Aggressive B-cell Lymphomas

Studies of Treatment, FDG-PET Evaluation and Prognostic Factors

MAGDALENA ADDE
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Abstract

To improve outcome in young, high-risk lymphoma patients, treatment was intensified, adding etoposide and rituximab to standard CHOP treatment. Granulocyte-colony stimulating factor (G-CSF) enabled treatment bi-weekly. Results were promising: overall (OS) and event-free survival (EFS) 79% and 60% respectively, median follow up 27 months. Single infusion Ara-C, contrary to expectations, did not prevent relapse in CNS.

DLBCL were classified as germinal center (GC) or non-GC derived, using immunohistochemical markers, CD10, BCL6 and MUM1. We investigated the outcome for both phenotypes after adding rituximab to chemotherapy. For 106 patients treated with CHOP alone, the GC phenotype displayed significantly better OS and EFS. In contrast, GC phenotype did not predict outcome in 95 patients treated with immunochemotherapy. Thus, addition of rituximab seems to eliminate the prognostic value of immunohistochemically defined GC phenotypes in DLBCL.

To improve evaluation and find non-responders, mid-treatment FDG-PET CT was incorporated into clinical routine for patients with high-risk aggressive lymphoma. For those with positive PET, biopsy followed by treatment intensification was recommended. Twenty-five patients were examined, five with positive PET. Two of these had lymphoma in the biopsy. Two had a negative biopsy, and one had a false positive investigation. Seven patients had increased uptake of uncertain significance. Two patients with uncertain PET, and two with negative PET have relapsed, giving a negative predictive value of 85%.

In case of relapse of aggressive lymphoma or if not obtaining CR, high dose chemotherapy with autologous stem cell support (HDT) is standard treatment. HDT outcome for 38 patients with transformed follicular lymphoma was compared to outcome for 79 patients with de novo B-cell lymphoma. At median follow-up of 11.5 years both OS and EFS were superior in the transformed group, OS 67% and 33%, EFS 55% and 27% respectively. Treatment related mortality was less than reported in other studies.

Keywords: High-risk aggressive B-cell lymphoma, R-CHOEP-14, Prognostic factors, Immunochemotherapy, FDG-PET, High-dose treatment, Transformed follicular lymphoma

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“The greatest glory in living lies not in never falling, but in rising every time we fall”
Nelson Mandela

To my children: Josefine, Johanna, Jesper and Petronella
List of Papers

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

I. Outcome for Young High-Risk Aggressive B-cell Lymphoma Patients Treated with CHOEP-14 and Rituximab (R-CHOEP-14).
   
   **Adde M**, Enblad G, Hagberg H, Sundström C, Laurell A
   Med Oncol. 2006;23 (2):283-93.

II. Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immuno-chemotherapy.


III. Little usefulness of mid-treatment FDG-PET and biopsy for treatment intensification in patients with aggressive lymphoma.

   Manuscript

IV Superior outcome in transformed follicular lymphoma compared to de novo aggressive B-cell lymphoma treated with high-dose therapy and autologous stem-cell support.

   Manuscript

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Abbreviations

aaIPI age-adjusted International Prognostic Index
ABC activated B-cell group
CD cluster of differentiation
CLL chronic lymphocytic leukaemia
CNS central nervous system
CR complete remission
CT computer tomography
DFS disease free survival
DLBCL diffuse large B-cell lymphoma
EFS event-free survival
FDG fluorine-18 fluorodeoxyglucose
FL follicular lymphoma
GC germinal center
G-CSF granulocyte-colony stimulating factor
HDT high dose therapy
HTLV1 human T-cell lymphoma virus type 1
Ig immunoglobulin
IPI International Prognostic Index
IT intrathecal
NHL non-Hodgkin lymphoma
NK natural killer
OS overall survival
PCP pneumocystis carinii pneumonia
PET positron emission tomography
PFS progression free survival
R rituximab
REAL Revised European-American Classification of Lymphoid Neoplasms
RT-PCR reverse transcriptase polymerase chain reaction
s-LDH serum lactate dehydrogenase
SUV standardized uptake value
TCR T-cell receptor
TRM treatment related mortality
WHO World Health Organization
1 Introduction

Lymphomas constitute a heterogeneous group of neoplastic disorders originating from lymphoid cells (B and T/NK cells) with varied underlying biology and clinical features.\(^1\) Lymphomas can occur at any age and involve any lymphatic tissue such as lymph nodes, Waldeyer’s ring and the spleen, and can also involve extranodal tissue such as the gastrointestinal tract, liver, brain, skin or lung either primarily or by haematogenous spread. The incidence of lymphoma has increased by 3-4% per year since the 1960's, although the rate of increase has declined during the last decade.\(^2\) Still lymphomas make up about 5% of all new cancers in Sweden (around 2,000 new cases per year).\(^5\) Some risk factors for developing lymphoma are known: The likelihood of developing lymphoma increases with age. Lymphomas are slightly more common in men than in women. A compromised immune system such as in HIV infection, autoimmune diseases and treatment with immunosuppressant drugs following an organ transplant all increase the risk for developing lymphoma.\(^6\) Certain viruses, such as the Epstein Barr virus (EBV), Hepatitis C and human T-cell lymphoma virus 1 (HTLV1) can contribute to the development of lymphomas. It is also known that exposure to chemicals found in pesticides, solvents and fertilizers increases the risk of developing lymphoma.\(^7\)

Lymphomas are divided according to the World Health Organization (WHO) classification into B-cell, T/NK-cell and Hodgkin lymphomas (table 1). Further, the disease is subdivided into around 50 different entities based on morphological, clinical, molecular and genetic grounds. B-cell lymphomas make up about 85% of all lymphomas, T/NK-cell lymphomas 5-10% and Hodgkin lymphoma 5-10%. In previous classifications, the term non-Hodgkin lymphoma (NHL) was used to include both B and T-cell lymphomas as opposed to Hodgkin lymphoma. Crudely, lymphomas can be divided into aggressive, high-grade malignant, and indolent, low-grade malignant lymphomas; although not all fit into these categories. Aggressive lymphomas have a rapidly progressive clinical course and are potentially curable with intensive chemotherapy. The most common type of aggressive lymphoma is diffuse large B-cell lymphoma (DLBCL). Indolent lymphomas are chronic diseases for which, in the majority of cases, there is no available cure. The most common types of indolent lymphoma are chronic lymphocytic leukaemia (CLL) and follicular lymphoma (FL). FL and to some extent CLL can transform to DLBCL.
T-cell lymphomas are an even more heterogeneous group of lymphomas with a varying clinical course. Some of the T-cell lymphomas have a clinical course similar to or worse than that of DLBCL. This group includes peripheral T-cell lymphoma unspecified, enteropathy-type T-cell lymphoma, anaplastic large cell lymphoma, and angioimmunoblastic T-cell lymphoma.

The present WHO classification of lymphoma was published in 2008 and is based on the Revised European American Lymphoma Classification of Lymphoid Neoplasms (REAL). The system classifies lymphomas by the cell type corresponding to a normal counterpart using morphological, immuno-logical and genetic definitions.

Table 1. WHO 2008 Classification of lymphoma

**B-CELL NEOPLASMS**

*Precursor B-cell neoplasm*
- B-lymphoblastic leukaemia/lymphoma

*Mature B-cell neoplasms*
- Chronic lymphocytic leukaemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukaemia
- Splenic B-cell marginal zone lymphoma (with/without villous lymphocytes)
- Hairy cell leukaemia
- Lymphoplasmacytic lymphoma
- Waldenström macroglobulinemia
- Heavy chain diseases
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmocytoma
- Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Nodal marginal zone lymphoma
- Follicular lymphoma
- Primary cutaneous follicle centre lymphoma
- Mantle-cell lymphoma
- Diffuse large B-cell lymphoma (DLBCL) NOS
  - T-cell/histiocyte rich large B-cell lymphoma
  - Primary DLBCL of the CNS
  - Primary cutaneous DLBCL, leg type
  - EBV positive DLBCL of the elderly
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- ALK positive large B-cell lymphoma
- Plasmablastic lymphoma
- Large B-cell lymphoma arising in HHV8-associated multicentric
Castleman disease
Primary effusion lymphoma
Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma

T-CELL AND NK-CELL NEOPLASMS

Precursor T-cell neoplasm
T-lymphoblastic lymphoma/leukaemia

Mature T-cell and NK-cell neoplasms
T-cell prolymphocytic leukaemia
T-cell large granular lymphocytic leukaemia
Aggressive NK-cell leukaemia
Systemic EBV positive T-cell lymphoproliferative disease of childhood
Hydroa vacciniforme-like lymphoma
Adult T-cell leukaemia/ lymphoma
Extranodal NK/T cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30 positive T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
Anaplastic large-cell lymphoma, ALK positive
Anaplastic large cell lymphoma, ALK negative

HODGKIN LYMPHOMA

Nodular lymphocyte-predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
   Nodular sclerosis classical Hodgkin lymphoma
   Lymphocyte-rich classical Hodgkin lymphoma
   Mixed cellularity classical Hodgkin lymphoma
   Lymphocyte-depleted classical Hodgkin lymphoma
1.1 Tumour pathology; diagnostic aspects of Diffuse Large B-cell lymphoma and Follicular lymphoma

Diffuse large B-cell lymphoma is a neoplasm with a diffuse growth pattern, consisting of large B lymphoid cells with nuclei as large as, or larger than, normal macrophage nuclei, or having a cell size more than twice that of a normal lymphocyte. The World Health Organization has used all available information and relied on a combination of morphologic, immunophenotypic, genetic and clinical features to classify DLBCL into several different groups. Common morphological variants are centroblastic, immunoblastic and anaplastic DLBCL. Further, there are four DLBCL subtypes and eight other entities of large B-cells. The subtypes are T-cell/histiocyte rich large B-cell lymphoma, primary DLBCL of the CNS, primary cutaneous DLBCL, leg type, and EBV positive DLBCL of the elderly. The eight other entities include among others mediastinal large B-cell lymphoma and plasmablastic lymphoma. The latest classification also recognizes cases on the borderline between DLBCL and Burkitt lymphoma on the one hand, and DLBCL and classical Hodgkin lymphoma on the other. In addition there is a group of DLBCL for which there are no clear and accepted criteria for subdivision, the DLBCL NOS (not otherwise specified) group.

