 95%-os, ami azt jeleneti, hogy csaknem az összes limfóma megbetegedés Svédországban, 2000 óta regisztrálva van. 2007-ben a regisztrálás kibővült az alkalmazott kezelésekkel és azóta mindez elektronikus úton történik. Az ilyenfajta, regiszter alapú retrospektív populációs tanulmányok nélkülözhetetlen kiegészítői a randomizált klinikai tanulmányoknak, amelyek csak kiválasztott betegcsoportokon alapulnak.

Az első tanulmány a 2000-2010 között diagnosztizált DLBCL-es betegeknek a prognosztikai faktorait és a betegség incidenciáját tanulmányozza. Az elvárásoknak megfelelően az idős kór és a nyirokrendszeren kívüli megbetegedés, a csontvelő, a csont és az idegrendszer érintettsége negatívan befolyásolja a prognózist. A férfi nem is negatív prognosztikai faktor. A megbetegedés incidenciája növekedett a vizsgált idöperiódusban, leginkább a férfiak esetén. Általánosan a betegség túlélése javult ebben az időperiódusban, kivéve a 78 év feletti betegek esetén és akiknek előrehaladottabb megbetegedése volt a diagnózis megállapításakor. A jövőbeli tanulmányokat ezekre az alcsoportokra javasolt irányítani.

A második tanulmány a DLBCL-es betegek kezeléseit hasonlítja össze, megvizsgálva a túlélést. A három kezelési forma R-CHOP-14, R-CHOP-21 vagy R-CHOEP között a túlélésben különbség nem mutatkozott, ami az egész betegpopulációt illeti. Viszont a 65 év alatti betegek esetén az etoposid hozzáadása az R-CHOP-hoz jobb túlélést mutatott.

A harmadik tanulmány azt vizsgálja, hogy a DLBCL és a Hodgkin-limfómás betegeknek milyen a túlélése attól függően, hogy melyik évszakban diagnosztizálják és kezdik el a kezelést. A tanulmány eredménye azt mutatja, hogy a Hodgkin-limfómás betegek esetén nincs különbség viszont a DLBCL-es betegek esetén a túlélés jobb, ha a megbetegedés a nyári hónapokra esik. Ez különösen kifejezett a férfi betegeknél. Ez az eredmény leginkább az évszaktól függö D-vitamin nívó különbségekre vezethető vissza és annak az immunrendszert befolyásoló hatására. További vizsgálatokra van szükség ezeknek a feltételezéseknek a bizonyítására.

A negyedik és egyben utolsó tanulmány a follikuláris limfómás (FL) betegek túlélését vizsgálja attól függöen, hogy milyen terápiás eljárásban részesültek. A várakozó álláspont stratégia összehasonlítva az azonnali kezeléssel nem mutatott túlélési különbséget. A rituximab kezelés ugyanolyan hatásosnak mutatkozott mint a rituximab kombinációja citosztatikummal (bendamusztin vagy CHOP).

Összefoglalva, ez a négy tanulmány a DLBCL és FL betegek prognosztikai faktorait, a betegség incidenciáját és az alkalmazott kezelési eljárásokat elemzi. Randomizált tanulmányokat, amelyek összehasonlítanák ezeket a kezelési lehetőségeket nem terveznek. Emiatt figyelemreméltóak és felértékelhetőek az itt ismertetett eredmények. A D-vitamin immunrendszerre kifejtett hatását genetikai szinten is érdemes lenne tanulmányozni valamint megfontolni a D-vitamin hozzáadását a tervezett limfóma kezeléshez. A regiszterekre alapozott populációs tanulmányok eredménye nagyban hozzájárul a limfóma kezelések terápiás irányvonalához, amelyek nélkülözhetetlenek a betegek hétköznapi ellátásában.

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ORIGINAL ARTICLE: CLINICAL

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

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Abstract

Our aim was to describe a large population-based cohort of diffuse large B-cell lymphoma (DLBCL) during the last decade, evaluating possible improvement in survival and to identify subgroups in need of novel treatment strategies. The study population encompassed all patients diagnosed with DLBCL in Sweden from 2000 through 2010. Altogether 5349 patients were identified. There was no increase in incidence for females, but for males there was an estimated yearly increase in incidence by 0.019 per 10 000. When adjusted for age and gender, the improvement in overall survival for the whole group was estimated at 4.5% per year, most prominent in the age group 60–78 years, and in patients with good performance status. In this large dataset, we were able to detect a clear improvement in overall survival in DLBCL, although restricted to specific prognostic subgroups, and to identify specific disease presentations that significantly affect overall survival.

Keywords: Diffuse large B-cell lymphoma, prognostic factors, chemotherapy, incidence

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma. In Sweden the incidence is approximately 5.5 patients per 100 000, corresponding to 25% of all malignant lymphomas. Since the introduction of modern chemotherapy (dose-dense regimens and the addition of immunotherapy), approximately 50% of patients with DLBCL are cured [1–4]. The prognosis is strongly dependent on clinical factors, as summarized in the IPI (International Prognostic Index). Within clinical trials, the outcome of patients with DLBCL has shown a gradual improvement, but less is known about the impact in the population at large, or what subgroups of patients have benefited most from the improvement in therapy. The Swedish Lymphoma Registry (SLR) started in 2000, and has almost full coverage of patients with malignant lymphoma in Sweden. This study describes a population based cohort of patients with DLBCL, evaluating the possible improvement in survival during the period 2000–2010, in relation to patient age, prognostic groups and disease presentation. Our aim was to identify specific subgroups in need of novel treatment strategies.

Materials and methods

Swedish Lymphoma Registry

This is a retrospective study based on the population-based Swedish Lymphoma Registry. As previously reported, this registry was established in 2000 by the national Swedish Lymphoma Group (SLG) to provide a more detailed registration of patients with malignant lymphomas [5]. Compared to the compulsory Swedish Cancer Registry, the Swedish Lymphoma Registry displays coverage of approximately 95–97% of all cases of lymphoma in Sweden. In total, 49 hospitals treat patients with lymphoma and report to the SLR.

Study population

The study population included all patients diagnosed with DLBCL in Sweden from 1 January 2000 to 31 December 2010, and included in the Swedish Lymphoma Registry. Patients with primary central nervous system (CNS) lymphoma or human immunodeficiency virus (HIV)-related lymphoma were excluded. Data collected were year of diagnosis, gender, age, Ann Arbor stage, serum lactate dehydrogenase (S-LDH) level, World Health Organization (WHO) performance status, number of extranodal sites, type of extranodal involvement, B-symptoms and the presence of bulky disease (maximum diameter >10 cm), and whether treatment with curative intent was delivered. Data on survival status were obtained from the Swedish Population Registry, and updated as of 14 May 2013.

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Statistical methods

Survival curves were estimated according to the Kaplan–Meier method and compared by log-rank test. The Cox regression model was used for uni- and multivariate analysis. For evaluation of the prognostic impact of clinical risk factors and disease presentations, a Cox model with one term for the sex dependence and two terms, one linear and one quadratic, accounting for the age dependence, was used. The assumption of proportional hazards was checked graphically. For the dependence on year of diagnosis, the assumption of proportional hazards could not be validated. Therefore, stratification by year of diagnosis (1-year classes) was used. For interrelationships among prognostic factors, χ^2 tests were used. χ^2 test and linear by linear association were used for comparisons of the time periods. Age-standardized incidence was calculated according to the direct method (general population Sweden 2000). Linear regression was used for the estimation of differences in age-standardized incidence. Statistical analyses were performed using SPSS v 18 and R version 2.12.0.

Results

Patient characteristics

In total, 5349 patients diagnosed with DLBCL were identified in the Swedish Lymphoma Registry during 2000–2010. The median follow-up-time of surviving patients was 80 months. Patients' characteristics are summarized in Table I.

Table I. Patients' characteristics.

	Number (%)
All patients	5349 (100)
Gender	
Male	2919 (55)
Female	2430 (45)
Age	
Less than 40 years	273 (5)
40–59 years	1038 (19)
60–78 years	2915 (50)
79 years and older	1337 (25)
Missing value	26 (1)
Ann Arbor stage	
I	1052 (20)
II	1286 (24)
III	935 (18)
IV	1893 (36)
Missing value	237 (4)
Extranodal sites	
0–1	4537 (85)
More than 1	812 (15)
LDH level	
Normal	2036 (38)
Elevated	2970 (56)
Missing	343 (6)
Performance status WHO	
0–1	3812 (71)
More than 1	1395 (26)
Missing value	142 (3)
IPI	
0	379 (7)
1	1257 (24)
2	1423 (27)
3	1198 (22)
4	557 (10)
5	115 (2)
Missing value	420 (8)
Bulky disease	
No	3987 (75)
Yes	1122 (21)
Missing value	240 (4)
B-symptoms	
No	2929 (55)
Yes	2211 (41)
Missing value	209 (4)

LDH, lactate dehydrogenase; WHO, World Health Organization; IPI, International Prognostic Index.

Age and gender

The median age for all patients was 70 years (range 16–99, quartiles 60–79). A majority were males, 2919 cases (55%). The median age for males was lower, 69 years (range 17–96, quartiles 59–77) compared to females, 72 years (range 16–99, quartiles 61–80) ($p < 0.001$). The relative risk (RR) for overall survival (OS) in univariate analysis between these groups was not statistically significant (1.02, 95% confidence interval [CI] 0.95–1.10, $p = 0.62$). However, if stratified for age, stage, S-LDH, extranodal sites and performance status, the risk for men was higher: RR = 1.17 (95% CI 1.06–1.28, $p = 0.001$). This difference was constant during the observation period.

As expected, a pronounced association between age at diagnosis and OS was noted. Dividing the population into quartiles, the estimated 5-year survival for the youngest quartile was 74% compared to 22% for patients in the oldest quartile (≥ 79 years) (Figure 1).

Age-standardized incidence

During the whole period, the incidence was higher for men. There was no increase noted for females, with a constant age-standardized incidence of 0.65/10 000. For males, however, there was an estimated yearly increase in incidence by 0.019 per 10 000, from 0.68 in 2000 to 0.90 in 2008 ($p = 0.005$) (Figure 1).

Prognostic factors

As expected, in addition to age, all factors included in the IPI, i.e. stage, WHO performance status (PS), number of extranodal sites and S-LDH, were strongly related to survival. IPI was possible to calculate in 4929 patients (92%), with most patients distributed in the groups with IPI 1–4. The distribution of IPI groups did not change during the time period. The presence of B-symptoms was also associated

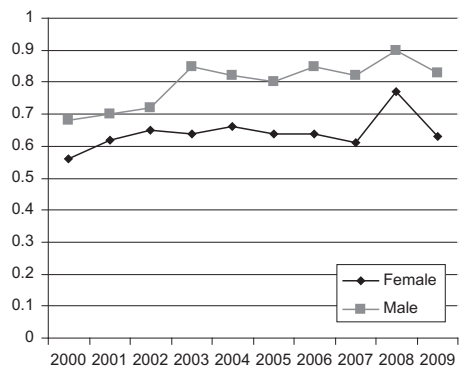

Figure 1. Age-standardized incidence of diffuse large B-cell lymphoma in Sweden by year of diagnosis and gender.

with adverse OS, after adjustment for age, gender and year of diagnosis; the relative risk was 1.64 (95% CI. 1.52–1.77, $p < 0.0001$). In addition, bulky disease (maximum diameter >10 cm) was associated with significantly higher relative risk, 1.40 (95% CI 1.28–1.53, $p < 0.0001$), after adjustment as above. When the prognostic impact of B-symptoms and bulky disease was evaluated in a Cox model together with IPI, their presence did not provide additional independent prognostic information.

Extranodal presentations

The most frequent extranodal presentation was bone marrow involvement, present in 634 (12%) cases, followed by skeletal in 407 (8%), gastric in 335 (6%), lung in 259 (5%) and liver in 242 (4%) cases. The frequencies of extranodal presentations were consistent across the regions in Sweden. When adjusted for age, gender, stage, S-LDH, performance status and number of extranodal sites, the RR for poor OS was highest for patients with CNS involvement, RR = 2.18 (95% CI 2.70–3.73), followed by bone involvement (ascites), RR = 1.70 (95% CI 1.43–2.03). In addition, involvements of bone marrow and urinary bladder were associated with adverse survival. Involvements of muscle, subcutaneous tissue, stomach and thyroid were associated with superior survival (Table II). Testicular involvement had no impact on OS.

Longitudinal survival analysis

By univariate Cox regression, the rate of improvement was similar for male and female patients. According to age at diagnosis, there was significant improvement (5.9–6.8% per year) in all age groups, except for the highest quartile (≥ 79 years) (Figure 2). Dividing the population according to IPI, significant improvement in OS was noted in all subgroups (Figure 3), except for the low risk group ($p = 0.20$), and was most prominent in the high intermediate risk IPI population (5.6% per year). There was no significant improvement in patients with poor PS (2–4). The rate of improvement was similar for patients with PS 0–1 or with more extranodal sites. No improvement was seen for patients with CNS or testicular involvement, but for patients with skeletal lesions, a significant yearly improvement of 5.6% was found.

