Poor-risk aggressive lymphoma
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1 General introduction
The non-Hodgkin’s lymphomas are a variety of malignant disorders of the immune system with distinct clinical and pathological characteristics. Incidence rates of lymphoma in 2002 were 18.4/100,000/year in the US, and approximately 2500 new cases were reported in 2003 in the Netherlands by the cancer registry. In general, as is the case in other malignancies, incidence rates increase with age, but 50% of patients will be younger than 60 years when first diagnosed with malignant lymphoma. Most lymphomas arise from B-cells, but T-cells or natural killer cells may also be involved as cell of origin. This thesis focuses on treatment and prognostic aspects of young adult patients with poor-risk aggressive B-cell lymphoma. In this chapter a general introduction to these aspects is presented.

1.1 Clinical presentation and pathological diagnosis

Lymph node enlargement is often the first clinical presentation of malignant lymphoma, but signs or symptoms of extranodal localizations, including organ or bone marrow failure can also determine the clinical picture. Extranodal involvement occurs as first presentation in up to 30% of cases of diffuse large B-cell lymphoma, the most common lymphoma entity. Some lymphomas tend to be restricted to specific sites, e.g. T-cell lymphomas of the skin, mucosa associated lymphoid tissue (MALT) lymphoma of the stomach, whereas others have a tendency to disseminate to specific extranodal sites, e.g. meningeal dissemination associated with lymphoblastic and Burkitt lymphomas. Other lymphoma entities present with widespread disease involving nodal as well as extranodal sites at diagnosis, e.g. mantle cell lymphoma.

Both, clinical presentation and the specific morphological, immunohistochemical and genetic features of the malignant lymphoma determine the approach to the patient as well as treatment and prognosis. Accordingly, the current World Health Organization (WHO) classification of malignant lymphoma is based on an integration of these characteristics. Thus, apart from a careful disease history and clinical assessment, the diagnosis of malignant lymphoma foremost relies on the availability of an adequate tumor biopsy specimen for examination by a hematopathologist.

Over 40 distinct lymphoma entities are currently recognized in the WHO classification. This classification, published in 2001 is a further refinement of the work set in motion by an international collaboration of expert hematopathologists that culminated in the revised European-American classification of lymphoid neoplasia (REAL classification) in 1994, integrating morphological, immunological and genetic features. This classification finally ended the application of dif-
ferent classification systems used by pathologists in Europe and the United States. More importantly, it also made the much used but frustrating translation between these different systems for clinical usage, the so called Working Formulation, 4 redundant.

For practical clinical reasons, the majority of the lymphomas have traditionally been subdivided into behaving either aggressively or indolently, as summarized in Table 1. Although scientifically redundant, given the distinct clinical-pathological lymphoma entities of the WHO classification, this segregation into lymphomas with aggressive or indolent behavior has been used by clinicians for a long period of time. It may still be practical for usage at initial diagnosis for the clinician working with restricted hospital facilities, who is less familiar with the WHO classification, in particular to guide timing of expert consultation, referral, staging and treatment.

Table 1. **Most frequent lymphoma subtypes.** 5

<table>
<thead>
<tr>
<th>B-cell lymphoma</th>
<th>% of cases</th>
<th>Clinical behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Diffuse large B-cell</td>
<td>31%</td>
<td>aggressive</td>
</tr>
<tr>
<td>- Follicular</td>
<td>23%</td>
<td>indolent</td>
</tr>
<tr>
<td>- MALT</td>
<td>8%</td>
<td>indolent</td>
</tr>
<tr>
<td>- Small lymphocytic/CLL</td>
<td>7%</td>
<td>indolent</td>
</tr>
<tr>
<td>- Mantle Cell</td>
<td>6%</td>
<td>aggressive</td>
</tr>
<tr>
<td>- Burkitt</td>
<td>3%</td>
<td>aggressive</td>
</tr>
<tr>
<td>- Primary Mediastinal B-cell</td>
<td>2%</td>
<td>aggressive</td>
</tr>
<tr>
<td>- Nodal marginal zone</td>
<td>2%</td>
<td>indolent</td>
</tr>
<tr>
<td>T-cell lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Mature T</td>
<td>8%</td>
<td>aggressive</td>
</tr>
<tr>
<td>- Anaplastic large cell</td>
<td>2%</td>
<td>aggressive</td>
</tr>
<tr>
<td>- Precursor T lymphoblastic</td>
<td>2%</td>
<td>aggressive</td>
</tr>
<tr>
<td>Other types (B and T)</td>
<td>7%</td>
<td></td>
</tr>
</tbody>
</table>

As can be seen in Table 1, 80% of the malignant lymphomas are of B-cell origin. Of these, the vast majority are either diffuse large B-cell lymphomas, which behave aggressively or follicular lymphomas, behaving indolently in the majority of cases.