The morphologic differential diagnosis of DLBCL includes other large-cell malignancies such as carcinoma and melanoma. The latter entities can easily be distinguished by immunohistochemical techniques with carcinoma being cytokeratin positive and melanoma being S100 positive.

The lymphoma cells express all or some of the pan B-cell markers CD19, CD20, CD22 and CD79a, and immunoglobulin is present in 50-75%. CD5 is expressed in 10% of DLBCL cases, most often primary (de novo) DLBCL, and in rare instances cases thought to represent Richter transformation from a low-grade B-cell lymphoma (CLL). CD10 is a marker of the germinal center (GC) and is expressed in 30-60% of DLBCL both in de novo and transformed FL cases. BCL6, another GC marker, and IRF4/MUM1, a post GC marker, are expressed in 60-90% and 33-65% respectively. The proliferation index, measured as Ki-67 expression, is usually high, above 40%, and values greater than 90% are associated with a worse outcome.

Several genetic abnormalities are seen in DLBCL, but no single genetic aberration is pathognomonic. Rearrangement of immunoglobulin heavy and/or light chain genes is present and hypermutation is seen in the majority of cases. There are aberrant somatic hypermutations targeting other genetic loci such as MYC and PAX5 encountered in more than 50% of DLBCL cases, possibly contributing to the oncogenesis of this lymphoma group. The t(14:18)(q32;21) translocation, usually associated with follicular lymphoma, is found in up to one third of DLBCL cases. The translocation juxtaposes the BCL2 gene of chromosome 18 with the immunoglobulin heavy chain gene.
on chromosome 14. Structural abnormalities involving the BCL6 gene at 3q27 are also frequent.10

Follicular lymphoma is a neoplasm composed of follicle center (germinal center) B-cells characterized by a predominantly follicular growth pattern with closely packed follicles that efface the nodal architecture. Areas of diffuse growth may be present. Centrocytes and centroblasts are randomly distributed in the germinal centers. A histological grade is assigned according to the number of blasts/high power field (grade 1-2<15 blasts, grade 3 >15 blasts). Follicular lymphoma grade 3b (with sheets of blasts) is considered an aggressive lymphoma and treated as such. The proportion of centroblasts (large cells) seems to be predictive of clinical outcome.11 Cases with a greater proportion of large cells behave more aggressively and have a greater likelihood of progression to DLBCL than those with a lesser proportion. Transformation of follicular lymphoma to an aggressive B-cell lymphoma is evidenced by coherent areas of centroblasts in diffuse areas, or complete effacement of the tissue by blastic cells.

The follicular lymphomas express B-cell associated antigens CD19, CD20, CD22, CD79a, and the proteins BCL2 and BCL6. They are usually CD10+ and CD5-. Some cases, especially grade 3b, may lack CD10 but retain BCL6 expression. BCL2 protein is expressed by 85-90% of the neoplastic cells in grade 1 and grade 2 tumors, but in only 50% of the neoplastic cells in grade 3 tumors. This may be useful in distinguishing reactive from neoplastic follicles. IRF4/MUM1 is typically absent in FL. The proliferation index generally correlates with histological grade.

The cells of follicular lymphoma express surface Ig. The heavy and light chains genes are rearranged and the variable region genes show extensive and ongoing somatic hypermutation. FL is characterized by the translocation t(14;18)(q32;q21), present in up to 70-95% of grade 1-2 tumors and involving BCL2 gene rearrangements. This results in deregulated expression of the anti-apoptotic protein BCL2. There are a number of additional and heterogeneous genetic alterations, increasing with grade and transformation suggesting that no single genetic mechanism is responsible for all the events of transformation.

1.2 Tumour biology

The lymphomas occur as a result of neoplastic transformation of B or T lymphocytes arrested at different stages of differentiation. B-cells express surface Ig, and T-cells express surface T-cell receptors (TCR), and both work as antigen receptors. The Ig molecule is composed of two heavy and two light chains (either kappa or lambda). The TCR, which is structurally similar to the Ig receptor, is composed of two types of receptors, alpha/beta or gamma/delta. In addition, a variety of surface and cellular antigens can be
detected and are specifically expressed at the different stages of B and T cell development. They are referred to as the cluster of differentiation (CD).

The variable heavy chain (VH) gene of Ig is rearranged and expressed as a unique, clonal surface-Ig receptor in B-cell lymphomas. Studies of DLBCL have demonstrated that the majority of these tumours contain mutated VH genes in a pattern that suggests antigen selection pressure. These mutations are considered to be the result of the somatic mutation process that occurs in germinal centers of secondary lymphoid organs.

Germinal centers are B-lymphoid blast cell areas arising eccentrically in primary lymphoid follicles in response to antigenic stimulation. They are the generally accepted sites of generation of memory B-cells undergoing isotype switching and somatic hypermutations in the Ig genes. They are localized in the internal region of the follicles in lymph-nodes, toward the submucosal layer in Peyer’s patches and at bifurcations of the arterioles traversing the white pulp in the spleen.

In 2000, Alizadeh et al published a paper on gene expression profiling in DLBCL where they showed that DLBCL could be divided into three subgroups according to gene expression profile similarities to normal B-cells. One group of DLBCL showed an expression pattern close to GC derived B-cells. Another group showed a closer relationship with activated post-GC cells, the activated B-cell group (ABC), and there was also a third, smaller group that could not be assigned to either of the two other groups. When survival was analyzed, patients in the GC group had a significantly better survival compared to the ABC and third group, which had a similar outcome, and formed the non-GC group. This finding has been confirmed in a study by Rosenwald et al who developed DNA micro-arrays composed of genes whose products are preferentially expressed in lymphoid cells and genes thought or confirmed to play a part in cancer and immune function, the Leukemia Lymphoma Molecular Profiling Project (LLMPP). Also in this project, patients whose tumours had a GC B-cell-like signature were associated with a good outcome, whereas tumours made up of the ABC subtype, were associated with a poor outcome. These different tumour subtypes exist within each clinical risk group. Thus the development and use of DNA microarray techniques and gene profiling have supplied us with more precise tools with which to explore the relationship between prognosis and the molecular features of the disease. Results, however, are still difficult to duplicate because of technical differences and different compositions of used microarrays.

Hans et al have shown that it was possible to use immunohistochemical staining to subdivide DLBCL into GC and non-GC phenotypes. In this paper, tumour samples from patients, whose B-cell subtype had already been determined using microarray technique, were used. It was found that antibodies recognizing molecules, whose mRNA expression was highly associated with the GC and non-GC subtypes, respectively, were reactive in forma-
lin-fixed paraffin embedded tissue. Based on the expression of BCL6, CD10 and MUM1/IRF4, i.e., using a panel of only three immunostainings, DLBCL could be subdivided into GC and non-GC subtypes. The cases were assigned to the immunohistochemically defined GC-group if CD10 alone or together with BCL6 was positive. If both CD10 and BCL6 were negative the cases were classified to the non-GC group. If CD10 was negative and BCL6 positive, the classification was based on MUM1 expression. If MUM1 was negative, the cases were assigned to the GC-group; whereas MUM1 positive cases were classified to the immunohistochemically defined non-GC group.

Figure 1. Results of immunoperoxidase staining. (A) A GC case that is positive for CD10 and BCL6 but negative for MUM1. (B) A non-GC case that is negative for CD10 but shows rare BCL6 positive cells and is positive for MUM1.

Five year survival for the GC B-cell group was 76% compared to 34% for the non GC B-cell group. Multivariate analysis showed that the high-risk International Prognostic Index (IPI) group, (3-5 factors) and non-GC B-cell phenotype were independent adverse predictors. However, data are conflicting, with some studies showing better survival for the GC B-cell group,24-26 while other studies have found no difference in survival between the two groups.27, 28
Figure 2. Decision tree for immunoperoxidase tissue microarray classification of DLBCL.\textsuperscript{23}

Lossos et al\textsuperscript{29} have shown that lymphomas derived from the GC phenotype had ongoing mutations in the Ig genes, and most cases classified as ABC phenotype had no ongoing mutations in the Ig genes. This shows that these lymphoid malignancies are derived from normal cells at discrete stages of normal lymphocyte maturation.

Transformation of follicular lymphoma to aggressive large cell lymphoma occurs in 25-60\% of patients. Studies have been carried out attempting to explain the molecular pathogenesis involved. In order to provide molecular characterization of the histological and clinical transformation, Berglund et al compared tumour samples from 30 patients before and after transformation using comparative genomic hybridization. The results were also compared with de novo DLBCL. Copy number changes were detected in 70\% of follicular lymphoma and in 97\% of transformed DLBCL. Clinical progression from follicular lymphoma to transformed DLBCL has been shown, on the genetic level, to be associated with the acquisition of an increasing number of genomic copy number changes, with non-random involvement of specific target regions. The findings support diverse genetic backgrounds for transformed and de novo DLBCL.\textsuperscript{30}

Lossos et al defined two different gene profiles associated with the transformation of FL to DLBCL involving c-MYC and the increased, alternatively, the decreased expression of the genes it regulates, thus demonstrating alternative pathways for transformation.\textsuperscript{31} Davies et al in a gene profiling and immunohistochemical study based on one patient with transformed FL found the tumour to be a germinal center B-like malignancy evolving by two pathways, one that is similar in proliferation rate to the antecedent FL, and the other that has a higher proliferation rate and is characterized by the presence of recognized oncogenic abnormalities.\textsuperscript{32}

Mukhopadhyay et al reported a case of FL containing a t(14;18) translocation transforming into a Burkitt-like lymphoma containing the original t(14;18) as well as an additional t(8;14) translocation. The latter translocation resulted in the phenotype of Burkitt lymphoma. The transformation from follicular lymphoma to Burkitt like lymphoma was demonstrated
within a single lymph node. This case illustrated the oncogenic stimulus resulting from the inhibition of apoptosis by BCL2 combined with the deregulation of cell growth by c-MYC.33

1.3 Staging of lymphoma and treatment response evaluation

The stage of a malignancy is a descriptor (usually I to IV) of the extent of the disease within the body. Knowledge regarding tumour extension is crucial in the management of lymphoma. The use of staging classifications facilitates comparison of treatment results between patients though it has not been as effective predicting long term survival. The Ann Arbor staging classification was published in 1971, and updated in 1989 in order to take into account the introduction of modern imaging techniques such as computer tomography (CT). This updated classification is still in use. It was originally developed for Hodgkin Lymphoma but subsequently applied to non Hodgkin Lymphoma.34-36

For the majority of patients DLBCL is a systemic disease at the time of diagnosis. At the completion of the initial staging evaluation, bulky stage II, stage III or stage IV disease is documented in approximately 75% of all DLBCL patients. One third of the patients have B-symptoms. More than 50% of the patients have an elevated serum lactate dehydrogenase (s-LDH). Bone marrow is involved in about 15% of the cases.