To further understand the interdependence of age and gender, the following Cox model was adjusted: relative risk = $\exp[-0.11 \times (\text{female gender}) + 0.057 \times (\text{age at diagnosis} - 70) + 0.000543 \times (\text{age at diagnosis} - 70)^2]$ [6], where the gender term, although significant ($p = 0.0069$), has a rather large estimated standard deviation of 0.04. The other terms are highly significant. The quadratic correction term with positive sign means that the age effect is most pronounced in the higher age groups.

By use of the Cox model above, a substantial improvement in OS was noted for the group as whole during the period 2000–2013, estimated as 4.5% per year ($p < 0.001$) (Figure 3).

Therapeutic intent

For 4404 patients (82%) there was available information on therapeutic intent. During this decade, in total, 572 patients

Table II. Hazard ratios for OS, unadjusted and adjusted for age, gender, stage, LDH, PS and number of extranodal sites.

	Univariate	Multivariate
Bone, $n = 407$ (7.6%)	$p = 0.062$	$p < 0.001$
No	1 (—, —)	1 (—, —)
Yes	1.15 (0.99, 1.32)	1.70 (1.43, 2.03)
Bone marrow, $n = 634$ (12%)	$p < 0.001$	$p < 0.001$
No	1 (—, —)	1 (—, —)
Yes	1.61 (1.47, 1.79)	1.29 (1.13, 1.46)
Breast, $n = 51$ (1%)	$p = 0.283$	$p = 0.337$
No	1 (—, —)	1 (—, —)
Yes	0.81 (0.55, 1.19)	1.36 (0.73, 2.54)
CNS, $n = 58$ (1%)	$p < 0.001$	$p < 0.001$
No	1 (—, —)	1 (—, —)
Yes	2.49 (1.84, 3.03)	2.18 (2.70—3.73)
Gastric, $n = 335$ (6%)	$p = 0.214$	$p = 0.042$
No	1 (—, —)	1 (—, —)
Yes	1.10 (0.95, 1.27)	0.82 (0.68, 0.99)
Kidney, $n = 111$ (2%)	$p = 0.026$	$p = 0.915$
No	1 (—, —)	1 (—, —)
Yes	1.32 (1.04, 1.70)	1.02 (0.76, 1.36)
Large bowel, $n = 150$ (3%)	$p = 0.599$	$p = 0.985$
No	1 (—, —)	1 (—, —)
Yes	0.94 (0.75, 1.18)	1.00 (0.75—1.32)
Liver, $n = 242$ (4.5%)	$p = 0.001$	$p = 0.731$
No	1 (—, —)	1 (—, —)
Yes	1.33 (1.11, 1.58)	1.04 (0.85, 1.27)
Lung, $n = 259$ (5%)	$p < 0.001$	$p = 0.107$
No	1 (—, —)	1 (—, —)
Yes	1.42 (1.20, 1.67)	1.17 (0.97, 1.42)
Muscle, $n = 111$ (2%)	$p = 0.087$	$p = 0.003$
No	1 (—, —)	1 (—, —)
Yes	0.78 (0.59, 1.04)	0.60 (0.43, 0.85)
Ocular, $n = 11$ (0.2%)	$p = 0.567$	$p = 0.307$
No	1 (—, —)	1 (—, —)
Yes	1.26 (0.57, 2.81)	1.81 (0.58, 5.69)
Ovarian, $n = 12$ (0.2%)	$p = 0.693$	$p = 0.536$
No	1 (—, —)	1 (—, —)
Yes	0.84 (0.35, 2.02)	1.37 (0.51, 3.68)
Pancreatic, $n = 72$ (1.3%)	$p = 0.006$	$p = 0.857$
No	1 (—, —)	1 (—, —)
Yes	1.50 (1.12, 1.99)	0.86 (0.60, 1.22)
Peritoneal, $n = 83$ (1.6%)	$p < 0.001$	$p = 0.533$
No	1 (—, —)	1 (—, —)
Yes	1.65 (1.26, 2.17)	1.10 (0.81, 1.51)
Pleural, $n = 186$ (3.5%)	$p < 0.001$	$p = 0.187$
No	1 (—, —)	1 (—, —)
Yes	1.55 (1.29, 1.86)	1.15 (0.93, 1.43)
Salivary gland, $n = 40$ (0.7%)	$p = 0.447$	$p = 0.887$
No	1 (—, —)	1 (—, —)
Yes	1.17 (0.79, 1.77)	1.05 (0.56, 1.97)
Sinus, $n = 84$ (1.6%)	$p = 0.739$	$p = 0.287$
No	1 (—, —)	1 (—, —)
Yes	0.95 (0.71, 1.27)	0.81 (0.54, 1.20)
Skin, $n = 155$ (3%)	$p = 0.006$	$p = 0.072$
No	1 (—, —)	1 (—, —)
Yes	1.32 (1.08, 1.61)	1.32 (0.98, 1.79)
Small bowel, $n = 186$ (3.5%)	$p = 0.484$	$p = 0.961$
No	1 (—, —)	1 (—, —)
Yes	1.07 (0.88, 1.30)	1.01 (0.78, 1.29)
Subcutaneous, $n = 93$ (1.7)	$p = 0.966$	$p = 0.049$
No	1 (—, —)	1 (—, —)
Yes	0.99 (0.75, 1.31)	0.69 (0.48, 1.00)
Testicular, $n = 141$ (3%)	$p = 0.655$	$p = 0.688$
No	1 (—, —)	1 (—, —)
Yes	0.95 (0.76, 1.19)	1.07 (0.76, 1.52)
Thyroid, $n = 67$ (1.3)	$p = 0.028$	$p = 0.050$
No	1 (—, —)	1 (—, —)
Yes	0.65 (0.44, 0.95)	0.66 (0.43, 1.00)
Urinary bladder, $n = 33$ (0.6%)	$p = 0.001$	$p = 0.049$
No	1 (—, —)	1 (—, —)
Yes	1.96 (1.231, 2.93)	1.60 (1.00, 2.57)
Uterus, $n = 24$ (0.4%)	$p = 0.698$	$p = 0.389$
No	1 (—, —)	1 (—, —)
Yes	1.10 (0.67, 1.83)	0.76 (0.40, 1.42)

(*Continued*)

Table II. (*Continued*)

	Univariate	Multivariate
Vagina, $n = 8$ (0.1%)	$p = 0.024$	$p = 0.936$
No	1 (—, —)	1 (—, —)
Yes	2.35 (1.12, 4.94)	0.96 (0.36, 2.59)
Other extranodal site, $n = 216$ (4%)	$p = 0.742$	$p = 0.011$
No	1 (—, —)	1 (—, —)
Yes	0.97 (0.80, 1.17)	0.74 (0.59, 0.93)

CNS, central nervous system; LDH, lactate dehydrogenase; PS, performance status.

(11%) were treated without curative intent. This fraction generally decreased during this time period in all age groups, except for the oldest (≥79 years), being 37% in 2000–2005, and 32% in the latter half of this decade ($p = 0.14$). In the youngest quartile, this fraction declined from 4.2 to 1.4% from 2000–2005 to 2006–2010 ($p = 0.007$), and was most pronounced among patients 70–78 years, 13 vs. 5.4% ($p < 0.001$).

Discussion

This retrospective study is the largest population based series of DLBCL published so far, and specifically addresses incidence patterns and temporal changes in survival, within age categories, gender and prognostic groups, and according to presentation.

The size of the present study enables us to provide an even more complete picture of DLBCL on a population level, as compared to previous population based series [7–9]. The incidence of DLBCL has previously been shown to increase among men up to the year 2000 [10]. In the United States, the incidence shows a plateau during the 1990s [11]. In this series, we could document an increase also within the last decade among men, but not among women. The reason why this is restricted to males is not clear, but may be due to gender differences in exposure to environmental toxic or infectious agents.

As expected, we found age, Ann Arbor stage, number of extranodal sites, serum LDH level, PS, B-symptoms and bulky disease to be statistically significant negative prognostic factors [12–14]. In addition, specific involvement of bone marrow, CNS, lung, peritoneum and skin was associated with inferior outcome. Thyroid DLBCL was the most

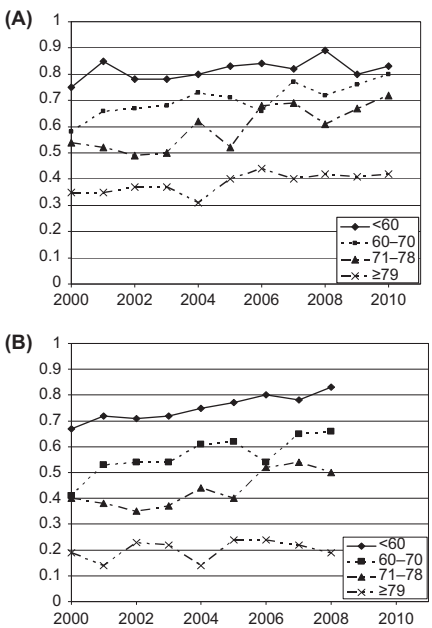

Figure 2. Estimated 2- (A) and 5-year (B) OS of diffuse large B-cell lymphoma in Sweden during the period 2000–2010 according to age quartile.

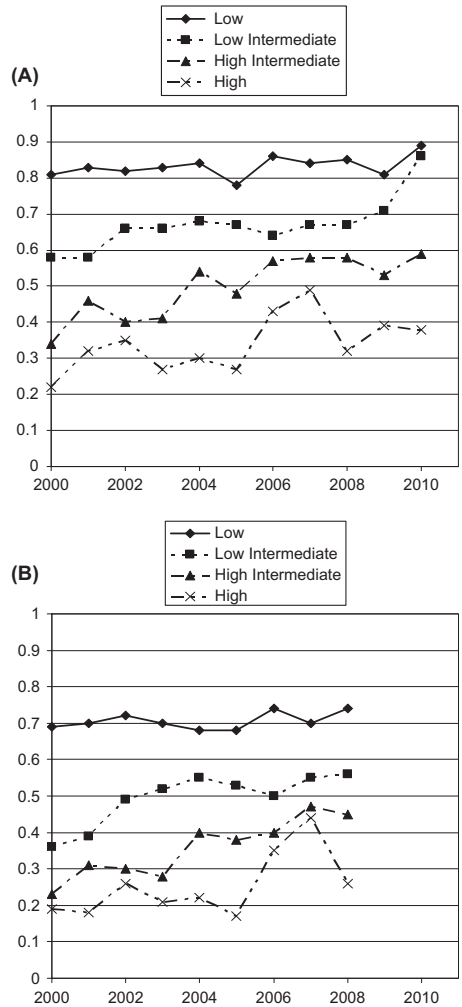

Figure 3. Estimated 2- (A) and 5-year (B) OS of diffuse large B-cell lymphoma in Sweden during 2000–2010 according to International Prognostic Index.

favorable extranodal presentation in this series, with an estimated 5-year survival of 64%, in line with a recent report from the International Extranodal Lymphoma Study Group [15]. In addition, bone and muscle involvement was associated with superior survival in multivariate analyses, confirming previous reports [16,17].

In a number of series, male sex has been shown to be associated with a negative impact on OS and progression-free survival (PFS) in DLBCL, also when treated with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) and rituximab [9,18,19]. One possible explanation has been provided by the German High Grade Non-Hodgkin Lymphoma Study Group, showing that the elimination half-life of rituximab was significantly prolonged in women compared to men [20–22]. However, male sex has also been associated with inferior outcome in Hodgkin lymphoma not treated with rituximab, indicating that other mechanisms may be present [23–25]. In this series, we were able to investigate the relationship of gender and age in more detail. If stratified for age, the risk for men was higher, which can be interpreted as the slightly lower median age of the men compensating for the worse prognosis. The higher risk for males was constant during the observation period, indicating that this may be unrelated to rituximab.

Note, however, that the gender effect is so small that it is compensated for by only 2 years of age, implying that the risk for a 72-year-old woman is very close to that of a 70-year-old man. This means that being about 2 years younger compensates for the disadvantage of male sex. In the general population as whole, a woman in the upper age groups has to be 3 or 4 years older than a man to have the same death rate. For example, the death rate in 2005 for Swedish women of age 76 was 2.6%/year, close to the 2.7%/year that was the death rate in 2005 for Swedish men aged 72. The estimated life expectancy of a 70-year-old male in Sweden in 2013 is 84.6 years, compared to 87.1 years for females. Given the large standard deviation of the gender term, it is most likely that the difference in background mortality is the cause of the effect seen in the analysis, and is most likely not a result of a gender difference in coping with the disease.

The changes in therapy during this decade are the addition of rituximab to chemotherapy [1,2,4], and the introduction of dose-dense regimens. A population based study from British Columbia showed that the addition of rituximab to anthracycline-based chemotherapy (CHOP) dramatically improved the outcome of DLBCL [26]. Dose-dense regimens, with a 14-day interval with granulocyte-colony stimulating factor (G-CSF) support, as well as rituximab addition, were introduced in Sweden around 2003. Other advances in the management of relapsed patients, including the use of high-dose chemotherapy with autologous stem cell transplant and improvement of supportive care, may also have contributed to the outcome during this decade.