Although most B-cell lymphomas are readily diagnosed based on their distinct morphology, clinical presentation, immunological and or genetic hallmarks, some clinically relevant difficulties in classification still remain. For instance, the distinction between Burkitt lymphoma and diffuse large B-cell lymphoma in
an adult patient, which is of utmost relevance for the choice of treatment, can still be very difficult even in the presence of a MYC gene rearrangement, pathognomonic for Burkitt lymphoma\(^6\) (this thesis chapter 2.3 and 3.1). Morphology of diffuse large B-cell lymphoma is highly variable and tumor cells can have variable cytological features and additional genetic abnormalities. Although not consistently correlated with distinct morphological or cytogenetic characteristics, this large group of aggressive lymphomas may in fact comprise several distinct clinically relevant subgroups (see also gene expression below and this thesis chapter 2.3).

T-cell lymphomas are rare entities and most of these lymphomas behave clinically aggressively, with the exception of some T-cell lymphomas of the skin. Diagnosis of T-cell lymphoma may be difficult due to their sometimes subtle clinical presentation. They often present at extranodal sites and diagnosis may present a considerable challenge. Without the presence of a clear morphologically malignant presentation in the biopsy, differential diagnosis, for instance with a reactive T-cell infiltrate, may be extremely difficult even in the hands of an expert hematopathologist. Moreover, in contrast to B-cell lymphomas, there are no techniques as yet to determine clonality of the antigen receptor in T-cells by immunohistochemistry or flowcytometry. In consequence, diagnosis of T-cell lymphomas is often dependent on aberrant (co-)expression or the absence of expression of specific T-cell antigens of the tumor cells. Clonality in T-cell lymphomas can only be determined by molecular techniques.

Post transplant lymphoproliferative disorders are rare lymphoid proliferations or lymphomas originating in transplant recipients. These lymphomas arise in the context of impaired function of the immune system as a result of immunosuppressive treatment to prevent allograft rejection. Although rare, these lymphomas are not infrequently encountered in a large transplant center such as our hospital (UMCG). Most of these lymphomas are of B-cell origin and EBV positive. Impaired T-cell mediated immune surveillance against EBV induced B-cell proliferation plays an important role in the etiology of these lymphomas. Post transplantation lymphoma frequently presents at extranodal sites, including the allograft and may behave very aggressively (chapter 4.1 this thesis).

1.2 Pathogenetic aspects

Although some lymphomas have a distinct etiology, i.e. may be associated with a particular pre-existing immune disorder, infectious agent, carcinogenic event or combinations thereof, the precise etiology of the majority of lymphomas is unknown. \(^2\)
Most lymphomas can be considered as clonal proliferations originating from a particular stage of lymphoid development. Immunoglobulin gene rearrangement in early B-cell development, or somatic hypermutation of immunoglobulin genes during antigen affinity proliferation and maturation of B-cells in the germinal center, provide a genetically unstable and promiscuous environment for the occurrence of genetic aberrations. These aberrations may ultimately lead to disruption of gene regulation and/or abnormal expression of gene products, including fusion genes coding for specific proteins in specific lymphoma subtypes. Among others, these aberrations may lead to increased proliferation (e.g. MYC gene deregulation in Burkitt lymphoma), inhibition of apoptosis (e.g. bcl2 protein over-expression in follicular lymphoma), disruption of cell cycle progression control (e.g. cyclin D1 over-expression in mantle cell lymphoma), or prevention of cell cycle arrest (e.g. bcl6 protein in diffuse large B-cell lymphoma). Key genes commonly involved in the histopathogenesis of mature B-cell lymphoma subtypes are summarized in Table 2.