Table 2. Staging adapted after Carbone et al, 1971; Lister et al 1989

<table>
<thead>
<tr>
<th>Stage I</th>
<th>Involvement of a single lymph node region or structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage II</td>
<td>Involvement of two or more lymph node regions or structures on the same side of the diaphragm.</td>
</tr>
<tr>
<td>Stage III</td>
<td>Involvement of lymph node regions or structures on both sides of the diaphragm</td>
</tr>
<tr>
<td>Stage IV A</td>
<td>No symptoms</td>
</tr>
<tr>
<td>Stage IV B</td>
<td>Presence of any of the following symptoms:</td>
</tr>
<tr>
<td></td>
<td>Unexplained weight loss of &gt; 10% of the body weight during the last 6 months</td>
</tr>
<tr>
<td></td>
<td>Unexplained fever with temperature above 38°C</td>
</tr>
<tr>
<td></td>
<td>Night sweats</td>
</tr>
<tr>
<td>Stage IV E</td>
<td>Involvement of a single extra nodal site, contiguous or proximal to nodal site</td>
</tr>
<tr>
<td>Stage IV X</td>
<td>Bulky disease: A peripheral lymph node or region &gt; 10cm or a mediastinal mass &gt; 1/3 of the mediastinum at Th 5-6 level on a chest X-ray</td>
</tr>
</tbody>
</table>

Response is currently assessed on the basis of clinical, radiological, and pathological (i.e. bone marrow) criteria. CT scans are still the standard for evaluation of nodal disease even though positron emission tomography (PET) is coming into more frequent use. It is essential to use uniform re-
response criteria in clinical trials of patients with lymphoma. Therefore American and European lymphoma experts met in 1998 and agreed upon standardized guidelines for response assessment in adult patients with indolent and aggressive lymphoma. Briefly, complete remission (CR) was defined as the absence of any clinical and radiological sign of disease, and complete remission unconfirmed (CRu) as a rest tumour, which has decreased in size more than 75%, and is believed to be of no clinical significance. Partial remission (PR) requires a ≥ 50% decrease in size of involved lymph nodes. Progressive disease (PD) is defined as the enlargement of known lymph nodes or appearance of new tumours.

PET has been increasingly used in evaluation of lymphoma treatment, and updated response criteria were published in 2007 by Juweid et al. A pre-therapy PET is not obligatory for assessment of response after treatment, however, it is strongly recommended because it can facilitate the interpretation of post-therapy PET. In order to avoid post-therapy inflammatory changes, PET should preferably be done four weeks after chemotherapy; and in case of radiotherapy eight to twelve weeks after therapy. Visual assessment alone appears to be adequate for determining whether or not PET is positive or negative at the conclusion of therapy.

1.4 The International Non-Hodgkin Lymphoma Prognostic Factor Index (IPI)

The International Non-Hodgkin Lymphoma Prognostic Factor Index (IPI) analyzed pre-treatment clinical and laboratory characteristics in a retrospective sample of 3,273 patients with aggressive lymphoma in order to develop a predictive model for the outcome for NHL. The majority of patients had received anthracycline-based chemotherapy. Five pre-treatment characteristics were found to be independent predictors of death due to lymphoma: Age over 60, stadium III or IV disease, more than one extranodal site of involvement, elevated s-LDH and ECOG performance status 2 or 3 (not ambulatory). This allowed a sample of 2,031 patients to be divided into four risk groups (low, low-intermediate, high-intermediate and high) each with associated 5-year survival rates (SR): low risk (0-1 factor) 73% SR, low-intermediate risk (2 factors) 51% SR, high-intermediate risk (3 factors) 43% SR and high risk (4-5 factors) 26% SR. Increasing risk corresponded both to a lower rate of CR and a higher relapse rate. For 1,274 younger patients, the simpler age-adjusted IPI (aIIIPI) using s-LDH, performance (ECOG) and stage have been used to divide patients into four different categories in which aIIIPI 2 and 3, high and high-intermediate, have been shown to have a worse outcome.
Table 3. The International Prognostic Index (IPI) and age-adjusted IPI (aaIPI)

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Risk factors</th>
<th>Distribution of cases (%)</th>
<th>CR rate (%)</th>
<th>2-year rate</th>
<th>5-year rate</th>
<th>RFS (%)</th>
<th>OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPI (all ages)</td>
<td>Low (L)</td>
<td>0-1</td>
<td>35</td>
<td>87</td>
<td>79</td>
<td>70</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Low-intermediate (LI)</td>
<td>2</td>
<td>27</td>
<td>67</td>
<td>66</td>
<td>50</td>
<td>66</td>
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<tr>
<td></td>
<td>High-intermediate (HI)</td>
<td>3</td>
<td>22</td>
<td>55</td>
<td>59</td>
<td>49</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>High (H)</td>
<td>4-5</td>
<td>16</td>
<td>44</td>
<td>58</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>Age-adjusted Index Patients &lt;60</td>
<td>Low (L)</td>
<td>0</td>
<td>22</td>
<td>92</td>
<td>88</td>
<td>86</td>
<td>90</td>
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<tr>
<td></td>
<td>Low-intermediate (LI)</td>
<td>1</td>
<td>32</td>
<td>78</td>
<td>74</td>
<td>66</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>High-intermediate (HI)</td>
<td>2</td>
<td>32</td>
<td>57</td>
<td>62</td>
<td>53</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>High (H)</td>
<td>3</td>
<td>14</td>
<td>46</td>
<td>61</td>
<td>58</td>
<td>37</td>
</tr>
<tr>
<td>Age-adjusted Index Patients &gt;60</td>
<td>Low (L)</td>
<td>0</td>
<td>18</td>
<td>91</td>
<td>75</td>
<td>46</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Low-intermediate (LI)</td>
<td>1</td>
<td>31</td>
<td>71</td>
<td>64</td>
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<td>68</td>
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CR, complete response; RFS, relapse-free survival; OS, overall survival.

1.5 Treatment of aggressive lymphoma

The clinical presentation of an aggressive lymphoma varies: a single or disseminated lymph node enlargement, presence or not of extranodal tumours, as well as presence or not of B-symptoms. Generally, a division into limited (stage I or early stage II) and advanced stage is made. The treatment decision is usually made taking into account the stage of the disease and IPI/aaIPI. Before the introduction of multi-agent chemotherapy, radiotherapy alone was given, whenever feasible, to locally symptomatic areas even in the presence of spread disease. At present, however, radiotherapy is most often
given combined with chemotherapy to patients with limited and/or bulky disease. More than 75% of patients with localized DLBCL can now be cured with a reduced number of chemotherapy courses and involved field irradiation.

Anthracyclines are the fundamental component of chemotherapy for aggressive lymphomas. Since 1976 the standard therapy has been CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) given every third week. Radiotherapy alone used to be the standard therapy for stage I disease. A retrospective analysis showed that combined therapy resulted in an improved 10-year outcome compared to radiotherapy alone, progression-free survival, (PFS) 83 vs 47% and OS 70 vs 43%.

Bonnet et al conducted a trial comparing chemoradiotherapy with chemotherapy alone. Patients older than 60 years with localized stage I or II histologically aggressive lymphoma, and no adverse prognostic factors (IPI), were randomized to receive either four cycles of CHOP plus involved field radiotherapy (299 patients), or chemotherapy alone with four cycles of CHOP (277 patients). With a median follow-up of seven years, EFS and OS did not differ between the two treatment groups (p= 0.6 and p = 0.5) respectively. The 5-year estimates of EFS were 61% for patients receiving chemotherapy alone, and 64% for patients receiving CHOP plus radiotherapy. The 5-year estimates of OS were 72% and 68%, respectively. In a multivariate analysis, OS was affected by stage II disease (p <0 .001) and male sex (p = 0.03). Thus, in this large prospective study, CHOP plus radiotherapy did not provide any advantage compared to CHOP alone for the treatment of low-risk localized aggressive lymphoma in elderly patients.

Reyes et al did a randomized trial, including patients less than 61 years old with localized stage I or II aggressive lymphoma and no adverse prognostic factors (IPI), comparing chemoradiotherapy with chemotherapy alone. Previously untreated patients were randomly assigned to three cycles of CHOP plus involved-field radiotherapy (329 patients), or chemotherapy alone with dose-intensified doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP) plus sequential consolidation (318 patients). With a median follow-up of 7.7 years, EFS and OS rates were significantly higher in the group given chemotherapy alone than they were in the group given CHOP plus radiotherapy (p<0.001 and p=0.001, respectively). The 5-year estimates of EFS were 82% for patients receiving chemotherapy alone and 74% for those receiving chemoradiotherapy. The respective 5-year estimates of OS were 90% and 81%. In a multivariate analysis, EFS and OS rates were affected by treatment group, independent of tumour stage and the presence or absence of bulky disease.