Here we were able to show that the OS of DLBCL has markedly improved during the last decade. The improvement was most prominent in the age group 60–78 years and for patients presenting with favorable performance status, and was significant for all IPI subgroups, except for the low risk category. Patients aged 60–78 years have to a higher degree been treated with curative intent, possibly due to the introduction of rituximab. Even patients receiving reduced chemotherapy doses may now be considered to be treated curatively, as rituximab improves efficacy without a major increase in toxicity. In contrast, survival for patients with poor PS, or age >78 years, has not significantly improved, indicating that the improvement of therapeutic regimens and supportive care has not been sufficient to overcome the adverse prognosis associated with these factors. An obvious limitation of our study was that we did not have access to data on treatment, as these data were not included in the registry until 2007. Another limitation is the lack of central pathology review, which was not feasible to perform in a cohort of this size.

In summary, this study presents a large population based cohort of patients with DLBCL with detailed data on prognostic factors and outcome over time. Involvement of CNS, bone, bone marrow and urinary bladder, as well as male gender, were shown to be negative prognostic factors, whereas subcutaneous, gastric, muscle and thyroid involvement were associated with a superior outcome. OS of these patients has markedly improved during the last decade, but to improve outcome further, our focus should be on patients with high risk features, who may benefit from upfront high-dose chemotherapy, as well as elderly patients and patients with poor performance status, who may require treatment with regimens specifically tailored to this population.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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Paper II

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Original Research Article

Impact on survival of addition of etoposide to primary chemotherapy in diffuse large B-cell lymphoma: a Swedish Lymphoma Registry study

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Abstract

No randomised study in the rituximab era has been performed specifically to evaluate addition of etoposide to treatment of diffuse large B-cell lymphoma (DLBCL). The aim of this study was to compare the outcome with three chemotherapy regimens (R-CHOP-21, R-CHOP-14 and R-CHOEP-14) in a population-based cohort in terms of overall survival, adjusted for clinical prognostic factors. Through the Swedish Lymphoma Registry, 3443 patients with DLBCL were identified 2007–2012. Among all patients, there was no evidence of a difference between the regimens, after adjustment for prognostic factors. However, when restricted to patients aged up to 65, R-CHOEP-14 was associated with superior outcome compared to both R-CHOP-21 (hazard ratio: 0.49, 95% confidence interval: 0.3–0.9, $p = 0.028$) and R-CHOP-14 (hazard ratio: 0.64, 95% confidence interval: 0.4–1.0, $p = 0.06$), when adjusted for prognostic factors. Results were consistent in an additional stratified analysis with patients grouped according to age and IPI-score. In conclusion, we could show that R-CHOEP-14 was associated with superior overall survival in patients with DLBCL aged up to 65 years, indicating that this may be a valid treatment option for this patient population. To further investigate which patient groups that may benefit the most from treatment intensification, R-CHOEP-14 should be compared to R-CHOP-21 in a randomised setting.

Keywords: **diffuse large B-cell lymphoma; etoposide; chemotherapy regimen; dose density**

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most commonly occurring lymphoma type and accounts for approximately one third of all cases of lymphoma and over 80% of all aggressive lymphomas [1–3]. DLBCL is a clinically, biologically and histopathologically heterogeneous entity [4]. Because of its aggressive nature, median survival is less than a year if untreated [1,5].

If adequately treated with appropriate chemotherapy, DLBCL is a curable neoplasm. Standard treatment has consisted of combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) since the 1970s [5,6]. Treatment improvement has been attempted by way of intensifying chemotherapy regimens but has led to considerable toxicity and failed to show additional benefit [6–8]. During the recent decade, survival has improved substantially because of the addition of the monoclonal antibody rituximab (R). The beneficial effect of adding rituximab to the conventional CHOP-regimen has been proven in multiple randomised trials including both the younger and older DLBCL population and R-CHOP is currently deemed standard therapy [2,5,9–13]. However, outcome among poor-prognosis subgroups who fail to be cured by first-line therapy is still unsatisfactory, and further treatment advances are required to improve survival among high-risk patients [5].

Recent discussions regarding optimal DLBCL treatment have included whether or not the addition of etoposide might be advantageous in certain subgroups, and if dose-dense chemotherapy with R-CHOP administered every 14 days instead of every 21 days can improve outcome. In the pre-rituximab era, these questions were addressed by the German High-grade Non-Hodgkin's Lymphoma Study Group (DSHNL). Two separate randomised studies were performed, concluding that dose-dense chemotherapy was favourable for patients aged over 60 and that dose-dense chemotherapy and the addition of etoposide (CHOEP)

improved survival among patients aged below 60 [14–16]. However, more recent trials, such as the MInT-study (Mabthera International Trial), have not been able to show an additive effect of etoposide in combination with rituximab in low risk patients [11,12]. In contrast, a Danish population based study indicated that a young, high-risk population may benefit from the addition of both etoposide and rituximab [17]. Furthermore, a small Swedish study indicated improved outcome with R-CHOEP [18]. As of yet, no randomised study in the rituximab era has been performed specifically to evaluate the addition of etoposide. Regarding the efficacy of rituximab containing dose-dense chemotherapy, two randomised trials have been unable to detect a beneficial additive effect of administering R-CHOP at a shortened 14-day interval in any prognostic subgroup [19,20].

The aim of this study was to compare the most commonly administered chemotherapy regimens used to treat DLBCL in Sweden and investigate if there is a beneficial effect of addition of etoposide and/or dose-dense chemotherapy in terms of overall survival in a population-based data set.

Methods

The population-based Swedish Lymphoma Registry (SLR) was established in 2000 by the Swedish Lymphoma Group in order to expand the data available in the Swedish Cancer Registry that was set up in 1958. Data from the SLR are presented in annual reports (www.swedishlymphoma.se) and cover approximately 95–97% of all lymphoma cases diagnosed in Sweden [21]. Detailed data regarding treatment was added to the registry from 1 January 2007. The study was approved by the ethics committee in Lund.

The study population in the current study consisted of all adult patients diagnosed with DLBCL in Sweden during a six-year period from 1 January 2007 to 31 December 2012. The cases of DLBCL in this series have been diagnosed according to the current World Health Organisation (WHO) classification [22].

The following variables from the SLR were extracted: gender, age, WHO performance status (PS), bulky disease, B-symptoms, extranodal presentation, year of diagnosis, Ann Arbor stage, serum lactate dehydrogenase (S-LDH), immunotherapy, radiotherapy, first-line chemotherapy regimen and number of chemotherapy cycles. Data regarding survival status was gathered from the Swedish Population Registry, without access to cause of death. All patients with central nervous system (CNS) involvement were excluded from the study. End point was overall survival (OS), defined as time from diagnosis until death from any cause. Chemotherapy regimens included in the registry which had been administered to less than 35 patients were all added to the 'other' group. Patients who solely received radiotherapy, steroids, surgery or various other regimens classified as 'other' in the SLR were also included in this category. Moreover, patients who had received the CHOP regimen but with no data on administration interval were added to the 'other' group as were all patients who had received a CHOP- or CHOEP-regimen without the addition of rituximab.

For estimation of OS, the Kaplan–Meier method was used. To compare survival curves the log-rank test was utilised. Crude and adjusted hazard ratios (HR) were calculated using Cox proportional hazards regression. In multivariable analyses the effect of chemotherapy was adjusted for WHO PS (linear), S-LDH, gender, bulky disease, stage (as a factor on four levels) and age. Age was modelled as a restricted cubic spline with five knots, to more truthfully allow the effect of increased age on survival to vary in impact among different ages. To test the stability of our results and to further reduce the risk of bias because of differences in age and prognostic factors between patients receiving versus not receiving etoposide, we performed stratified Cox regression, thus allowing for different baseline hazards across strata. The strata were defined by age in eight groups, including patients up to 65 years (analysis adjusting for S-LDH, PS, stage, gender and bulky disease as above) as well as age in eight groups separated for age-adjusted IPI (analysis adjusting for gender and bulky disease). Data was analysed in STATA version 13 (for Kaplan–Meier estimation and Cox regression) and SPSS version 22 (for patient characteristics).

Results

Patient characteristics

A total of 3443 patients were diagnosed with DLBCL in Sweden from 1 January 2007 to 31 December 2012, excluding patients with CNS involvement ($n = 173$). A total of 1395 (40.5%) patients in the study population died. The median follow-up time for surviving patients was 47.4 months. Median age at diagnosis was 70 (range: 18–105). There was a slight male predominance of 55%. Patient characteristics according to chemotherapy regimen are presented in Table 1.

Chemotherapy regimens

Data on treatment were available for 2838 patients (82%), with chemotherapy regimens distributed as follows: R-CHOP-21 $n = 910$ (32%), R-CHOP-14 $n = 1196$ (42%), R-CHOEP-14 $n = 158$ (6%), other regimens $n = 373$ (13%) and no treatment $n = 201$ (7%). The distribution of the chemotherapy regimens R-CHOP-21, R-CHOP-14 and R-CHOEP-14 overall and in relation to prognostic factors is summarized in Table 1. Age differed between the

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Table 1. Patient characteristics, overall survival rates and response rates according to chemotherapy regimens

	All patients			Patients ≤65		
***N* (%)**	**R-CHOP-21**	**R-CHOP-14**	**R-CHOEP-14**	**R-CHOP-21**	**R-CHOP-14**	**R-CHOEP-14**
N	910	1196	158	201	657	155
Median age (range)	76 (26–99)	64 (18–90)	50 (18–78)	58 (26–65)	58 (18–65)	49 (18–65)
Age						
≤65	201 (22)	657 (55)	155 (98)	201 (100)	657 (100)	155 (100)
>65	709 (78)	539 (45)	3 (2)	0 (0)	0 (0)	0 (0)
Gender:						
Male	461 (51)	709 (59)	93 (59)	120 (60)	383 (58)	92 (59)
Female	449 (49)	487 (41)	65 (41)	81 (40)	274 (42)	63 (41)
Median follow-up (years)	3.2	3.6	3.8	4.1	4.0	3.9
Five-year OS (%)	56	70	84	85	78	84
Response						
CR	531 (58)	812 (68)	109 (69)	163 (81)	479 (73)	106 (69)
CRu	129 (14)	135 (11)	26 (16)	17 (8)	77 (12)	26 (17)
PR	99 (11)	114 (10)	13 (8)	9 (5)	44 (7)	13 (8)
SD	6 (1)	8 (1)	0 (0)	0 (0)	2 (0)	0 (0)
PD	38 (4)	63 (5)	5 (3)	5 (2)	34 (5)	5 (3)
Missing	107 (12)	64 (5)	5 (3)	7 (3)	21 (3)	5 (3)
S-LDH:						
<ULN	494 (54)	360 (30)	23 (14)	141 (70)	202 (30)	22 (14)
>ULN	390 (43)	821 (69)	134 (85)	56 (28)	447 (69)	132 (85)
Missing	26 (3)	15 (1)	1 (1)	4 (2)	8 (1)	1 (1)
WHO PS:						
0	443 (49)	587 (49)	74 (47)	148 (74)	365 (56)	73 (47)
1	308 (34)	406 (34)	56 (35)	43 (21)	211 (32)	55 (36)
2	80 (9)	106 (9)	15 (9)	4 (2)	44 (7)	15 (10)
3	54 (6)	71 (6)	10 (6)	4 (2)	25 (4)	9 (6)
4	20 (2)	20 (2)	3 (2)	2 (1)	9 (1)	3 (2)
Missing	5 (1)	6 (1)	0 (0)	0 (0)	3 (0)	0 (0)
Ann Arbor stage:						
I	246 (27)	169 (14)	14 (9)	95 (48)	108 (16)	14 (9)
II	263 (29)	283 (24)	28 (18)	60 (30)	159 (24)	28 (18)
III	130 (14)	263 (22)	29 (18)	23 (11)	142 (22)	28 (18)
IV	243 (27)	471 (39)	87 (55)	22 (11)	244 (37)	85 (55)
Missing	28 (3)	10 (1)	0 (0)	1 (0)	4 (1)	0 (0)
Bulky disease:						
No	768 (85)	912 (76)	90 (57)	196 (98)	500 (76)	88 (57)
Yes	130 (14)	267 (22)	65 (41)	4 (2)	149 (23)	64 (41)
Missing	12 (1)	17 (1)	3 (2)	1 (0)	8 (1)	3 (2)
IPI						
0–1	353 (39)	319 (27)	36 (23)	144 (72)	249 (38)	36 (23)
2	216 (24)	337 (28)	65 (41)	38 (19)	198 (30)	64 (41)
3	172 (19)	316 (26)	41 (26)	6 (3)	130 (20)	40 (26)
4–5	113 (12)	193 (16)	15 (9)	8 (4)	65 (10)	14 (9)
Missing	56 (6)	31 (3)	1 (1)	5 (2)	15 (2)	1 (1)

Data are presented as *n* (%) unless otherwise indicated. OS, overall survival; CR, complete response; CRu, complete response unconfirmed; PR, partial remission; SD, stable disease; PD, progressive disease; S-LDH, serum lactate dehydrogenase; ULN, upper limit of normal; WHO PS, World Health Organization performance status; IPI, international prognostic index.

chemotherapy regimens, with the lowest median age among patients who received R-CHOEP-14 (50 years) and R-CHOP-14 (64 years). Moreover, there was a significant disparity in the distribution of other indicators of prognosis among the various chemotherapy regimens with a lower proportion of patients with elevated S-LDH, Ann Arbor stage III–IV and presence of bulky disease in the R-CHOP-21 group compared to the other regimens. As expected, the most intensive regimen, R-CHOEP-14, was more frequently administered to younger, poor-prognosis patients (Table 1). Ninety-five percent of patients treated with R-CHOEP-14 received at least six cycles.

In the 'other' and 'no treatment' groups, patients were older compared to patients receiving the other chemotherapy regimens. Median age in the no treatment group was 83 (range 44–105) and 80 (range 18–96) in the other group. Moreover, 5-year OS rates were lower, 3.7% in the no treatment and 34.2% in the other group. The focus

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in this article is on the comparison of R-CHOP-21, R-CHOP-14 and R-CHOEP-14. Thus, the 'no treatment' and 'other' group were not included in the remaining analyses.

Data on treatment were missing for 605 (17.6%) patients. Compared to the group with treatment data available, patients with missing treatment data were slightly older and had a marginally higher incidence of WHO PS score >1. Furthermore, the subgroup without treatment data had an inferior 5-year OS (52.7% compared to 57.1% for the cohort with treatment data available). There was no difference in distribution of any other prognostic factors in this group compared to the cohort with treatment data available.

Response rate and overall survival

In this series, patients receiving R-CHOEP-14 had a superior 5-year OS rate of 84% compared to 70% for R-CHOP-14 and 56% for R-CHOP-21 (Figure 1). In the univariable Cox regression analysis, a strong association between chemotherapy regimen and survival was found, with strong evidence of lower HR rates for both R-CHOEP-14 and R-CHOP-14 compared to R-CHOP-21 (Table 2). However, after adjustment for standard prognostic factors, the HRs approached 1 (no effect), and there was no remaining evidence of an overall difference between the studied chemotherapy regimens ($p = 0.15$), indicating that the unadjusted differences to a large extent was because of confounding with other prognostic factors (Table 2). To adjust for the variations in age as truthfully as possible, age was adjusted for as splines in the multivariable analysis, allowing the impact of age as a prognostic factor to vary among ages (Figure 2).

Among high-risk prognostic subgroups (elevated S-LDH, PS > 1, Stage III–IV, presence of bulky disease), a trend towards superior OS rates was found among those who received R-CHOEP-14 (results not shown). However, after adjustment for prognostic factors, there was no apparent indication of a discrepancy in efficacy between R-CHOP-14, R-CHOEP-14 and R-CHOP-21 in any of the suggested subgroups ($p \geq 0.11$ unadjusted for multiple testing, results not shown).

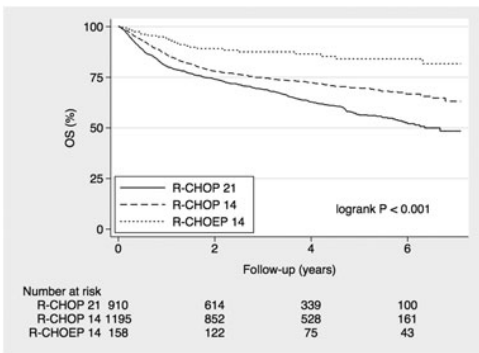

Figure 1. Overall survival (%) according to chemotherapy regimen

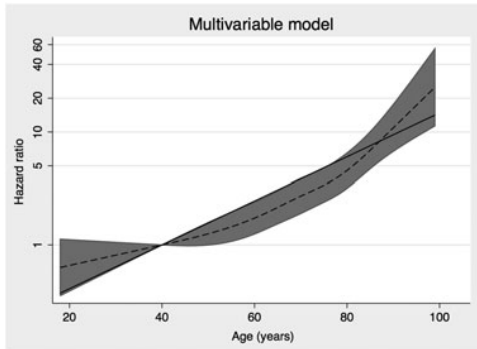

Figure 2. Comparison of the effect (hazard ratio) of age in a multivariable analysis when modelled as a linear covariate (whole line) compared to when modelled as splines (dotted line)

Table 2. Univariable and multivariable analysis of overall survival according to chemotherapy regimen

				Univariable analysis			Multivariable analysis[a]		
	Chemotherapy regimens	Deaths/person years	Rate/100 person years (95% CI)	HR	95% CI	P	HR	95% CI	P
All patients	R-CHOP-21[b]	356/2949	12.1 (10.9–13.4)	1		<0.001	1		0.15
	R-CHOP-14	345/4233	8.1 (7.3–9.1)	0.69	0.6–0.8	<0.001	0.91	0.8–1.1	0.3
	R-CHOEP-14	23/629	3.7 (2.4–5.5)	0.32	0.2–0.5	<0.001	0.63	0.4–1.0	0.06
Patients ≤65	R-CHOP-21[b]	27/828	3.3 (2.2–4.8)	1		0.02	1		0.06
	R-CHOP-14	137/2509	5.5 (4.6–6.5)	1.6	1.1–2.5	0.02	0.76	0.5–1.2	0.3
	R-CHOEP-14	23/620	3.7 (2.5–5.6)	1.1	0.7–2.0	0.6	0.49	0.3–0.9	0.028

[a]Multivariable analysis adjusted for WHO performance status (linear), S-LDH, gender, bulky disease, stage (as a factor on 4 levels) and age. Age included as a spline with five knots (4df) (Figure 2).
[b]R-CHOP-21 was used as the reference category. Overall *p*-value from LR-test for difference between chemotherapies shown.
HR, hazard ratio; CI, confidence interval.

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R-CHOP-21, R-CHOP-14 and R-CHOEP-14 all exhibited similar response rates according to the chi-square test, with 58%, 68% and 69% of the patients receiving these regimens achieving complete remission (CR) respectively ($p = 0.11$). Response rates are presented in Table 1.

Analysis restricted to patients aged ≤65

To more accurately analyse the effect of addition of etoposide, a subgroup analysis was performed consisting only of the patients eligible in terms of toxicity tolerance to receive etoposide, which in our population-based data was among patients aged up to 65 years. This cohort consisted of 1304 patients. Patient characteristics are demonstrated in Table 1. In this cohort, 1013 had received either R-CHOP-21 ($n = 201$), R-CHOP-14 ($n = 657$) or R-CHOEP-14 ($n = 155$). Five-year OS rates were 85%, 78% and 84% for the patients who received R-CHOP-21, R-CHOP-14 and R-CHOEP-14 respectively. Also in this population, there was a disparity in the distribution of prognostic factors among patients receiving the various chemotherapy regimens, with a lower proportion of patients with elevated S-LDH, PS > 1, stage III–IV and presence of bulky disease in the R-CHOP-21 group compared to the other regimens. In a multivariable analysis containing R-CHOP-21, R-CHOP-14, R-CHOEP-14 and prognostic factors, the overall evidence of a difference between the three groups did not reach significance at the 5-percent level ($p = 0.065$). However, focusing on the most intensive regimen, R-CHOEP-14, HR was lower compared to R-CHOP-21 (HR: 0.49 95% CI: 0.3–0.9 $p = 0.028$, Table 2) and in a direct comparison with R-CHOP-14 (HR: 0.64 95% CI: 0.4–1.0 $p = 0.06$). Treating intensity of chemotherapy regimen as a linear covariate (1 = R-CHOP 21, 2 = R-CHOP-14, R-CHOEP-14) also indicated evidence of an association with increased survival (HR = 0.7 per step, 95% CI: 0.51–0.95, $p = 0.023$) when adjusted for the other prognostic factors. To further decrease the risk of bias because of differences in age and distribution of prognostic factors, and to test the stability of our results, we also performed stratified analyses with patients grouped according to age (in eight groups) and IPI-score. The results were consistent with the primary analysis and showed that R-CHOEP-14 was associated with superior outcome (HR: 0.65 95% CI: 0.4–1.0 $p = 0.072$ stratified for age in 8 groups, HR: 0.63 95% CI: 0.4–1.0 $p = 0.059$ stratified for age-adjusted IPI plus age in 8 groups) in multivariable analysis.

Discussion

DLBCL is a curable disorder if properly treated with appropriate chemotherapy [11,12,23]. However, outcome among poor-prognosis patients warrants further improvement [5]. In this study we aimed to evaluate the efficacy of the most commonly administered chemotherapy regimens used to treat DLBCL in Sweden and investigate whether dose-dense chemotherapy and/or addition of etoposide provide a beneficial effect in a population-based setting.

Real-world data, as presented in this study, may contribute valuable information regarding optimal treatment for various subgroups of patients with DLBCL because of the comparably large study population in this series, and function as a complement to randomised studies performed.

Median age in this population was 70, which is in accordance with population-based data previously reported [1,3,5,24], but higher than the median age often presented in clinical trials [19]. Furthermore, other patient characteristics and presence of adverse prognostic factors in this study population were representative for DLBCL patients [1,3,24].

In a multivariable analysis restricted to patients aged up to 65 years the most intensive regimen, R-CHOEP-14, was found to be associated with superior outcome compared to R-CHOP-21 and R-CHOP-14. Moreover, evidence of a linear association between increased treatment intensity and survival was indicated in this population. Because of current treatment guidelines in Sweden stating that R-CHOEP-14 should be administered to young, high-risk patients, the fact that the cohort who received R-CHOEP-14 or R-CHOP-14 exhibited a higher incidence of patients with elevated S-LDH, bulky disease and Ann Arbor stage III + IV, in this series is not surprising. To decrease the potential risk of bias we adjusted for all relevant prognostic factors in the multivariable analysis. Additionally, the stability of our results was tested in a stratified analysis with patients grouped according to age and IPI-score. Results were consistent, indicating that we have, as far as possible, satisfyingly corrected for potential confounders when comparing outcome between the chemotherapy regimens. Still, we cannot rule out differences in comorbidity or use of salvage therapies between the different treatment groups, as such data were not available. Because the majority of patients administered R-CHOEP-14 belonged to high-risk subgroups, we were precluded from identifying potential prognostic subsets within the cohort aged under 65 especially benefitting from addition of etoposide in this series.

These results are in accordance with a Danish population-based study which found a superiority of R-CHOEP-14 compared to R-CHOP-14 among patients aged 60 and below with an age-adjusted IPI-score of at least two or three [17]. Recently, a retrospective study of this cohort, with patients stratified according to cell of origin, indicated that treatment with R-CHOEP-14 was associated with superior survival compared to R-CHOP only in patients with the germinal center (GCB) phenotype [25]. In the

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pre-rituximab era, addition of etoposide demonstrated a beneficial effect in patients aged 60 and below [14,15]. Moreover, the high efficacy and tolerable toxicity of R-CHOEP-14 have been demonstrated in both prospective and retrospective studies [7,18], although toxicity is increased compared to original CHOP-regimens and etoposide is associated with a higher risk for secondary acute myeloid leukemia and myelodysplastic syndrome. In contrast, results from a rituximab era study on low-risk patients showed that the improved efficacy with addition of etoposide to the CHOP regimen diminished when combined with rituximab [11,12]. Currently, a randomised clinical trial comparing the etoposide containing chemotherapy regimen DA-EPOCH-R (dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab) with R-CHOP-21 among adult DLBCL patients is being performed [26]. However, as of yet, no randomised study evaluating etoposide in the rituximab era has been performed.

In this study, no difference in outcome between patients receiving R-CHOP-14 compared to R-CHOP-21 was found regardless of age or other prognostic factors. This is in line with data from two prospective, randomised trials that have been unable to detect a significant difference in outcome for R-CHOP administered in 14- versus 21-day cycles in any subgroup [19,20].

A central pathology review was not feasible to perform on a cohort of this size. This is a limitation even though uniform pathological criteria have been applied throughout the study period. Additionally, in order to perform a full comparison of the various chemotherapy regimens, information regarding toxicity and health-related quality of life, as well as comorbidity and use of salvage therapies, would have been valuable. Although the R-CHOP-21 group had a numerically smaller proportion of patients achieving CR, no significant difference in response was seen between groups. As we have no data regarding relapse rates or salvage therapy we are precluded from comparing regimens regarding these parameters, thus this may constitute a potential bias. Another limitation in a population-based study, such as this, is missing data. In this series, data on treatment was missing for 17.6% of the study population. The group with missing treatment data had an inferior survival as well as a higher median age, indicating that some of the patients where data on treatment was not available may have received no treatment. However, apart from treatment, the missing data is unlikely to introduce bias because of the random distribution of missing data.