In recent years rituximab has been incorporated into the treatment of limited stage DLBCL. A pivotal study showed 88% PFS at four years and 92% OS when rituximab was added to treatment, compared to 78% PFS and 88% OS in a historic group of patients treated without rituximab.
Many attempts to improve the treatment of advanced stage aggressive lymphoma have been made by adding different cytostatics to CHOP, creating new regimens such as m-BACOD, ProMACE-CytaBOM, and MACOP-B; but randomized trials have demonstrated that they offer little advantage over CHOP as first line therapy.\textsuperscript{45-48} A meta-analysis of results from five major randomized trials, including a total of 1,982 patients, comparing MACOP-B, m-BACOD, ProMACE-CytaBOM with CHOP confirmed that there was no survival advantage.\textsuperscript{49} Furthermore these new regimens were much more toxic. Therefore CHOP still remains the best available treatment for patients with advanced-stage aggressive NHL. However, approximately 50-60\% of patients are not cured using CHOP alone; and improvement of treatment is still necessary. One study showed slightly improved results when etoposide was added to the CHOP treatment, though this treatment proved to be too toxic for use in elderly patients.\textsuperscript{50}

The most important improvement in therapy during the last decade has been the introduction of the anti CD20 monoclonal antibody rituximab. CD20 is expressed by the vast majority of B-cell lymphomas. Rituximab was developed for 'low-grade' or indolent lymphomas. It is an unmodified, naked, monoclonal antibody. The chimeric human-mouse antibody fixes the antigen on the surface membrane of lymphoma cells with the murine-antibody part, and stimulates the immune host mechanisms through the human Fc part. Fixing the antigen on the cell surface may also trigger a cascade of biological events leading to cell death through the apoptotic process. Three large randomized trials, GELA, ECOG and MInT, and one population based study have shown the efficacy of rituximab in combination with chemotherapy without the addition of any acute toxicity.

In the GELA study, 399 previously untreated patients, 60-80 years old, with DLBCL received either eight cycles of CHOP every three weeks or eight cycles of CHOP every three weeks plus rituximab given on day one of each cycle. Complete response rates of 76\% and 63\% (p=0.005) and 2-year OS of 70\% and 57\% (relative risk for CHOP-R vs CHOP; 0.64, p=0.007) were achieved by CHOP-R and CHOP respectively. Clinically relevant toxicity was similar in the two arms.\textsuperscript{51}

In the ECOG study, 632 elderly patients were randomized to receive either CHOP or CHOP with rituximab. Responding patients were randomized to receive rituximab maintenance, or no maintenance. A suggestion of a PFS benefit for patients treated with CHOP plus rituximab (p=0.059) was shown, however, no survival benefit was reported. One explanation for this might be the fact that 40\% of the patients treated with CHOP alone received maintenance rituximab. A subset analysis has shown that only patients not receiving rituximab as part of induction therapy benefited from rituximab maintenance. The trial is therefore difficult to analyze for overall survival. Therefore this study shows a benefit of combining rituximab with CHOP as either induction therapy or maintenance therapy but not both.\textsuperscript{52}
The MInT trial (Mab Thera International trial) included younger patients, aged 18-60 with DLBCL, in either stage II-IV, or stage I with bulk, and having an IPI of 0 or 1. They received six cycles of CHOP or CHOP-like treatment with or without rituximab. Chemotherapy was followed by radiation therapy (30-40 Gy) to bulky disease or E lesions. After a median follow-up of 34 months, patients assigned to chemotherapy and rituximab showed an improved 3-year EFS compared to those who received chemotherapy alone (79% vs 59%)(p<0.0001), and had increased 3-year OS (93% vs 84%)(p=0.0001). EFS was affected by treatment group, the presence of bulky disease, and age-adjusted IPI. The frequency of adverse events was the same in both groups. After chemotherapy and rituximab, a favourable subgroup (ie, IPI=0, no bulk) could be differentiated from a less-favourable subgroup (ie, IPI=1 or bulk, or both), laying the foundation for a more refined therapeutic approach for these patients. Thus rituximab added to six cycles of CHOP is an effective treatment for young patients with good-prognosis DLBCL.53

In British Colombia, a population-based analysis was conducted to assess the impact of the combination of CHOP plus rituximab on adult patients with DLBCL. They compared outcomes during a 3-year period: 18 months before (pre-rituximab) and 18 months after (post-rituximab). CHOP and rituximab were recommended, after new policy recommendations, for all patients with newly diagnosed advanced-stage (stage III or IV or stage I or II with "B" symptoms or bulky disease) DLBCL. In all, 292 patients were evaluated: 140 in the pre-rituximab group (median follow-up 42 months), and 152 in the post-rituximab group (median follow-up 24 months). Both PFS (risk ratio, 0.56; 95% CI, 0.39 to 0.81; p=0.002) and OS (risk ratio, 0.40; 95% CI, 0.27 to 0.61, p < 0.0001) were significantly improved in the post-rituximab group. After controlling for age and IPI score, era of treatment remained a strong independent predictor of PFS. The benefit of treatment in the post-rituximab era was present regardless of age. Thus this study showed dramatic improvement in outcome for DLBCL patients after rituximab was added to the CHOP treatment.54

Dose intensification is aimed at improving the treatment of advanced stage DLBCL by overcoming resistance to chemotherapy. Intensification may be expressed in different ways depending on the parameter chosen, among others: by increasing the dose per time unit by decreasing the interval between treatment courses, by increasing milligrams per square meter of delivered drug per week of therapy, by increasing the peak level with a single very high dose of cytostatic agent, or by adding a new drug.

Two studies suggest a possible benefit from dose intensification. One pilot study from SWOG (Southwest Oncology Group) evaluated dose intensified CHOP (cyclophosphamide 1600mg/m², doxorubicin 65mg/m² and vincristine 1.4mg/ m²). The treatment was given on a two-week schedule and
made possible with filgrastim support. The treatment resulted in a survival rate 14% better than that found in a group of historical controls.\textsuperscript{55}

The NHL-B2 trial from Germany randomized patients, 61 to 75 years old, to six cycles of CHOP-21, CHOP-14, CHOEP-21 (CHOP plus etoposide 100mg/ m\textsuperscript{2} day 1-3), or CHOEP-14. In addition they received G-CSF starting from day four, as well as radiotherapy (36 Gy) to sites of initial bulk or extranodal disease. The 5-year survival was 40% for CHOP-21 and 53% for CHOP-14. The combination with etoposide proved to be too toxic for elderly patients. This study recommends CHOP-14 as the standard treatment for elderly patients.\textsuperscript{56}

In the NHL-B1 study the same treatment was given but to patients between 18-60 years old. Patients treated with CHOEP achieved better CR rates (87.6% vs 79.4%; p = 0.003) and 5-year EFS rates (69.2% vs 57.6%; p = 0.004, primary end point) than those treated with CHOP, whereas interval reduction improved OS regardless of regimen (p = 0.05; p = 0.044 in the multivariate analysis). Although the CHOEP regimens induced more myelosuppression, all regimens were well tolerated. The study was not designed to compare the difference between CHOEP-14 and CHOEP-21. This was the first study to show that the addition of etoposide to CHOP improved the outcome for young patients with good prognosis (normal s-LDH) aggressive lymphoma.\textsuperscript{50}

The use of high dose therapy followed by autologous stem-cell transplantation (HDT) in the treatment of haematological malignancies is based on the principle that a more intensive cytoreductive therapy may result in an increase of the complete remission rate. The role of HDT in the primary treatment of lymphomas is controversial, increasingly so since the introduction of rituximab.

A dose-response relationship has been observed for many anti-tumour agents regarding both therapeutic and toxic effects. A certain degree of drug sensitivity is required so that the transplant plays the final role in disease eradication. The ideal preparative regimen for HDT should achieve high degree of destruction of tumour cells while generating manageable toxicity to normal tissues. Most agents used in preparative regimens are highly toxic to bone marrow stem-cells and require stem-cell support to rescue the patient from potentially lethal aplasia. In practice there are two main advantages to combing the use of chemotherapy with stem-cell support: First, the duration of acute myelosuppression is dramatically reduced; and second, a broader array of non-cross resistant agents is available. As HDT relies on the ability to eradicate the tumour with doses that do not cause unmanageable toxicity in non hematopoetic organs, only a few drugs have been found suitable. Alkylating agents have been found to exhibit three properties important to an effective HDT drug: 1) Doses can be intensified with confidence as their main toxicity is hematological. 2) They have a significant anti-tumoural
activity in lymphoma. 3) Their drug-resistance mechanism is not attributable to the multidrug resistant gene.

Three different settings of preparative regimens have been used: combination of drugs with or without total body irradiation (TBI), and sequential high dose therapy.

The sole conditioning agent in early preparative regimens was TBI. TBI could either be given as a maximum single dose of 10 Gy or up to 16 Gy fractionated. Later TBI was combined with one or more cytostatics. Cyclophosphamide was the first to be used, followed by other drugs such as cytarabine, etoposide and melphalan. A commonly used regimen was cyclophosphamide and etoposide combined with 12 Gy fractionated TBI.

A combination of drugs without TBI is preferred as this avoids the long-term side-effects of radiation. BEAM (BCNU, etoposide, cytarabine, and melphalan), BEAC (BCNU, etoposide, cytarabine, and cyclophosphamide) and CBV (cyclophosphamide, BCNU and etoposide) are the most frequently used regimens for lymphoma patients.