Conclusion

From these real-world data, we can conclude that there is no significant difference in outcome between patients receiving R-CHOP-14 and R-CHOP-21 among all age groups, but that the more intensive regimen R-CHOEP-14 was associated with superior overall survival in patients aged up to 65 years, indicating that this may be a valid treatment option for this patient population. R-CHOEP-14 should preferably be compared to R-CHOP-21 in a randomised setting in order to further elucidate which patient groups that may benefit the most from treatment intensification.

Conflict of interest

The authors have no competing interest.

Authorship and disclosures

T.W. analysed data and wrote the manuscript. M.J. designed the study. M.J., E.S. and L.H. reviewed data and provided critical input during manuscript writing. L.H. performed the statistical analysis.

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Paper III

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ORIGINAL ARTICLE

Season of diagnosis is associated with overall survival in patients with diffuse large B-cell lymphoma but not with Hodgkin's lymphoma – A population-based Swedish Lymphoma Register study

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Abstract

Objective and methods: The aim of this study was to investigate the effect of season of diagnosis on the outcome of patients with diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL). In this study, we included curatively treated DLBCL (n = 5875) and HL (n = 1693) patients, diagnosed between 2000 and 2011, based on data from the Swedish Lymphoma Register. *Results:* Overall survival was significantly better for patients diagnosed with DLBCL during the summer months, but not for patients diagnosed with HL. The difference remained in a multivariable analysis adjusted for age, stage, performance status, number of extra nodal sites and year of diagnosis (HR 1.08; 95% CI 1.02–1.14, P = 0.0069). When analyzing the DLBCL patients according to gender in the multivariable model, the effect of season was shown to be restricted to male patients (HR = 1.09, 95% CI 1.01–1.17, P = 0.0269. *Conclusions:* In summary, season of diagnosis was shown to have impact on overall survival in male patients with DLBCL. Possible explanations of our results are the higher vitamin D level during the summer months, the effects of sunlight on the circadian rhythm and the immune system, or the lower risk of infectious disease during the summer. Further investigations are needed to explore these hypotheses.

Key words diffuse large B-cell lymphoma; seasonal variation; gender

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Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma. The incidence of DLCBL has risen rapidly, especially among men, during the past decade, but the reason for this is unclear (1). Since the introduction of modern chemotherapy (dose-dense regimens and the addition of immunotherapy) approximately 50% of patients with DLBCL are cured. Hodgkin lymphoma (HL) is another highly curable malignant lymphoproliferative disease, of which approximately 80% can be cured with chemotherapy alone, or with combined modality strategies employing chemotherapy followed by consolidating involved field radiotherapy. Younger patients generally have a more favorable prognosis than older ones.

Only limited data are available on the variation in prognosis of DLBCL and HL with season of diagnosis (2, 3). It has been suggested that the better prognosis associated with diagnosis in the summer months may be related to lower exposure to infectious agents and/or the favorable effect of sunlight during the summer. The seasonal variation in the UV radiation in Sweden (latitude: ~60°N) is very marked. During the winter, it is not possible to produce previtamin D (25-hydroxyvitamin D) from UVB radiation. Several reports suggest that low serum 25-hydroxyvitamin D levels may be associated with increased cancer incidence and mortality. The most investigated cancer diagnoses in this respect are colorectal (4) and breast cancer (5). Using data from the Swedish Lymphoma Register (SLR), we investigated the hypothesis that patients with DLBCL and HL diagnosed and treated during the summer season have a better outcome. The study was restricted to patients receiving treatment with curative intent.

Methods

This is a retrospective study based on data obtained from the SLR for all patients diagnosed with DLBCL or HL in Sweden, from January 1, 2000 to December 31, 2011. The Swedish Lymphoma Registry (SLR) started in 2000, and has almost full coverage of patients with malignant lymphoma in Sweden. Patients with primary CNS lymphoma, or HIV-related lymphoma were excluded. Survival data were extracted from the Swedish Population Database.

Statistical methods

Cox regression was employed for the analysis of overall survival. The impact of light exposure was defined as a periodical, continuous variable: cos ((month of diagnosis -φ)/ 12 × 2π) – where φ is the phase. For example, a value of 6 implies that the function varies between −1 in December and +1 in June. The phase was estimated over the integers as given by the 12 months. In addition, age, gender, disease stage, year of diagnosis, performance status, number of extranodal sites and lactate dehydrogenase (LDH) were included as cofactors. Potential time trends were investigated by introducing the interaction between the terms for light exposure and the time periods 2000–2004, 2005–2007, and 2008–2011. Data from patients with the two diagnoses, DLBCL or HL, were analyzed separately. Analysis of distribution of time from diagnosis until start of treatment was performed by Kruskal–Wallis test.

Results

Patient characteristics

The patients' characteristics are summarized in Table 1. In total, 5875 cases of DLBCL and 1693 cases of HL were identified in the SLR between 2000 and 2011. Of these, 4812 (82%) of the patients with DLBCL and 1510 (90%) of the patients with HL received treatment with curative intent, and 56% and 54%, respectively, of these patients were men. Curative intent was defined at the discretion of the treating physician. The median age of the patients with DLBCL was 67 years (range 16–99) and for the HL patients 38 years (16–93). The number of cases diagnosed per month was equally distributed in both groups. As expected, the factors included in the International Prognostic Index (IPI) for DLBCL: age, stage, performance status, and s-LDH were strongly related to overall survival (data not shown).

Table 1 Patient characteristics

	DLBCL treatment		HL treatment	
Variable	Curative (%)	Not curative (%)	Curative (%)	Not curative (%)
N (%)	4812 (82)	1063 (18)	1510 (90)	183 (10)
Median age	67 (16–99)	80 (18–105)	38 (16–93)	72 (16–95)
Sex				
Male, *N* (%)	2697 (56)	518 (49)	818 (54)	89 (48)
Female, *N* (%)	2115 (44)	545 (51)	692 (46)	94 (52)
WHO performance status *N* (%)				
0	2105 (44)	198 (18)	1038 (69)	65 (36)
1	1704 (35)	307 (29)	371 (25)	49 (27)
2	498 (10)	184 (18)	62 (4)	15 (8)
3	369 (8)	213 (20)	33 (2)	36 (20)
4	136 (3)	161 (15)	60	18 (9)
Missing values	0	0	0	0
Number of extranodal sites *N* (%)				
0–1	4111 (85)	889 (84)	1455 (97)	179 (98)
> 2	701 (15)	174 (16)	53 (3)	4 (2)
Missing values	0	0	0	0
Ann Arbor stage				
I	1069 (23)	171 (18)	215 (14)	28 (17)
II	989 (21)	152 (16)	703 (47)	51 (31)
III	959 (20)	194 (20)	330 (22)	43 (27)
IV	1726 (36)	417 (44)	257 (17)	40 (25)
Missing values	69	129	5	21
LDH level				
Normal	2016 (43)	281 (31)	Not relevant	Not relevant
Elevated	2689 (57)	635 (69)		
Missing values	107	147		
Month of diagnosis				
Jan	382	90	112	17
Feb	366	77	134	11
Mar	437	98	152	12
Apr	372	90	142	13
May	416	83	127	17
Jun	421	76	121	13
Jul	430	75	117	20
Aug	381	103	98	17
Sept	405	92	114	10
Oct	387	86	134	15
Nov	402	91	129	26
Dec	413	102	130	12
Periods				
2000–2004	1775	499	592	104
2005–2007	1290	251	397	35
2008–2011	1747	313	521	44

Time from diagnosis until start of treatment

For patients diagnosed from 2007 and forward, data on start of treatment was available. The median time was 20 days for patients with DLBCL, and 18 days for HL. There was no difference between males and females, and the distribution was the same regardless of month of diagnosis.

Seasonal impact on prognosis

To model the impact of season, we used a periodical continuous variable, henceforth called the season variable. Univariate

Cox regression showed that the season variable was associated with overall survival in patients with DLBCL, with improved survival of patients diagnosed during the summer ($P = 0.02$) (Fig. 1). However, no statistically significant association was found for patients with HL (Fig. 2). The season variable was then analyzed with multivariable Cox regression, adjusted for age, gender, stage, performance status, S-LDH, number of extranodal sites, and year of diagnosis.

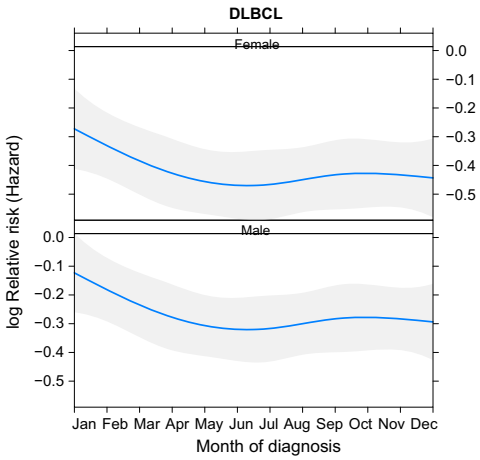

Figure 1 The relative risk for overall survival per month of diagnosis in patients with diffuse large B-cell lymphoma, with January as the reference category. Females are shown in the upper panel, males in the lower.

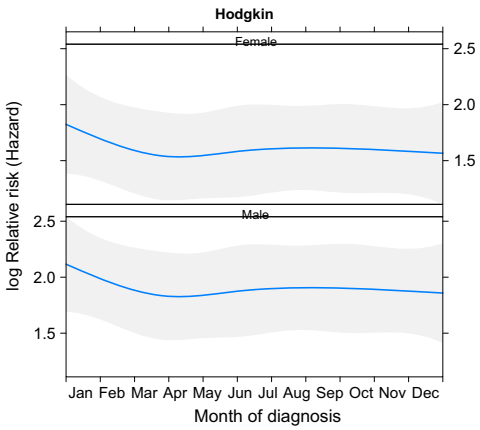

Figure 2 The relative risk for overall survival per month of diagnosis in patients with Hodgkin lymphoma, with January as the reference category. Females are shown in the upper panel, males in the lower.

According to this analysis, diagnosis during the summer was associated with significantly better overall survival in patients with curatively treated DLBCL (HR 1.08, 95% CI 1.02–1.14, $P = 0.0069$) (Table 2). When analyzing the DLBCL patients according to gender with the multivariable model, improved overall survival was observed for male patients diagnosed during the summer period (HR = 1.09, 95% CI 1.01–1.17, $P = 0.0269$) (Fig. 1), whereas no significant association was found for female patients (Table 3). We also investigated whether the impact of season varied during the time period studied (2000–2011) in patients with DLBCL, but no statistically significant differences were found between the periods 2000–2004, 2005–2007 and 2008–2011.

Discussion

Both DLBCL and HL are highly curable lymphoid malignancies. As previously reported, the overall survival of DLBCL has improved during the past decade (6), while male gender has been shown to be associated with poorer outcome in both HL and DLBCL (7–11). This retrospective study demonstrates that the season of diagnosis has an impact on the prognosis of DLBCL, and that the effect is mainly restricted to male patients. We were unable to reproduce earlier findings of a seasonal impact in HL (3).

Several studies have been carried out on the effect of sunlight on the development of lymphoma (12, 13) and solid tumors (14), but there are only a few publications on the effect of sunlight on DLBCL prognosis. In a recent review, van der Rhee *et al.* (15) reported that almost all epidemiological studies suggest that chronic (continuous) sun exposure is associated with a reduced risk of colorectal, breast, and prostate cancer, and NHL (non-Hodgkin′s lymphoma). Other sunlight-potentiated and vitamin D-independent pathways may play a role in reducing cancer risk, particularly prostate cancer and NHL. Exposure to sunlight has also been found to modulate subclinical immunosuppression (16) and circadian rhythm (17), and to be associated with increased degradation of folic acid (18). Chronic exposure to UV irradiation induces local and systemic immunosuppression involving Langerhans and dendritic cells, natural killer cells, macrophages, and mast cells (16, 19). Previous studies have shown that patients with solid tumors and lymphomas with high inflammatory activity have poorer overall survival (20). Thus, immunomodulation induced by sunlight may have a favorable effect in this respect.

Most studies concerning the effects of sunlight have been focusing on the seasonal variation in vitamin D levels. It has been suggested in two prospective investigations that low serum 25-hydroxyvitamin levels are not associated with the overall risk of lymphoid cancer (21, 22). In addition, findings of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancer failed to support the hypothesis that an elevated vitamin D level is associated with a reduced risk of NHL (23).

Table 2 Results of Cox regression analysis for overall survival in curatively treated DLBCL and HL patients, adjusted for season of diagnosis, age, gender, stage, LDH, WHO performance status (PS) and number of extranodal sites

	DLBCL, *N* = 4785			HL, *N* = 1501		
	HR	CI (95%)	*P*-value	HR	CI (95%)	*P*-value
Season	1.08	1.02; 1.14	0.0069	0.93	0.79; 1.11	0.4681
Age	1.04	1.04; 1.05	<0.0001	1.06	1.06; 1.07	<0.0001
Stage II vs. I	1.02	0.89; 1.18	0.6819	1.21	0.80; 1.82	0.3604
Stage III vs. I	1.09	0.95; 1.25	0.1966	1.57	1.05; 2.36	0.0278
Stage IV vs. I	1.32	1.16; 1.51	<0.0000	1.94	1.23; 3.04	0.0039
Year of diagnosis	0.94	0.93; 0.95	<0.0001	0.99	0.95; 1.02	0.6273
WHO PS						
1 vs. 0	1.66	1.50; 1.85	<0.0001	1.98	1.51; 2.62	<0.0001
2 vs. 0	2.19	1.90; 2.52	<0.0001	2.55	1.62; 4.01	<0.0001
3 vs. 0	2.98	2.56; 3.47	<0.0001	2.72	1.59; 4.65	0.0002
4 vs. 0	4.97	4.04; 6.12	<0.0000	3.23	0.77; 13.51	0.1075
Extranodal sites						
>1 vs. 0–1	1.02	0.90; 1.15	0.7328	1.34	0.81; 2.19	0.2435
LDH						
Elevated vs. normal	1.34	1.22; 1.47	<0.0001	1.25	0.98; 1.61	0.0683
Gender						
Female vs. Male	0.81	0.74; 0.88	<0.0001	0.68	0.53; 0.87	0.0027

Table 3 Results of Cox regression analysis for overall survival, by gender, in curatively treated DLBCL patients, adjusted for season of diagnosis, age, stage, LDH, PS and number of extranodal sites

	Male, *N* = 2681			Female, *N* = 2104		
	HR	CI (95%)	*P*-value	HR	CI (95%)	*P*-value
Season	1.09	1.01; 1.17	0.0269	1.07	0.97; 1.17	0.1381
Age	1.04	1.04; 1.05	<0.0001	1.05	1.04; 1.05	<0.0001
Stage II vs. I	1.08	0.89; 1.30	0.4050	0.97	0.79; 1.20	0.8302
Stage III vs. I	1.16	0.97; 1.40	0.0977	1.01	0.82; 1.25	0.8853
Stage IV vs. I	1.34	1.34; 1.60	0.0007	1.34	1.09; 1.63	0.0037
Year of diagnosis	0.94	0.92; 0.95	<0.0001	0.94	0.92; 0.96	<0.0001
WHO Performance status						
1 vs. 0	1.71	1.49; 1.96	<0.0001	1.61	1.36; 1.90	<0.0001
2 vs. 0	2.21	1.84; 2.66	<0.0001	2.15	1.72; 2.69	<0.0001
3 vs. 0	2.53	2.07; 3.10	<0.0001	3.75	2.99; 4.71	<0.0001
4 vs. 0	4.89	3.67; 6.53	<0.0001	5.15	3.80; 6.96	<0.0001
Extranodal sites						
<1 vs. 0–1	1.02	0.87; 1.19	0.7499	0.99	0.82; 1.21	0.9968
LDH-level						
Elevated vs. normal	1.35	1.19; 1.53	<0.0001	1.31	1.13; 1.52	0.0002

The main determinants of previtamin D status in serum are dietary intake of vitamin D, such as fatty fish, fortified reduced-fat dairy products, dietary supplements, and vacations in sunny regions during the autumn or winter (24). Low vitamin D levels in the population of Norway, another Nordic country at latitude 60°N, indicate the importance of sun exposure for optimal vitamin D status (25–27). Serum vitamin D levels in the population of Norway have been found to be 15–50% lower in winter than in summer (3), and sunbed exposure has been found to be more effective in treating vitamin D deficiency than daily oral intake of vitamin D (25, 28). There is very little data on the effects of vitamin D insufficiency on prognosis in NHL and HL (2, 3). Drake *et al.* found that vitamin D insufficiency was associated with poorer event free and overall survival in DLBCL and T-cell lymphoma patients. It has also been found in a Norwegian study (3) on Hodgkin lymphoma that the season of diagnosis was a strong prognostic factor, particularly for patients younger than 30 years. Vitamin D insufficiency has also been found to impair rituximab-mediated cellular cytotoxicity, and was associated with poorer outcome in elderly DLBCL patients treated with rituximab (29). Due to lack of data on disease progression, and details on treatment, we were unable to investigate whether the prognostic impact of season was limited to patients treated with rituximab. However, for the time period under study, there was no interaction between year of diagnosis and the prognostic impact of season.

In conclusion, in this study we have found the prognosis of DLBCL to be correlated with the season of diagnosis, particularly for male patients. It can be speculated that this seasonal effect is due to vitamin D synthesis in the skin by sun exposure, but other explanations are possible, such as a

decreased risk of infections during the summer, or the effect of sunlight on the immune system and circadian rhythm. Further investigations are required to investigate the effect of vitamin D status during the treatment of DLBCL.

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Paper IV

First-line treatment of follicular lymphoma: A retrospective population-based study from the Swedish Lymphoma Registry

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Key Points:

- Rituximab as single agent is not inferior to immunochemotherapy in terms of overall survival
- Rituximab maintenance therapy may be associated with prolonged overall survival in a population-based setting

Abstract

The aims of this study were to identify the initial management of follicular lymphoma (FL), and investigate the efficacy of different treatment options in a population-based series from the Swedish Lymphoma Registry. Out of a total number of 4679 registered patients with FL, information on initial treatment was available in 2251 cases. In 617 (28%) of the cases of totally 2251, the primary option was watchful waiting, whereas treatment was initiated at the time of diagnosis in 1634 (72%) patients. The most common forms of initial therapy were chemoimmunotherapy (736 or 46%), single-agent rituximab (347 or 22%) and radiotherapy (342 or 21%). Among systemic therapies, the most common treatment was R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone) in 486 (32%) of cases. The overall survival (OS) of patients treated with the watch and wait approach did not differ significantly from that of patients treated immediately, adjusted for prognostic factors (HR=0.91, 95% CI 0.69-1.2, p=0.49). Patients treated with single-agent rituximab had a similar OS as patients treated with R-CHOP or R-bendamustine. Maintenance rituximab was associated with improved overall survival (HR 0.56; 95% CI 0.37-0.87, p=0.010), also when corrected for immortal time bias. This study highlights the role of watchful waiting in the management of FL and the non-inferiority of rituximab monotherapy compared to chemoimmunotherapy. For patients with FL with symptoms requiring early treatment, rituximab monotherapy should be considered as one of the first-line treatment options. Rituximab maintenance may be associated with prolonged survival.

Introduction

Follicular lymphoma (FL) is the second most common form of lymphoma [1], and accounts for approximately 10-20% of all lymphomas in the Western world [2]. The disease is associated with long-term survival, and a variety of initial treatment strategies are used. Its clinical presentation is characterised by asymptomatic peripheral adenopathy, and the growth and reduction of lymph node enlargements over several years is a common feature [3].

Several attempts have been made to identify the biological and clinical features that may predict early risk of progression indicating the need for the initiation of therapy. The most internationally recognized prognostic instrument is the FLIPI (Follicular Lymphoma International Prognostic Index). The FLIPI is based on a number of clinical and biological factors: age >60 years, Ann Arbor stage III/IV, haemoglobin level <120 g/L, >4 nodal involvement areas and serum lactate dehydrogenase levels above the normal range [4]. Studies over the past two decades have provided evidence that overall survival does not appear to be affected by immediate treatment of an asymptomatic patient. Due to this, watchful waiting is generally recommended for patients with asymptomatic low-tumour-burden lymphoma, although immediate rituximab monotherapy has shown a significant delay in the need to start chemotherapy[5-7].

In the case of asymptomatic high-tumour-burden disease, which is not a very common scenario, either watch and wait [3,8] or a combination of rituximab and chemotherapy have been recommended [9]. The recommended therapy for patients with symptomatic high-tumour-burden disease in most parts of the world is chemoimmunotherapy, such as rituximab plus bendamustine or rituximab plus CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) [10-12].

However, results from the Nordic Lymphoma Group [13] and from the Swiss Group for Clinical Cancer Research (SAKK) [14] suggest that rituximab monotherapy may also be an appropriate option for patients with symptomatic high-tumour-burden disease, sparing a large proportion of patients the side effects of chemotherapy.

Radiation therapy (RT) plays an important role in the treatment of FL. Involved-field irradiation (IFRT) with a radiation dose of 24-30 Gray delivered in 12-15 fractions is a curative option for stage I-II disease, so-called “limited stage” disease [15]. Evidence suggests that radioimmunotherapy (RIT) is one of the most effective options for the treatment of FL. RIT has shown excellent efficacy in phase II and phase III studies, but extensive bone marrow involvement is a major contraindication to RIT[16-19]. In cases of relapse, RIT has been shown to be associated with a higher response rate and longer period of remission than single-agent rituximab[20].

In summary, immediate treatment with rituximab monotherapy or the combination of rituximab and chemotherapy is recommended in cases of organ impairment or high tumour burden. In addition, IFRT is an option for immediate treatment of stage I-II, limited disease.

The primary objective for this study was to compare outcome for patients treated with between single-agent rituximab and chemoimmunotherapy in terms of overall survival. Secondary objectives were to evaluate overall survival according to initial watchful waiting and radiotherapy, as well as the impact of maintenance therapy in a population based setting.

Patients and methods

This population-based study was based on the Swedish Lymphoma Registry (SLR). The SLR was established in 2000 by the Swedish Lymphoma Group to collect detailed information on patients with malignant lymphoma. Data from the SLR are presented in annual reports (www.swedishlymphoma.se), and cover approximately 95-97% of the cases of lymphoma diagnosed in Sweden. The SLR excludes patients under 18 years old and cases diagnosed at post-mortem examinations. The following variables from the SLR were extracted: gender, age, date of diagnosis, WHO performance status, bulky disease, B-symptoms, extranodal presentation, Ann Arbor stage, serum lactate dehydrogenase, the use of an initial watchful waiting approach, immunotherapy, radiotherapy, chemotherapy regimen, maintenance treatment and response to initial therapy. Validation of registry data has shown 95% agreement between the diagnoses reported to the SLR and the actual diagnosis in the patient's medical records (www.swedishlymphoma.se). Survival data were obtained from the National Causes of Death Registry.

Study population

The study population included all patients diagnosed with FL grade I-IIIA, from 1 January 2000 until 2 December 2015. Out of a total number of 4679 registered patients with FL, information on initial treatment was available in 2251 cases.

Patients with FL grade IIIB or with confirmed transformation at the time of diagnosis were excluded. Detailed information on treatment was included in the registry from 1 January 2007. In the case of patients registered before 2007, information on treatment was collected from patient records.

Immunotherapy included all cases treated with rituximab monotherapy. Chemoimmunotherapy was defined as rituximab combined with chemotherapy, including bendamustine, CHOP, CVP (cyclophosphamide, vincristine, and prednisone) or chlorambucil. Patients treated with radiotherapy alone received doses from 4 to 30 Gy. Combined modality therapy included both systemic therapy (immunotherapy, chemoimmunotherapy or chemotherapy) and radiation therapy.

The assessment of clinical response was performed and reported by the treating physician, without central review of imaging. According to national guidelines, the evaluation was based on computed tomography (CT), according to the Cheson 2007 criteria[21].

Statistical analysis

Multivariable Cox regression was employed for the analysis of overall survival (OS), using 95% confidence intervals (CI). The analyses were adjusted for gender, FLIPI, bulky disease, and date of diagnosis. Survival curves were estimated according to the Kaplan-Meier method, and compared with the log-rank test. Statistical analyses were performed using SPSS version 22. The chi-squared test was used to test for interrelationships between prognostic factors. To minimize immortality bias, in the analysis of maintenance therapy, data were conditioned to reach 200 days overall survival.

Results

Patient characteristics

In total, 4679 patients with follicular lymphoma were identified in the Swedish Lymphoma Registry between January 1, 2000, and December 2, 2015. Information on initial treatment was available in 2251 (48%) cases. Table 1 presents a summary of the characteristics of the patients for whom treatment data were available compared to patients for whom no such data were available. The median follow-up time for surviving patients with treatment data was 48 months, the median age at the time of diagnosis was 65 years (range: 18-100 years), and a slight majority were female (1177, 52%). In the group lacking treatment data, the incidence of FLIPI=2 was statistically significantly lower ($p=0.018$), and the incidence of bone marrow involvement was higher ($p<0.001$). Otherwise, there

were no differences between the two groups. The 5-year OS for patients with available treatment data was 75%, vs. 72% for patients for whom no treatment data were available.