The third concept used is high-dose sequential chemotherapy, followed by myeloablative therapy and hematopoietic stem-cell support. The high-dose regimen entails the administration of several non-cross-resistant drugs, each at the maximum tolerated dose, mainly as single agents given within the shortest possible interval. The purpose of this high-dose sequential regimen is to prevent the emergence of drug-resistant lymphoma cells. This model was used by Gianni et al in a study comparing MACOP-B and high-dose sequential therapy.57

Treatment related mortality (TRM) has been a major concern when using HDT, with earlier studies reporting TRM above 10%. However progress has been made in supportive care and management of aplasia reducing TRM to 3.5%.58

HDT is the treatment of choice for patients with relapsed aggressive lymphoma still responding to chemotherapy. This was shown in the PARMA study which enrolled 109 patients who had relapsed from CR and responded to two cycles of DHAP (dexamethasone, cytarabine, cisplatin), who were either randomized to continued treatment with DHAP or to HDT. The FFS was 12 vs 51 % and OS 32 vs 53% at five years.59

The use of HDT as part of first line treatment is controversial. This strategy has been used in several phase-two studies and in some randomized trials. The LNH87-2 study which compares consolidative sequential chemotherapy ACVBP (ifosfamide plus etoposide, asparaginase, and cytarabine), with HDT, using the CBV regimen. With a median follow up of eight years, and in the population of 236 randomized patients, HDT was superior to sequential chemotherapy.60 This result has been confirmed by another French group.61 However, several randomized trials have not been able to support these results, among them a study by Olivieri et al showing no improved outcome for patients receiving sequential HDT compared to those receiving...
VACOP-B, although in this study, patients in the control arm not obtaining CR received HDT.\textsuperscript{62} Other randomized trials designed with an abbreviated standard induction therapy followed by early HDT have given negative results.\textsuperscript{63, 64} The abbreviated induction therapy before HDT in these studies might explain why no advantage was shown. A meta-analysis by Greb et al, of HDT upfront, showed no evidence of improved results when used to treat good risk patients. The evidence of benefit for poor risk patients was inconclusive; and for this subgroup, high quality studies are warranted.\textsuperscript{65}

Aggressive B-cell lymphoma can occur either \textit{de novo} or as the result of transformation of an indolent lymphoma, most often a follicular lymphoma (FL). The median survival after transformation is reported to be as short as 1.7 years.\textsuperscript{66} Whether or not HDT improves outcome after transformation remains unclear, with small and heterogeneous series reported. These studies also report high TRM to which the frequent use of TBI might have contributed.\textsuperscript{67-70} The European randomized CUP Trial compared HDT to conventional treatment in 89 patients with relapsed, not transformed, FL to determine whether or not HDT was more effective than standard treatment with regard to PFS and OS. It also assessed the additional value of B-cell purging of the stem-cell graft. The patients received three cycles of chemotherapy. Responding patients with limited bone marrow infiltration were eligible for random assignment to three further cycles, either chemotherapy (C), unpurged HDT (U) or purged HDT (P). This study showed that HDT significantly improved PFS and OS. There was no clear evidence of benefit attributed to purging.\textsuperscript{71}

Very little is known about how patients with transformed FL do, compared to those with \textit{de novo} aggressive B-cell lymphoma when treated with HDT. A study from the EBMT (European Bone Marrow Transplant) registry reported 50 patients with histological transformation of FL treated with HDT resulting in 50% and 33% OS and PFS, respectively, at five years. The median PFS was 13 months. Of note, TRM was 18%. The benefit of the treatment was most evident for patients with chemosensitive disease and for those in whom CR was achieved. A comparison of the patients with transformed FL and matched patients with \textit{de novo} high or intermediate-high grade lymphoma showed no significant difference in OS (p = 0.438).\textsuperscript{72}

**Purging**

A major concern when using autologous stem-cells for transplantation is contamination of the graft with lymphoma cells that might contribute to relapse. Therefore, efforts have been made to eliminate lymphoma cells either directly, by purging the B-cells, or indirectly, by enriching the stem-cell content of the graft.

Purging can be done \textit{in vitro} with anti-B-cell monoclonal antibodies (CD10, CD19, CD20, CD22, CD23 and CD37). Some studies have shown
no positive effect of *in vitro* purging\textsuperscript{71, 73} whereas others report a favourable outcome.\textsuperscript{74, 75} *In vivo* purging by the infusion of rituximab prior to the stem-cell harvest, is easier and at least as efficient as the *in vitro* method. The use of *in vivo* purging with rituximab seems to improve the outcome.\textsuperscript{76, 77} As rituximab is now used in combination with chemotherapy before harvesting, contamination of the harvest by tumour cells has probably diminished. Indirect decontamination has most often been carried out by enrichment of CD34 positive stem-cells.

**CNS relapse and prophylaxis**

Central nervous system (CNS) relapse in patients with aggressive non-Hodgkin lymphoma is a serious complication, difficult to treat and with a poor prognosis. The frequency of CNS relapse is reported to range from 4\% to 30\% depending on histology and stage of lymphoma.\textsuperscript{78} In a retrospective study of 2,514 patients with NHL treated in Norway between 1980 and 1996, it was reported that 106 patients (4.2\%) developed CNS involvement, either during primary treatment or at relapse.\textsuperscript{79} There is a lack of high-quality evidence for the efficacy of CNS prophylaxis as well as to what constitutes the best prophylactic treatment.\textsuperscript{80} The most frequently used prophylactic treatment is intrathecal (IT) methotrexate sometimes given in combination with IT Ara-C; but this has only a meningeal effect and parenchymal relapses occur as well. The duration of conventional IT treatment is short, but a new drug, liposomal Ara-C, with long duration after IT administration, seems promising.\textsuperscript{81-83} In the subset of DLBCL there is no clear consensus as to which patients would benefit from CNS prophylaxis. Some primary sites, such as bone marrow involvement with large-cell lymphoma, liver, testicles, bone/vertebrae, nasal/paranasal sinuses and multiple extranodal disease sites, as well as age > 60, elevated s-LDH and low s-albumin (< 35g/l) are considered to indicate an increased risk of CNS relapse.\textsuperscript{84, 85} Chua et al reported, in a small retrospective study of 25 patients with newly diagnosed "intermediate-grade" NHL who received either IT methotrexate or IT methotrexate in combination with IT Ara-C, that this did not prevent CNS relapse.\textsuperscript{86} However Tomita et al found that prophylactic IT methotrexate and hydrocortisone reduced CNS recurrence and improved survival in a retrospective study of 68 adults with aggressive NHL.\textsuperscript{87} Haioun et al studied the incidence of and risk factors for CNS relapse in 974 patients in CR following treatment for aggressive lymphoma (excluding Burkitt and lymphoblast lymphoma). They received ACVBP and as CNS prophylaxis IT methotrexate in week 0, 2, 4 and 6. Patients also received intravenous methotrexate at week 10 and 12 as part of the consolidation regimen. The overall incidence of CNS and isolated CNS relapse was 2.2\% and 1.6\% respectively. In this study multivariate analysis identified male sex, involvement of
testis, and high/high-intermediate IPI as risk factors for CNS relapse. S-LDH and more than one extranodal site were also independent prognostic factors for CNS relapse. This study provided evidence that intrathecal and intravenous methotrexate reduce the incidence of CNS relapse.

Tilly et al conducted a randomized trial of 635 eligible patients in order to compare the intensive conventional chemotherapy regimen ACVBP with standard CHOP in previously untreated patients with poor-risk aggressive lymphoma. The patients were 61 to 69 years old and had at least one adverse IPI prognostic factor. ACVBP consisted of an induction phase of intensified chemotherapy and CNS prophylaxis followed by a sequential consolidation phase. The complete response rate was 58% in the ACVBP group and 56% in the CHOP group (p = 0.5). TRM was 13% in the ACVBP group and 7% in the CHOP group (p = 0.014). At five years, the EFS was 39% in the ACVBP group and 29% in the CHOP group (p = 0.005). Cases of CNS progression, and relapse, were more frequent in the CHOP group, 26 patients (8%) vs nine patients (3%) treated with ACVBP (p = 0.004).

The RICOVER-60 trial is one of the few sources of prospective data on CNS relapse in NHL. In this study, 1,222 patients with CD20-positive DLBCL (aged 61-80 years) were randomized to receive one of four chemoradiation regimens based on bi-weekly CHOP (CHOP-14), consisting of six or eight cycles of CHOP-14 with or without eight courses of rituximab. OS was highest in the group receiving six cycles of CHOP-14 plus rituximab. CNS prophylaxis was given to patients with involvement of bone marrow, testis, upper neck, or head and consisted of it methotrexate (day one and five of the first two courses). Fifty-eight cases of lymphoma in the CNS were observed: 36/609 patients in the CHOP-14-arm and 22/608 patients in the R-CHOP-14-arm. Median time to CNS relapse was eight months (range 1-39 months) and median survival from relapse was three months (0.1-38 months). The estimated 2-year incidence of CNS disease was 6.9% after CHOP-14 and 4.1% after R-CHOP-14. R-CHOP reduced the relative risk for CNS disease to 0.58 (p=0.046). Cox regression analysis identified involvement of more than one extranodal site and B-symptoms as significant risk factors for CNS disease. This study concluded that elderly patients with aggressive CD20-positive lymphoma showed a significantly lower incidence of CNS disease if treated with R-CHOP-14 instead of CHOP-14. IT methotrexate has no role in preventing CNS disease for patients treated with combined immunochemotherapy (R-CHOP-14) with the possible exception of patients with testicular involvement.

1.6 FDG-PET and the evaluation of treatment response

For many years a CT scan at mid-treatment and after completed therapy has been the standard procedure in evaluating treatment response. CT, however,
is unable to prove or disprove the existence of viable lymphoma in a still enlarged tumour/lymph node that has decreased in size. Previous studies have shown that 10-20% of residual tumours/enlarged lymph nodes found on CT contain viable disease.91-93

Positron emission tomography (PET) utilizing a radioactive tracers is increasingly used to diagnose, stage and follow-up various malignancies.94, 95 Detection of coincidence photons emitted during positron annihilation is the key to PET imaging, providing images of physiologic processes. This metabolic imaging technique uses a radiopharmaceutical tracer to target a specific physiologic process (e.g., glucose metabolism, amino acid metabolism, DNA synthesis). It was observed in the 1930s that malignant transformation of cells is associated with an increased glucoyltic rate.96 The most widely used pharmaceutical tracer is the radiolabeled glucose analog fluorine-18-deoxyglucose (FDG). FDG is transported into cells and phosphorylated in the same way glucose is. However, because FDG-6-phosphate is not a substrate for glucose-6-phosphate isomerase and because FDG-6-phosphate is typically not dephosphorylated in tumours, it becomes trapped in the cell and reaches a near equilibrium state approximately 60 minutes after injection. The positron-emitting 18F isotope to which FDG is linked decays, and the emitted positron annihilates after “bumping” into an electron, generating two 511-keV photons emitted in nearly opposite directions that are detected by the same scanner.97 Clinically, a semi-quantitative index of glucose metabolism is used: the standardized uptake value (SUV). The SUV is obtained by placing a region of interest over the lesion and dividing the value (in microcuries per cubic centimetre) by the injected dose (in microcuries) divided by the patient’s body weight (in grams).

To make it easier to interpret the PET scan, PET-CT was described by Beyer et al in which precisely co-registered functional and anatomic images could be obtained by performing a PET study and a CT study on the same scanner without moving the patient.98 This combination of the PET scanner and a CT scanner makes the localization of areas of increased tracer activity much easier and more reliable.