Table1. Patient characteristics according to whether data on treatment were available or not

Variable	Treatment data available	Treatment data not available
N(%), N=4679	2251 (48%)	2428 (52%)
Gender **Female, N=2408** **Male, N=2271**	 1177 (52%) 1074 (48%)	 1231 (50%) 1197 (50%)
Agegroups **< 70** **70-80** **=>80**	 1428 (63%) 553 (25%) 270 (12%)	 1599 (65%) 556 (23%) 273 (12%)
Bulky disease **Yes** **No** **Unverified** **Missing N=61 (2%)**	 320 (13%) 1875 (83%) 52 (2%)	 393 (16%) 1949 (81%) 29 (1%)
Date of diagnosis **<2010** **>=2010**	 1106 (50%) 1145 (50%)	 1655(68%) 773(32%)
FLIPI **0** **1** **2** **3** **4** **Unverified**	 170 (8%) 633 (28%) 537 (24%) 247 (11%) 59 (2%) 605 (27%)	 82 (4%) 316 (13%) 332 (14%) 130 (5%) 31 (1%) 1537 (63%)
Bone marrow involvement **Yes** **Missing**	 536 (24%) 1715 (76%)	 710 (30%) 1718 (70%)
5-year OS	75-77%	72%

Prognostic factors

Prognostics factors, including gender, age (divided into three groups: <70 years, 70-79 years and ≥80 years), FLIPI, the presence of bulky disease (>10 cm), and date of diagnosis (before 2010 and from 2010 to December 2, 2015) were analysed using univariable and multivariable Cox regression. We found that all prognostic factors except gender were highly significant ($p<0.001$) in univariate analyses. In adjusted multivariate analyses, all factors except bulky disease were significant (Table 2). Data on beta-2-microglobulin and lymph node diameter were not available.

Table 2. Prognostics factors. Univariate and multivariate analyses adjusted for FLIPI, age groups, bulky disease and date of diagnosis

Variable	Reference	Univariate analysis HR (95% CI)	p-value	Multivariate analysis HR (95% CI)	p-value
Gender	Male	0.96 (0.86-1.06)	0.410	0.80 (0.65-0.97)	0.025
Age groups	<70 years				
70-80		3.64 (3.21-4.12)	<0.001	3.18 (2.48-4.07)	<0.001
80+		8.16 (7.10-9.38)	<0.001	8.06 (6.25-10.4)	<0.001
FLIPI	FLIPI 0				
1		7.90 (2.91-21.4)	<0.001	3.89 (1.42 -10.6)	0.008
2		11.0 (4.08-29.8)	<0.001	5.33 (1.96-14.5)	0.001
3		22.1 (8.14-59.8)	<0.001	8.84 (3.22-24.2)	<0.001
4		45.3 (16.4-125)	<0.001	16.4 (5.80-46.2)	<0.001
Bulky disease	No	1.35 (1.17-1.55)	<0.001	1.14 (0.88-1.47)	0.328
Date of diagnosis After 2010	2010	0.68 (0.58-0.77)	<0.001	0.76 (0.62-0.94)	0.010

Immediate treatment versus watch and wait

The characteristics of patients receiving immediate treatment versus those in which watch and wait was applied are summarised in Table 3. In 617 (28%) cases of newly diagnosed FL, watchful waiting was the primary option. No significant difference was found between female and male patients in the choice of immediate treatment or watch and wait. However, significantly more patients <70 years ($p<0.001$), with bulky disease ($p<0.001$) and with verified bone marrow involvement ($p<0.001$) were given immediate treatment. The 5-year OS for the immediate treatment group was 77% vs. 75% for the watch and wait group. As can be seen in Figure 1, according to univariate analysis there was no difference in survival between patients receiving immediate treatment and those in which watch and wait was applied. Neither was there any significant difference in survival (HR, 0.909; 95% CI 0.691-1.195, $p=0.492$) after adjustment for prognostic factors.

Table 3. Patient characteristics according to whether they were given immediate treatment or watchful waiting was applied

Variable	Immediate treatment	Watch & wait
N (%)	1634 (72%)	617 (28%)
Gender **Female** **Male**	 838 (51%) 796 (49%)	 339 (55%) 278 (45%)
Age **(<70)** **(70-80)** **(≥80)**	 1091 (67%) 383 (23%) 160 (10%)	 337 (55%) 170 (27%) 110 (18%)
Bulky disease **Yes** **No** **Unverified** **Missing N=4**	 307 (19%) 1293 (79%) 31 (2%)	 13 (2%) 582 (95%) 21 (3%)
Date of diagnosis **<2010** **=>2010**	 821 (50%) 813 (50%)	 285 (46%) 332 (54%)
FLIPI **0** **1** **2** **3** **4** **Unverified**	 126 (8%) 415 (25%) 380 (23%) 202 (13%) 55 (3%) 456 (28%)	 44 (7%) 218 (36%) 157 (25%) 45 (7%) 4 149 (25%)
Bone marrow involvement **Missing** **Yes**	 1212 (75%) 422 (25%)	 503 (82%) 114 (18%)
5-year OS	77%	75%

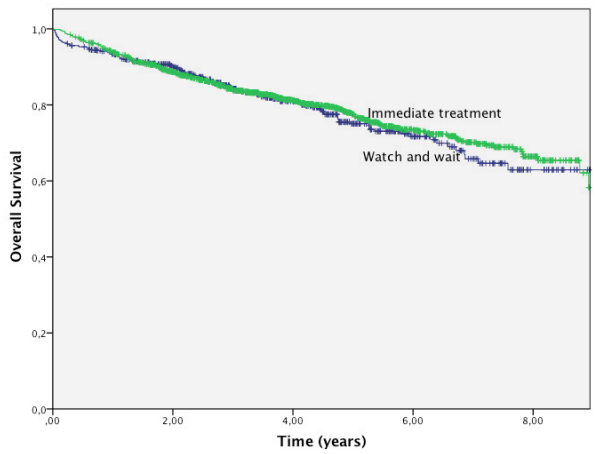

Figure 1. Survival curves estimated using the Kaplan-Meier method (unadjusted analyses, all patients), for immediate treatment and watch and wait.

Treatment modalities

Data on initial treatment modality were available for 1603 patients. In 12 cases the patients were treated by surgery, steroids, etc. and in 19 cases, data on initial treatment was not available. The distributions of therapy modalities, both overall, and for specific patient subsets, are summarised in Table 4.

Table 4. Patient´s characteristics according to different therapy modalities

Variable	Immuno therapy	Chemoimmuno therapy	Radio therapy	Combined modality therapy	Chemo therapy	All
N(%)	347	736	342	30	148	1603
Gender						
Female	195 (56%)	341 (46%)	192 (56%)	16 (53%)	72 (49%)	816 (51%)
Male	152 (44%)	395 (54%)	150 (44%)	14 (47%)	76 (51%)	787 (49%)
Agegroups						
<70	263 (75%)	489 (67%)	239 (70%)	26 (86%)	58 (39%)	1075 (67%)
70-80	61 (18%)	193 (26%)	69 (20%)	2 (7%)	46 (32%)	371 (23%)
=>80	23 (7%)	54 (7%)	34 (10%)	2 (7%)	44 (29%)	157 (10%)
Bulky disease						
Yes	29 (8%)	239 (33%)	11 (3%)	7 (23%)	21 (14%)	307 (19%)
No	302 (89%)	487 (66%)	328 (96%)	23 (77%)	117 (79%)	1264 (79%)
Unverified	9 (3%)	10 (1%)	3 (1%)	0	10 7%)	32 (2%)
Date of diagnosis						
<2010	176 (51%)	373 (51%)	138(40%)	16 (53%)	93 (62%)	796 (50%)
>=2010	171 (50%)	363 (49%)	204 (60%)	14 (47%)	55 (38%)	807 (50%)
FLIPI						
0	25 (7%)	24 (3%)	68 (20%)	6 (20%)	2 (1%)	125 (8%)
1	94 (27%)	135 (19%)	147 (43%)	7 (20%)	27 (18%)	410 (26%)
2	98 (28%)	202 (27%)	30 (9%)	4 (14%)	39 (27%)	373 (23%)
3	32 (9%)	127 (17%)	3 (0,5%)	3 (11%)	35 (23%)	200 (12%)
4	6 (2%)	38 (5%)	2 (0,5%)	1 (4%)	7 (5%)	54 (3%)
Missing	92 (27%)	210 (29%)	92 (27%)	9 (31%)	38 (26%)	441 (28%)
Bone marrow involvement						
Yes	96 (28%)	260 (35%)	7 (2%)	5 (17%)	52 (35%)	420 (26%)
Missing	251 (72%)	476 (65%)	335 (98%)	25 (83%)	96 (65%)	1183 (74%)
5-year OS	84%	75%	89%	78%	53%	

Significantly more females (56%) than males received immunotherapy (single-agent rituximab) (p=0.025) or radiotherapy (p=0.028). Correspondingly, significantly more men (54%) were treated with chemoimmunotherapy (p<0.001). Regarding age, we found that significantly more patients <70 years of age were

treated with immunotherapy (p<0.001). In total 87 (26%) of limited stage patients received rituximab monotherapy. Otherwise, there were no differences in the treatment modalities among the age groups.

Patients with bulky disease or bone marrow involvement were treated significantly more often with chemoimmunotherapy than with other therapies (p<0.001), which was expected. Regarding the choice of therapy before and after 2010, we found that chemotherapy was used significantly more often before 2010 (p<0.001), and that RT became more frequent after 2010 (p<0.001). The majority of the patients treated with radiotherapy were stage I (N=218, 74%) or II (N=45, 15%). The radiation dose was 30 Gy in 168 patients (57%), 24 Gy in 84 patients (29%) and 4 Gy in 41 patients (14%). Patients with FLIPI 2-4 were treated significantly more often with chemoimmunotherapy (p<0.001) or chemotherapy (p<0.001), compared to patients with low-risk FLIPI.

The 5-year OS for patients treated with immunotherapy was 84% vs. 75% and 89% for those treated with chemoimmunotherapy or radiotherapy, respectively. Figure 2 shows the OS of patients treated with each modality. In multivariate analyses, the only significant finding was that chemotherapy was inferior to immunotherapy (HR= 1.91, 95% CI 1.17-3.11, p=0.009). Otherwise, no differences were seen in the overall survival of patients treated with immunotherapy vs. chemoimmunotherapy (HR, 1.28; 95% CI 0.85-1.92, p=0.283) or immunotherapy vs. radiotherapy (HR, 0.91; 95% CI 0.53-1.56, p=0.737).

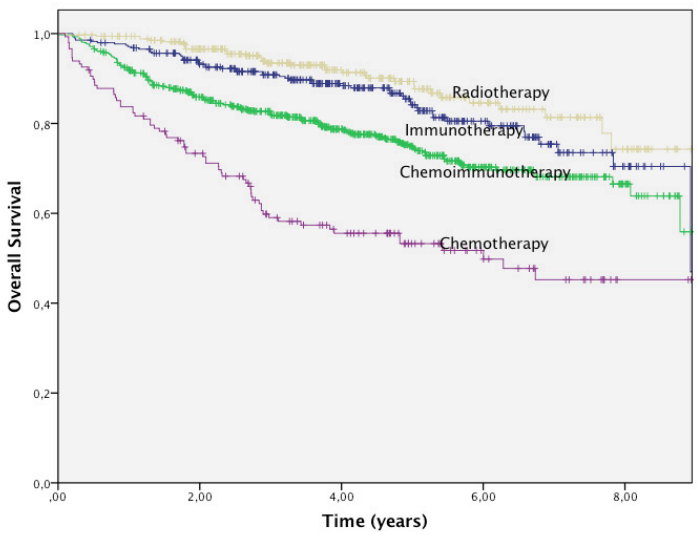

Figure 2. Survival curves estimated using the Kaplan-Meier method (unadjusted analyses) for radiotherapy, immunotherapy, chemoimmunotherapy and chemotherapy.

Systemic therapies

A total of 1146 patients with detailed data on medical treatment were identified. In 342 cases, the patients were treated with RT, and in 30 cases with combined modality therapy. Table 5 summarises the systemic treatment options, overall and in different subgroups. R-CHOP (rituximab and CHOP) was the most frequently used systemic regimen (N=486, 32%), and rituximab monotherapy the second most common (N=347, 23%). Rituximab monotherapy was significantly more common in women (38%) than in men (20%) ($p<0.001$), whereas R-CHOP was more often used in men (34%) than in women (29%) ($p=0.01$).