FDG-PET is increasingly being used in lymphoma diagnosis, staging and therapy evaluation. However, not all lymphomas are FDG avid, i.e., metabolically active and visualized on PET-CT. FDG uptake has been shown to be related to the tumour grade, and indolent lymphomas are frequently only mildly FDG avid; whereas aggressive lymphomas usually are readily visualized.99, 100

PET and CT are concordant in staging 80 to 90% of patients with DLBCL, FL and probably also mantle cell lymphoma.101, 102 In the 10 to 20% of patients in whom a discordance is observed, PET typically results in upstaging, due to additional presumed sites of disease detected by PET alone, such as lymph nodes of 1 cm or smaller, and splenic and hepatic infiltration. PET can detect focal or multifocal bone/bone marrow involvement.
However PET alone is unreliable in detecting bone marrow involvement, particularly of limited extent. A meta-analysis estimated the PET sensitivity for detecting bone marrow involvement in NHL to be 43%. Thus PET cannot substitute for bone marrow biopsy in lymphoma staging.97

Lymphomas often respond rapidly to given therapy, and PET should theoretically be attractive to use to evaluate response. Relative tumour activity can be estimated by measuring the SUV before, during and after treatment. FDG-PET has also been shown to predict tumour viability (or nonviability) in residual masses with an accuracy approaching 80-90%,103-105 suggesting that FDG-PET is superior in evaluating treatment response compared to CT. In one comparative study, PET was found to be 89% sensitive and 100% specific for the presence of lymphoma in patients with NHL, in contrast to CT alone which had a sensitivity and specificity of 81% and 41%, respectively.106 Changes in FDG uptake have been seen as early as a few days after the initiation of therapy, and the SUV at 42 hours after treatment has been found to accurately reflect the patient’s overall tumour status.107 Meta-analysis of the literature has shown that after treatment, a still metabolically active tumour was a strong predictor of relapse, with up to 100% of patients relapsing within two years.108, 109 The opposite was also shown with very few relapses among patients with a negative FDG PET.
A number of studies, carried out prior to the addition of rituximab to treatment regimens, have found mid-treatment PET to be a powerful predictor of relapse and survival in NHL patients.\textsuperscript{110-113} However, the role of PET-CT in the management of aggressive lymphoma remains unclear. Studies made after the addition of rituximab to treatment regimens do not fully support the earlier findings.\textsuperscript{114, 115}

FDG is not tumour specific and increased FDG accumulation may be seen in a variety of benign entities, some of the most common being infection, sarcoidosis, G-CSF therapy, physiologic activity, post-biopsy changes and bone fractures.\textsuperscript{116, 117} PET results must be interpreted in correlation with clinical and other radiological factors allowing improved diagnostic accuracy and avoiding false positive findings.
The clearest role for PET is in restaging patients following the completion of therapy. PET is more accurate than CT in this setting, largely due to its superiority in distinguishing between viable tumour, necrosis and fibrosis in residual masses. In general, PET has a consistently high negative predictive value (NPV), averaging about 85% across studies including patients with HL and/or DLBCL. The 15% false-negative rate with PET is mostly related to its inability to detect microscopic disease resulting in future relapses. The positive predictive value (PPV) is generally lower, averaging 70 to 80%. One explanation for this is that radiotherapy causes an inflammatory reaction with an increased FDG uptake. There are also reports that rituximab might contribute to an inflammatory reaction. Still, the PPV of PET is substantially higher than that of CT, which has a reported PPV in patients with aggressive NHL of approximately 40 to 50%. In the recently published revised response criteria for lymphomas, FDG-PET is recommended after treatment of PET-avid lymphomas, i.e., metabolically active lymphomas like DLBCL and HL. According to these recommendations, the PET should be performed at least four weeks, and preferably six to eight weeks after chemotherapy or chemoimmunotherapy, and 8 to 12 weeks after radiation or chemoradiotherapy. A positive PET is defined as a focal or diffuse FDG uptake above background activity. However, the following exceptions are made: 1) Mild and diffusely increased FDG uptake at the site of moderately sized or large residual masses (i.e. ≥ 2 cm in diameter), regardless of location, with intensity lower than or equal to that of mediastinal blood pool structures, should be considered negative for the presence of residual lymphoma, whereas diffuse or focal uptake exceeding that of the mediastinal blood pool structures should be considered indicative of lymphoma.2) Be-
cause of the effect of partial-volume averaging, any increased uptake above surrounding background activity in lymph nodes or nodal masses less than 2 cm in diameter, including normal sized lymph nodes at CT, should be considered positive for lymphoma. There are special recommendations for interpreting lymphoma of the lung, liver and spleen. Bone marrow biopsy still remains the standard procedure for assessment of the bone marrow.
2 Aim of the thesis

- To evaluate response and toxicity of R-CHOEP-14 in aggressive lymphomas.

- To define, immunohistochemically, the GC or non-GC phenotype, in DLBCL, and to investigate the influence of the phenotype on outcome in patients treated with chemotherapy with or without rituximab.

- To evaluate the use of mid-treatment PET and biopsy in aggressive lymphomas, in order to find non-responders early on, making early treatment intensification possible.

- To explore the role of HDT in transformed follicular lymphoma and de novo aggressive B-cell lymphoma, and to compare the outcome in the two groups.
Outcome for Young High-Risk Aggressive B-Cell Lymphoma Patients Treated with CHOEP-14 and Rituximab (R-CHOEP-14)

Patients and Methods
Between July 2001 and June 2003, 38 consecutive patients, 20 men and 18 women aged between 27 and 65 years (median 58) with newly diagnosed, biopsy proven CD20 positive aggressive B-cell lymphomas were treated with R-CHOEP-14. A majority of these patients had DLBCL (n=31). The patients underwent standard procedures for staging of lymphoma before treatment started. High-risk factors defined as aaIPI 2 and 3 were present in 86% (n= 33) of the patients. Of the remaining five patients, three had a bulky tumour in the abdomen, known to be associated with an increased risk of relapse, and two had extensive stage IV disease but normal s-LDH. Bone marrow involvement was present in 12 patients, six with large-cell involvement. One patient had lymphoma of the liver, with meningeal spread at diagnosis.

Treatment consisted of six courses of R-CHOEP given at two-week intervals supported by G-CSF, followed by one Ara-C infusion to prevent CNS relapse. The chemotherapy dose was reduced in case of neutropenia with fever requiring hospitalization or repeated grade 4 neutropenia or thrombocytopenia. The treatment outcome was evaluated with a CT-scan after three courses of R-CHOEP, and approximately six weeks after the end of treatment. In case of bone marrow involvement a second marrow-biopsy was done. Response was evaluated according to the Cheson criteria.37

The study was approved by the ethics committee of Uppsala University.

Statistical Methods
Overall survival was calculated from the day of diagnosis to the time of death by any cause. Event-free survival was calculated from the day of diagnosis until relapse, progression or death. Survival curves were calculated using the Kaplan and Meier method. Statistica 6.1 software was used for all calculations.
Results

All patients were considered responders after three courses. One patient progressed at the end of treatment; and another six patients did not obtain a CR or CRu; but four of these obtained CR with additional treatment. Of the 31 patients (82%) with a primary CR/Cru, eight relapsed. The majority (n=6) of these relapses occurred shortly after the end of treatment and all the patients have died, except for one with progressive disease. One patient had an isolated CNS-relapse and two showed a combined nodal and CNS relapse. Only two of the relapsing patients, relapsing more than a year after treatment, had a second CR after HDT. Four out of six patients with large-cell lymphoma in the bone marrow have relapsed. Of six patients with small-cell lymphoma in the bone marrow five obtained CR and are free from relapse, one had progressive disease.

Figure 5. Overall survival after treatment with R-CHOEP-14
CNS-prophylaxis

In the present study, all patients were recommended prophylaxis with intravenous infusion of Ara-C after six courses of R-CHOEP, presuming that it would give a cytotoxic concentration of the drug in the CNS. In spite of this strategy, three patients had relapses involving the CNS, all of whom had received Ara-C. For various reasons, twelve patients did not receive any Ara-C. None of these patients developed lymphoma in the CNS.

Side Effects

Fifteen patients had at least one period of fever with neutropenia requiring hospitalization. Chemotherapy doses were reduced due to hematological toxicity in seven patients. Neutropenia was the most common reason for treatment delay. Twelve patients had at least one course delayed due to neutropenia. Repeated blood transfusions were needed in sixteen patients. Three patients developed late neutropenia between one and three months after treatment. This was interpreted as a late effect of rituximab. All these patients responded well to G-CSF treatment.

There was no unexpected toxicity and no treatment related mortality. Treatment could be given on an outpatient basis. No Pneumocystis carinii pneumonia (PCP) (jiroveci) prophylaxis was given, and one patient contracted a PCP infection.
Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy

Patients and Methods
The study population consisted of 201 de novo DLBCL patients treated in Helsinki and Uppsala between 1994 and 2004. The patients were divided into two groups: patients treated before the advent of rituximab (n=106) and patients who received rituximab (n=95). The majority of patients received anthracycline containing chemotherapy, mainly CHOP, CHOEP or other CHOP-like regimens. Only eleven patients received treatment without anthracyclines. Disease characteristics including age, gender, stage and IPI score were well balanced between the groups.

Patient tissue samples were stained for CD10, BCL6 and MUM1, and the immuno-reactivity was determined without any knowledge of survival or other clinical data. The scoring was based on the algorithm described by Hans et al.23 Accordingly, the samples were evaluated for CD10, BCL6, and MUM1, respectively, and considered positive if 30% or more of the tumour cells were stained with the antibody. The cases were assigned to the immunohistochemically defined GC group if CD10 alone or together with BCL6 was positive. If both CD10 and BCL6 were negative, the cases were assigned to the non-GC group. If CD10 was negative and BCL6 positive, the classification was based on MUM1 expression. If MUM1 was negative, the cases were assigned to the GC-group, whereas MUM1 positive cases were assigned to the immunohistochemically defined non-GC group.

The study was approved by the ethics committee of Uppsala University.

Statistical Methods
The chi-square test was used to assess differences in the frequency of individual prognostic factors. Analyses of the reproducibility of immunohistochemical stainings were carried out with a kappa statistic. Survival rates were estimated by the Kaplan-Meier method and the differences were com-
pared by the log-rank test. Overall survival was measured from the date of diagnosis until the last follow-up or death from any cause. Failure-free survival was determined as an interval between the date of diagnosis and relapse, or death. Cox multivariate analysis was used to test the prognostic impact of identified genes on OS and FFS. Statistical data processing was carried out with SPSS software for Macintosh (SPSS, Chicago, IL). Probability values less than 0.05 were considered statistically significant. All $p$ values were two-tailed.

**Results**

The outcome for patients treated with the addition of rituximab to chemotherapy was better than the outcome for patients treated with chemotherapy only. OS was 76% in the rituximab group and 57% in the group receiving only chemotherapy ($p=0.002$). The same was seen with PFS rates that were 70% and 44% for the rituximab and the control group, respectively ($p=0.0006$). The improvement was seen for all ages and risk groups.

The distribution of GC and non-GC phenotypes was similar in the two treatment groups. The clinical outcome according to treatment, and GC versus non-GC phenotypes, was also analyzed. In the non-rituximab group the GC-type had a superior outcome, OS 69% vs 47% ($p=0.014$) and PFS 59% vs 33% ($p=0.002$). However in the rituximab treated group there were no significant differences found in survival parameters. OS was 77% vs 76%, for GC and non GC-type respectively. PFS was 71% vs 65% respectively ($p=\text{ns}$). This was confirmed by analyzing outcome in the non-GC group according to treatment. Non-GC patients receiving rituximab had a significantly better OS than non-GC patients treated with chemotherapy alone 76% vs 47% ($p=0.0005$). The PFS was estimated to be 68% in the rituximab group versus 33% in the control group ($p=0.0003$). On the other hand patients with GC-phenotype did not improve their outcome when treated with rituximab.

The results were confirmed in the Cox multivariate analysis in which both treatment and IPI were independent prognostic factors in the non-GC group whereas, in the GC-group, only IPI was a statistically significant prognostic factor.
Figure 7. The overall survival rates for control and immunochemotherapy treated DLBCL patients according to molecular and clinical factors.
Little usefulness of mid-treatment FDG-PET and biopsy in patients with aggressive lymphoma

Patients and Methods
Twenty-five consecutive patients, 13 men and 12 women, have been evaluated with mid treatment FDG-PET between January 2005 and December 2006. Median age was 59 (23-66) years. The vast majority of patients had DLBCL (n= 21). The others had Burkitt lymphoma, follicular lymphoma grade 3, transformed follicular lymphoma and one anaplastic alk negative lymphoma. Fifteen patients had an aaIPI of 2 or 3, and all patients were considered to have a high risk for relapse.

Treatment was given every second week either with R-CHOP-14 or R-CHOEP-14. The patient with a peripheral T-cell lymphoma received CHOEP-14. FDG-PET CT evaluation was done after three courses (five weeks after treatment start). For logistical reasons three patients had their investigations after four courses. A needle biopsy was recommended in case of increased uptake with intention to change therapies for those with a positive biopsy.

The FDG-PET investigations were performed at the Uppsala PET center (n=20) or in an ambulatory PET unit (n=5). All PET-investigations have been retrospectively evaluated. In this paper the original PET reports, the clinical interpretation and the retrospective evaluation are compared to the clinical outcome.

The PET interpretation was visual. PET findings were defined as positive when regional uptake was nodular and clearly higher than background levels, uncertain when a slight heterogeneous uptake was present and not fully explained by a known benign process, and negative when uptake was not elevated compared to backgrounds levels or when only faint uptake was present in residual lymphoma masses. Increased homogeneous bone marrow uptake found in patients examined during G-CSF treatment was considered caused by this treatment and therefore lymphoma negative. The Juweid recommendation of treatment evaluation was not used in this study which was planned and underway before these recommendations were published.

The study was approved by the ethics committee of Uppsala University.
Results

Five patients had positive FDG uptake according to the initial reports. Seven patients had an uncertain FDG uptake, and 13 patients had a negative FDG-PET. Four patients have relapsed, but only one at a site of pathological FDG-uptake.

The mean follow-up was 26 months (20-42 months). Two patients with a negative mid-treatment FDG-PET have relapsed, 18 and 35 months after treatment. One patient relapsed in a previously uninvolved site; and one patient relapsed after 35 months, in a site adjacent to his primary, PET-negative abdominal tumour. The other 11 patients with a negative FDG-PET are still in CR giving a negative predictive value (NPV) of 85%.

Five patients (20%) had a pathological FDG-uptake. Two of them had biopsy-proven viable tumour but did not complete the planned salvage treatment, one due to chemotherapy toxicity and one due to progressive disease during salvage therapy. Two patients had a negative biopsy. One had no needle biopsy due to biopsy difficulties at primary diagnosis. Positive FDG-PET, but negative biopsy was explained by G-CSF stimulation of the spleen showing an increased granulopoiesis in a patient with a focal spleen lesion prior to treatment. One patient had an increased uptake in a healing fracture. The positive uptake in the patient without a biopsy was due to patient movement causing anatomical mis-registration of the PET and CT images. What had been initially interpreted as tumour was normal kidney uptake registered at the tumour site.

Seven patients had varying degrees of slightly elevated, uncertain, heterogeneous metabolic uptake. Two of them have relapsed, one in sites with uncertain FDG uptake four months post therapy, and one in negative sites, eight months post therapy. Thus 5/7 (71%) in the uncertain group are relapse free. The uncertain FDG uptake in six patients without relapse in the uncertain areas was determined to be tumour necrosis with inflammation in large bulky mediastinal tumour masses, (three patients), benign ventricular ulceration, (one patient), and increased para-aortic uptake but no corresponding lymph-node (one patient).

In one patient the increased uptake could have been caused by pharyngeal movement in a patient with lymphoma of the oropharynx. Because of the uncertainty this patient had a follow-up PET that showed no uptake in the pharyngeal wall but findings consistent with sarcoidosis and a thyroid oncocytoma that on reevaluation were present at both PET investigations. The patient with a benign ventricular ulceration relapsed in PET negative lymph-nodes.
Figure 8. Uncertain FDG uptake explained by a ventricular ulceration

All patients that had their FDG-PET investigation during the influence of G-CSF had an increased bone marrow uptake. Seven patients had CT-verified focal skeletal lymphoma. They were all but one mid-treatment negative at the tumour site though there was FDG marrow uptake secondary to G-CSF treatment. One patient had increased uptake due to a healing pathological fracture but with no evidence of lymphoma in the biopsy. All these seven patients are in continuous CR.
Figure 9. Patient with G-CSF uptake in all but the previously lymphoma affected vertebrae.
Superior outcome in transformed follicular lymphoma compared to de novo aggressive B-cell lymphoma treated with high-dose therapy and autologous stem-cell support.

Patients and Methods
Between 1986 and 1999, 117 patients, 52 women and 65 men, aged between 21 and 65, median age 50 years, were treated with HDT at two Swedish university hospitals, Lund and Uppsala. The indication for HDT was either transformed follicular lymphoma (n=38) or de novo aggressive B-cell lymphoma (n=79) with adverse prognostic factors such as high risk criteria, relapse or failing to obtain CR. There was no significant difference in age or sex distribution between the two groups. Fourteen patients were treated with HDT upfront (in first CR). Sixty-seven patients were treated in a second CR, after relapse to first line treatment; and four patients were treated in a third CR. Twenty-four patients were in first partial remission (PR1) at time of transplantation. Six patients were in PR but had obtained CR after earlier treatments. Two patients had progressive disease. Thirteen (34%) of the patients with transformed follicular lymphoma had received chlorambucil or prednimustine before transformation.

The most frequent first line treatment was CHOP (n=50). MACOP-B and VACOP-B were given to 18 and 11 patients respectively. The most frequent second line treatment was MIME alone or in combination (n=79). Thirty-six patients had received radiotherapy at some time before HDT, not including eight patients receiving radiotherapy before transformation.

In 83 patients stem-cells were collected from the peripheral blood. In 22 patients bone marrow was harvested. Both methods were used in 12 patients. The most frequently used conditioning regimen was BEAC (n=109). Nine patients had TBI as part of the conditioning therapy. Thirty-four patients received radiotherapy after the high-dose procedure with fields including persisting or initially bulky tumour.

Purging or selection was used in 33 patients. The most frequently used method was CD-34-enrichment of stem-cells (n=21). Seven patients were
purged *in vitro* with anti B-cell monoclonal antibodies. *In-vivo* rituximab purging was used in five patients, given as a single dose before the stem-cell harvest. Fourteen (37%) of the patients with transformed FL and 19 (24%) of those with *de novo* aggressive lymphoma were subject to some kind of purging.

The study was approved by the ethics committee of Uppsala University.

**Statistical Methods**

Overall survival was calculated from the day of HDT to the time of death by any cause. Event-free survival was calculated from the day of HDT until relapse, progression, or death related to lymphoma or treatment. Survival curves were calculated using the Kaplan Meier method and survival differences between curves with log-rank test. The Chi-square test was used to calculate differences between groups. Uni-and multi-variate analyses were performed with the Cox proportional hazard method. Statistica 8.1 software was used for all calculations.

**Results**

After a median follow-up of 11.5 years (8-20 years) 46/117 patients are still alive (39%). The 10-year EFS in the entire group was 35% and the OS was 44%. Sixty patients (51%) are dead due to lymphoma relapse. Five patients with transformed follicular lymphoma and four patients with *de novo* B-cell lymphoma are alive after relapsing. Of these, one received allogenic transplant. Two received more aggressive therapy (CHOP, MIME). Five patients were treated with rituximab alone or in combination with chlorambucil. One patient with a *de novo* aggressive B-cell lymphoma and a biopsy proven relapse had a spontaneous remission.
**Figure 10.** Overall survival in patients with transformed FL compared to *de novo* aggressive B-cell lymphoma

EFS and OS were significantly better in patients with transformed FL compared to patients with *de novo* B-cell lymphoma. EFS was 55% and 27% (p<0.009) and OS 66% and 33% (p<0.0005) respectively.