Table 5. Patient characteristics and response assessment according to the choice of induction therapy

Variable	R-CVP	R-CHOP	Rituximab monotherapy	R-bendamustine	R-chlorambucil	Chlorambucil	All
N	36	486	347	144	31	102	1146
Gender							
Female	16 (46%)	225 (46%)	295 (85%)	68 (47%)	16 (51%)	49 (48%)	568 (50%)
Male	20 (54%)	261 (54%)	152 (15%)	76 (53%)	15 (49%)	53 (52%)	578 (50%)
Age groups							
<70	9 (25%)	353 (73%)	263 (76%)	97 (67%)	8 (26%)	30 (29%)	760 (66%)
70-80	17 (47%)	111 (23%)	61 (18%)	40 (28%)	9 (29%)	39 (38%)	277 (24%)
=>80	10 (28%)	22 (5%)	23 (6%)	7 (5%)	14 (45%)	33 (33%)	109 (10%)
Bulky disease							
Yes	4 (11%)	178 (37%)	29 (8%)	41 (29%)	7 (23%)	9 (9%)	268 (23%)
No	32 (89%)	301 (62%)	309 (89%)	102 (71%)	22 (71%)	86 (84%)	852 (75%)
Unverified	0	7 (1%)	9 (3%)	1	2 (6%)	7 (7%)	26 (2%)
Rituximab maintenance							
Yes	1 (2%)	210(43%)	26(7%)	55(38%)	6(19%)	0	298(26%)
No	35(98%)	253(52%)	310(90%)	81(56%)	25(81%)	94(92%)	798(70%)
Unverified	0	23(5%)	11(3%)	8(6%)	0	8(8%)	50(4%)
Date of diagnosis							
<2010	26 (72%)	294 (60%)	176 (51%)	14 (10%)	16 (51%)	61 (60%)	587 (51%)
=>2010	10 (28%)	192 (40%)	171 (49%)	130 (90%)	15 (49%)	41 (40%)	559 (49%)
FLIPI							
0	1 (3%)	13 (3%)	25 (7%)	5 (3%)	2 (6%)	2 (2%)	48 (4%)
1	2 (6%)	100 (21%)	94 (27%)	29 (20%)	1 (3%)	21 (21%)	247 (22%)
2	6 (17%)	124 (26%)	98 (28%)	53 (37%)	8 (26%)	22 (21%)	311 (27%)
3	4 (11%)	85 (17%)	32 (9%)	25 (17%)	5 (16%)	28 (27%)	179 (15%)
4	7 (19%)	17 (3%)	6 (2%)	8 (6%)	2 (6%)	3 (3%)	43 (4%)
Missing	16 (44%)	147 (30%)	92 (27%)	24 (17%)	13 (43%)	26 (26%)	318 (28%)
Bone marrow involvement							
Yes	15 (42%)	170 (35%)	96 (28%)	50 (35%)	11 (35%)	34 (33%)	376 (33%)
Missing	21 (58%)	316 (65%)	251 (72%)	94 (65%)	20 (65%)	68 (67%)	770 (67%)
Response evaluation							
ORR (CR/PR)	22(61%)	445(92%)	255(73%)	129(90%)	18(58%)	71(70%)	940(82%)
CR	9(26%)	232(48%)	103(30%)	72(50%)	7(23%)	14(14%)	437(38%)
PR	13(36%)	213(44%)	152(44%)	57(40%)	11(36%)	57(56%)	503(44%)
SD	2(5%)	7(1%)	34(10%)	2(1%)	1(3%)	10(10%)	56(5%)
PD	2(5%)	19(4%)	29(8%)	7(5%)	2(6%)	7(6%)	66(6%)
Missing	10(28%)	15(3%)	29(8%)	6(4%)	10(32%)	14(14%)	84(7%)
5-year OS	18%	80%	84%	82%	53%	53%	

Patients less than 70 years of age were significantly more commonly treated with R-CHOP (p=0.001) and with single-agent rituximab (p<0.001). In contrast, patients over 70 years were significantly more often treated with R-CVP (rituximab and CVP) (p<0.001) and (R)-chlorambucil (chlorambucil +/- rituximab) (p<0.001). R-CHOP (p<0.001) and R-CVP (p=0.026) were more commonly used before 2010 (p<0.001), while after 2010, the use of R-bendamustine increased significantly (p<0.001).

Compared to patients with low-risk disease (FLIPI 0-1), patients with FLIPI 2-4 were treated significantly more often with R-CHOP (34% vs. 26%) (p=0.007), R-bendamustine (13% vs 8%) (p=0.009) or (R)-chlorambucil (10% vs. 6%) (p=0.016), whereas rituximab monotherapy was more often used (27% vs. 20%) (p=0.005) in patients with FLIPI 0-1 than in those with FLIPI 2-4.

Response and overall survival

In the majority of patients 1062 (93%), receiving systemic induction therapy, data on initial response are available (Table 5). In total, an initial response to treatment was obtained in 445 (92%) of eligible patients treated with R-CHOP, in 129 (90%) of 144 patients with R-bendamustine, in 255 (73%) of 347 patients with rituximab monotherapy, in 71 (70%) of 102 patients with chlorambucil, in 22(61%) of 36 patients with R-CVP and in 18(58%) of 31 patients with R-chlorambucil. In total, the overall response rate was 940 (82%) among the 1146 patients receiving immediate systemic treatment. The complete remission rates were documented as 50% for R-bendamustine, 48% for R-CHOP and 30% for rituximab monotherapy.

The 5-year OS for the R-CHOP group was 80%, vs. 84% and 82% for single-agent rituximab and R-bendamustine, respectively (Figure 3). Multivariate analysis for overall survival, adjusted for gender, FLIPI, bulky disease, and date of diagnosis, showed no difference in efficacy between rituximab monotherapy and R-CHOP (HR 1.03; 95% CI 0.65-1.64, p=0.891), or between rituximab monotherapy and R-bendamustine (HR, 1.24; 95%CI 0.66-2.32, p=0.501).

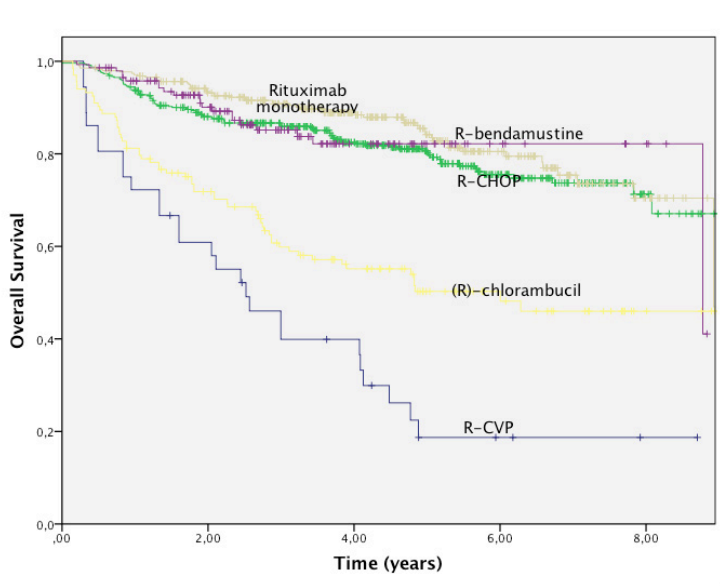

Figure 3. Survival curves estimated using the Kaplan-Meier method (unadjusted analyses) for single-agent rituximab, R-CHOP, R-bendamustine, chlorambucil, R-chlorambucil and R-CVP.

Rituximab maintenance

A minority of patients 298(26%), receiving different chemoimmunotherapy regimens, also received rituximab maintenance therapy. Data on schedules and duration of maintenance therapy are not available in the registry. The most common schedule and duration of maintenance treatment, according to the Swedish guidelines, is a single rituximab infusion every 2 months for up to 2 years.

Before maintenance, patients received induction therapy with R-CHOP (70%), R-bendamustine (18%), rituximab monotherapy (9%), R-CVP (1%) or R-chlorambucil (2%). Significantly more patients <70 years ($p<0.001$) were treated with rituximab maintenance. The 5-year OS for the patients treated with rituximab maintenance was 83% vs 75% for the group with no further treatment. In multivariate analysis, corrected for immortal time bias, and adjusted for gender, FLIPI, bulky disease, quality of initial response, and date of diagnosis rituximab maintenance treatment was associated with superior overall survival (HR 0.56; 95% CI 0.37-0.87, $p=0.010$).

Discussion

There is still no consensus regarding the optimal initial treatment of FL. In this Swedish population-based study, in which we surveyed the initial management of patients with FL, we hope to contribute to this body of evidence.

The primary objective of this study was to compare overall survival for patients treated with rituximab monotherapy, compared with chemoimmunotherapy (R-CHOP and R-bendamustine). After adjustment for prognostic variables, we found no difference between these two populations. Rituximab as a single agent is an easily administered and well-tolerated treatment with few side effects, without the need for central catheters, and without risk for long term cardiotoxicity and secondary malignancies. The data presented here indicate that initial treatment with rituximab does not seem to impair long term outcome. In addition long term follow-up of patients treated with rituximab only, suggest that a considerable proportion of patients, about 30%, will not need additional chemotherapy. [22] To the best of our knowledge, no randomised trials have been performed, or are planned, to compare the efficacy of rituximab to that of chemoimmunotherapy, but both this study and others provide support for rituximab monotherapy as a reasonable initial choice for patients requiring treatment [5,23,24]. However, without a randomized trial, we cannot exclude a bias, in that the patients receiving rituximab monotherapy in our series, may have been associated with more favourable characteristics.

R-CHOP was the most frequently used systemic treatment before 2010. Bendamustine was introduced in Sweden in 2010, and its use was also recommended in the Swedish treatment guidelines for FL. Accordingly, we observed a marked increase in the use of bendamustine in the later years. We found that both R-bendamustine and R-CHOP to be appropriate options for patients in need of rapid tumour reduction. These findings are in line with those from two randomised trials; the StiL (Study group Indolent Lymphoma) trial [10] and the BRIGHT study[11]. R-bendamustine has fewer toxic effects than R-CHOP, and is a well-tolerated treatment also for elderly patients[25]. Patients over 70 years were significantly more often treated with R-CVP in the present study. However, the 5-year OS for patients receiving R-CVP was only 18%, supporting that R-bendamustine may be a more suitable choice for elderly patients in need of rituximab combined with chemotherapy.

In the group studied here, we found no difference in OS between patients with FL who were treated immediately after diagnosis and those managed by watchful waiting. These results are in line with those from previous studies, including a population-based study in Denmark, showing that the watch and wait strategy was associated with a favourable outcome[26]. A large prospective, observational US

study based on the NLCS (National LymphoCare Study) also showed that an initial management strategy of watch and wait led to a meaningful OS, and remains a viable option in the modern era[27]. The FLIPI score was strongly related to OS in our study, as was age[28], confirming a previous report from the NLCS [29].

When comparing patients receiving different treatment modalities, we found that males, patients with bulky disease and patients with bone marrow involvement were treated significantly more often with chemoimmunotherapy. However, no significant difference was found in terms of OS between patients treated with chemoimmunotherapy, immunotherapy or radiotherapy.

Gender was found to be a significant prognostic factor for overall survival in multivariate analysis. A North American study based on the NLCS showed subtle differences in progression-free survival (PFS) and OS between males and females aged <60 years, treated either with rituximab monotherapy or chemo-immunotherapy [30]. Another study carried out in Sweden, also based on the SLR, showed improved survival of women over 60 years treated with rituximab monotherapy[31].

Radiotherapy is recommended as a possible curative treatment option for patients with Ann Arbor stage I and limited stage II disease [32,33]. Historical series support the use of RT at diagnosis for limited-stage FL, showing PFS rates at ten years ranging from 40 to 59%, and OS rates from 58 to 86% [15,34-38] despite a significant decline in the use of radiotherapy over time, according to the NLCS study [32]. In contrast, the use of radiotherapy has increased since 2010 in Sweden, and OS was found to be equivalent to immunotherapy and chemoimmunotherapy in both univariate and multivariate analyses (Fig. 2). The Stanford report[39], the study from the NLCS [40] and the present study all indicate that primary treatment with RT should be considered as a standard treatment option, despite the absence of randomised trials.

Contrary to our expectations, in this study we found a significant difference in OS, between patients treated with induction and rituximab maintenance compared with patients receiving induction therapy and no further treatment. In the large randomized PRIMA trial, the strategy of maintenance rituximab after induction, was shown to prolong PFS and delay the time to next antilymphoma treatment, but was not associated with a prolonged OS.[12]. The median follow-up in the PRIMA trial was one year shorter than in the present study, which may have been of importance. However, we are unable to draw definite conclusions regarding the survival benefit of maintenance rituximab since we do not have information on the schedule or duration of maintenance. We expect that the schedule was in accordance with our guidelines recommendation, one infusion every 8 weeks for 2 years. Thus, these results should be interpreted with caution.

Among the strengths of the present study is the high coverage rate in the SLR (95-97%). We have collected detailed information about risk factors, although, data on beta-2-microglobulin and lymph node diameter are missing. A limitation is that treatment data were missing in 52%, but this population was not significantly different from the patients for whom information on treatment was available. Other limitations are the lack of data on progression-free survival, duration of response, further lines of treatment and co-morbidity, which will also affect the OS in cases of this indolent disease. The median follow-up was relatively short for this type of study (48 months). The lack of central pathology review, which was not feasible in a cohort of this size, is also a limitation, as is the lack of central evaluation of imaging.

Our data support that rituximab monotherapy is a valid first-line treatment option, associated with a similar OS in comparison to chemoimmunotherapy, which raises intriguing questions regarding the choice of therapy for symptomatic patients. Our data also indicate that overall survival may be prolonged by rituximab maintenance therapy, although these results need to be interpreted with caution.

Authorship

Contribution: E.S., M.J. designed the research; E.S., S.P. and B.W. collected data; E.S., M.J. and S.P. analysed data; and E.S., M.J., S.P. and B.W. wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interest.

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