**Figure 11.** Event-free survival in patients with transformed FL compared to *de novo* aggressive B-cell lymphoma
There was no significant difference in OS based on sex, age at time of transplantation or source of the stem-cells. Fourteen patients transplanted in first CR, nine with transformed FL and five with *de novo* aggressive lymphoma, had a tendency towards better OS (p=0.09). The survival benefit seems to be present in the *de novo* group only.

![Figure 12](image) Overall survival in transformed FL and *de novo* aggressive B-cell lymphoma depending on treatment lines

The patients that received purged stem-cells (27%) had a significantly better EFS and OS (p=0.025) and (p=0.045) respectively, than those receiving unpurged stem-cells.

Nine patients had TBI as part of conditioning. Only two patients receiving TBI are alive. They both had transformed FL. Five patients receiving TBI died in progressive disease, and two died in late toxicity.

Seventy patients received radiotherapy, 36 patients before HDT and 34 patients after HDT. There was no difference in OS or EFS for these patients compared to patients not receiving radiotherapy.

Multivariate analyses, including factors significantly related to outcome univariately, that is purging and follicular *vs* *de novo*, showed that follicular *vs* *de novo* was the only significant factor (p= 0.003)
Side Effects

Five patients (4%) died within six months from acute/subacute complications related to HDT. Three patients died of heart failure, including one patient with veno-occlusive disease. Two patients died of infections. Seven patients died for reasons other than lymphoma between one and thirteen years post-transplant. One patient died 14 months post HDT in a Pneumocystis carinii infection. One patient who had received TBI developed a progressive respiratory failure after nine years and died after 13 years. Two patients died due to myelodysplastic syndrome (MDS) five years after HDT, one with transformed FL who had received TBI. The other one had received extensive alkylating chemotherapy. This gives a late transplant related mortality of 3.5%. Three late deaths were obviously not related to treatment.
Discussion & Conclusions

Discussion

Patients with aggressive lymphoma and high aaIPI score (2-3) have a poor prognosis when treated with conventional CHOP. The expected 5-year OS rate is as low as 30%. In paper I we reported an improvement in outcome with our intensified therapy, adding rituximab and etoposide as well as shortening the treatment interval. With a median follow-up of 27 months, 23/38 (61%) of the patients are relapse free and 30/38 (76%) are alive, one with progressive disease. The relapses generally occurred within a few months after the end of treatment but two patients relapsed after more than a year. They achieved a second CR after HDT.

Ara-C was given as CNS prophylaxis but as a consequence of its failure to prevent relapse in three cases, intravenous methotrexate has been added to later care programmes. The inclusion of five patients with an aaIPI score of 1 has not improved the results as only one of these patients is free from relapse. The fact that these patients had large tumour masses in the abdomen (3 patients) or stage IV disease but normal s-LDH (2 patients) might explain the poor outcome.

No large randomized trial has, to our knowledge, been carried out with young high risk patients treated with CHOP in combination with rituximab. The MInT trial included only patients with aaIPI score of 0 or 1. The results in our study using R-CHOEP-14 are comparable to the results in the Haion study with high risk lymphoma patients in which HDT was used as consolidation.60 The side-effects were manageable; and in case of relapse, HDT is still an option. In this small material we could see that bone marrow involvement with large cell lymphoma was an adverse prognostic sign with 4/6 patients relapsing and only one patient being salvaged with HDT.

Transformation of an indolent lymphoma to an aggressive lymphoma is a serious event that requires treatment as intensive as that given to patients with de novo aggressive lymphoma. In case of relapse, HDT is the treatment of choice and offers a second chance to cure these patients. The HDT treatment evaluated in paper IV was given before rituximab, in combination with chemotherapy, became the standard treatment for aggressive lymphoma.

Our study, showing a superior outcome for patients with transformed follicular lymphoma after HDT, supports this choice of treatment.
There is no obvious explanation for the better outcome in the transformed group compared to the *de novo* group. There are no differences regarding age or sex distribution between the groups. Most patients had received two chemotherapy regimens before HDT. Fourteen patients were transplanted in CR 1, and there is a tendency toward an improved outcome for this small group; but in a multivariate analysis, there is no significant difference in the outcome depending on the number of treatment regimens given before HDT. The degree of remission has repeatedly been associated to outcome after HDT. In this study we could not detect any differences in survival between patients in CR or PR. This is probably explained by a selection bias that refers only patients with a highly sensitive treatment response (either CR or very good partial remission) to HDT.

There are nine patients (13%), five with transformed FL, and four with *de novo* B-cell lymphoma, still alive after relapse: one treated with allogenic transplant, but the majority without intensive treatment, treated with rituximab alone or in combination.

Despite a very long follow-up, the treatment related mortality was lower compared to that found in other studies. There are studies, in which TBI has been more frequently used, that have shown a worse outcome. The limited use of TBI in this study might explain the low incidence of treatment related early and late deaths. TBI was given to nine patients. Seven have died, five due to lymphoma and two due to late treatment toxicity (MDS and respiratory failure).

Purging was used in 28 patients (33%). These patients have a superior outcome considering EFS and OS. Five patients received *in vivo* rituximab purging, and all but one of these patients are alive. Today, when rituximab is routinely combined with chemotherapy, the impact of purging has probably declined.

This study supports the use of HDT in case of relapse, both for transformed follicular lymphoma and for *de novo* B-cell lymphoma. However, there is a not yet explained difference in outcome between the two groups. Maybe future analyses of tumour biology will be able to explain this difference. Since FL is derived from the germinal center, transformed FL lymphoma would be expected to be of the GC type, indicating a better prognosis. Another possible explanation for the difference in outcome might be that the *de novo* aggressive lymphomas are selected for HDT on the basis of chemotherapy resistance and relapse, and logically would be a group dominated by the poor risk ABC type.

The future treatment of lymphoma patients might be more individually tailored as new techniques give us new tools for decision making. Immunohistochemical analyses of GC phenotypes, reported in paper II, allowed us to identify patients with different outcomes depending on treatment. It is important to note that these findings are restricted to immunohistochemically defined GC and non-GC subtypes, and cannot be extrapolated to the result
identified by gene expression-based microarrays as there is a misclassification rate of about 20%. Our study is not a concurrent comparison of treatment options, but there is no other obvious factor besides phenotype to explain the difference in treatment results. It clearly appears that rituximab treatment is most beneficial for non-GC patients, but this must be confirmed in randomized trials before changing treatment strategy. The mechanism by which the addition of rituximab to chemotherapy improves outcome significantly only in the immunohistochemically defined non-GC group, is unknown, but may represent a chemosensitizing effect of the antibody. One possible mechanism for this is to perturb BCL2-related antiapoptotic proteins thereby leading to increased sensitivity of lymphoma cells to chemotherapy. This is supported by a study showing that the adverse impact of BCL2 expression is associated with the non-GC phenotype in chemotherapy-treated patients.118

The new and more complex treatments are very costly, and it is of great importance to evaluate treatment response in an optimal way. We expected that mid-treatment PET-CT would give us the required information and enable us to change treatment strategy early on. However, our experience reported in paper III was disappointing. There were five clearly positive investigations, and only two of these were consistent with remaining lymphoma. Thirteen patients, two of whom have relapsed, were PET negative, giving an NPV of 85%. The difficulty arising from the high number of “uncertain” investigations made the early PET-CT evaluation less useful in clinical routine. Two of the patients with uncertain result have relapsed, and one of these in lymph-nodes with slightly increased uptake. There are indications that the use of rituximab might contribute to an inflammatory reaction therefore giving a “false” increased FDG-uptake.

Patients with bone involvement were all but one (with a healing pathological fracture) FDG-PET negative, valuable information as both CT- and MR-investigations are difficult to interpret when evaluating treatment response in bone lesions.

In a recently published article by Juweid et al, it was recommended that FDG-PET be done 6-8 weeks post treatment in order to avoid FDG-uptake due to treatment induced inflammatory reaction.

Based on our experience we cannot recommend mid-treatment PET-CT in clinical routine. The patients with obvious progressive disease will be detected with conventional CT and often clinically as well. Therefore, we now recommend that a PET-CT be performed after the completion of treatment. Hopefully this new recommendation will optimize the use of PET.
Conclusion

- Dose-intensified treatment with six cycles of R-CHOEP-14 is a well tolerated regimen with a promising treatment outcome, inducing a 2-year event-free survival of 60% in patients with aggressive B-cell lymphoma with poor prognosis. There was no treatment-related mortality and the treatment could be delivered on an outpatient basis. Whether or not this regimen is superior to other strategies, for example consolidation with standard induction high-dose therapy and stem-cell support remains to be shown in a randomized setting. Prophylaxis with a single intravenous infusion of high-dose Ara-C did not prevent CNS-relapse, therefore we have added intravenous infusion of methotrexate to later care programs.

- We replaced routine mid-treatment CT with mid-treatment FDG-PET CT expecting to find the patients not responding to standard treatment, thereby enabling early treatment intensification after a positive biopsy. The number of true positive PET investigations was few however and several uncertain investigations were reported. Therefore, early investigation with FDG-PET in order to identify patients with a biopsy-proven viable lymphoma who might benefit from treatment intensification, was not feasible in clinical routine; and a PET-CT is currently recommended 6-8 weeks after completion of treatment.

- The prognostic value of immunohistochemically defined GC phenotype in chemotherapy treated patients was eliminated with the addition of rituximab. The results have to be confirmed prospectively in an independent cohort of immunochemotherapy-treated DLBCL patients. This study illustrates that the molecular prognostic factors in the post rituximab era have to be re-evaluated in order to obtain additional tools for risk assessment in the current practice of DLBCL treatment.

- When treating both de novo aggressive B-cell lymphoma and transformed follicular lymphoma with HDT, an expected outcome in the de novo group was seen, but an unexpected, more favorable outcome, was seen for the patients with transformed FL. Treatment related toxicity was acceptable. Despite a very long follow-up, few late sequelae and relapses occurred, suggesting possible cure. The favorable outcome for patients with transformed follicular lymphoma supports the use of HDT for these patients at least in case of relapse after first line aggressive treatment.
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