**Table 2. Common genetic aberrations associated with mature B-cell lymphoma.**

<table>
<thead>
<tr>
<th>Mature B-cell lymphoma</th>
<th>Chromosomal aberration</th>
<th>Involved genes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular lymphoma</td>
<td>t(14;18)(q32;q21)</td>
<td>IgH and BCL2</td>
</tr>
<tr>
<td>- 80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 15%</td>
<td>3q27</td>
<td>BCL6</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 30%</td>
<td>t(14;18)(q32;q21)</td>
<td>IgH and BCL2</td>
</tr>
<tr>
<td>- 20%</td>
<td></td>
<td>BCL6</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>t(11;14)(q13;q32)</td>
<td>Cyclin D1 and IgH</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>t(8;14)(q24;q32)</td>
<td>MYC and IgH</td>
</tr>
<tr>
<td></td>
<td>t(2;8)(q11;q24)</td>
<td>Ig-kappa and MYC</td>
</tr>
<tr>
<td></td>
<td>t(8;22)(q24;q11)</td>
<td>MYC and Ig-lambda</td>
</tr>
<tr>
<td>Gastric MALT lymphoma</td>
<td>t(11;18)(q21;q21)</td>
<td>API2 and MLT</td>
</tr>
<tr>
<td>Splenic Marginal Zone lymphoma</td>
<td>t(2;7)(q11;q21)</td>
<td>Ig-kappa and CDK6</td>
</tr>
<tr>
<td>Lymphoplasmacytic lymphoma</td>
<td>t(9;14)(p13;q32)</td>
<td>PAX-5 and IgH</td>
</tr>
</tbody>
</table>

* IgH = immunoglobulin heavy chain gene; Ig-kappa = immunoglobulin kappa light chain gene; Ig-lambda = immunoglobulin lambda light chain gene.

Although analysis of chromosomal aberrations may be helpful in pinpointing the diagnosis of specific lymphoma subtypes, the specificity of some of these aberrations may vary as they can be observed in several distinct lymphoma entities. Moreover, additional specific and nonspecific genetic abnormalities may be
observed (not shown). The prognostic value of specific chromosomal breakpoints within these lymphoma entities is not clearly defined (chapter 2.3 this thesis).

Although a potential promiscuous environment for genetic events is also present during antigen-receptor gene-rearrangement in T-cell development, much less is known about molecular pathogenetic events in T-cell lymphomas, due to their infrequent occurrence. An exception is ALK protein expression positive anaplastic large cell lymphoma. In this lymphoma, translocation of the ALK gene on chromosome 2p23 results in a fusion gene with aberrant expression and gain of function of an oncogenic ALK gene-encoded tyrosine kinase receptor, not normally expressed in lymphoid cells.

1.3 Staging and prognostic factors

To select the appropriate treatment, if any, for a patient with malignant lymphoma, evaluate its result, and to stratify prognostic subgroups, careful staging of all involved sites is paramount. In addition, the International Prognostic Index (IPI) should be documented at diagnosis in all patients with aggressive lymphoma, because of its importance for prognosis. FDG-PET scanning (2-(F-18)-fluoro-deoxy-glucose positron emission tomography) has become an important tool in staging and treatment evaluation. In addition, analysis of gene expression or protein expression may help to further define subgroups of lymphomas with different prognosis.

International Prognostic Index

Before the IPI was in common use, most study groups had their own clinical risk classification to stratify patients for prospective studies. For instance, in the Netherlands, HOVON (Stichting Hemato-Onclogie voor Volwassenen Nederland) developed a risk classification based on treatment results of 285 patients up to 65 years included in a randomized study for stage II-IV aggressive lymphoma. Based on stage and serum LDH, young patients could be divided into three HOVON risk groups: low-risk (stage II, normal serum LDH), intermediate-risk (stage III or IV with normal LDH; stage II with elevated LDH), or high-risk (stage III or IV with elevated LDH), with corresponding 5 year survival rates of 77%, 49%, and 23%, respectively. Inclusion of patients in subsequent HOVON trials for patients below 65 years of age was based on risk classification according to this model.

The IPI is based on multivariate analysis of clinical data from 2031 patients with aggressive lymphoma treated with CHOP-like chemotherapy (cyclophosphamide, hydroxylaudiomycin (doxorubicin), vincristine (Oncovin®) and prednisone) in 16 centers in Europe and North-America. Five clinical risk factors at
diagnosis were identified that were independently associated with treatment outcome: Ann Arbor stage (I-II v. III-IV), age (younger or older than 60 years), performance status (ECOG 0-1 v. 2-4), serum LDH (lactate dehydrogenase) (normal v. elevated) and number of extranodal sites of involvement (0-1 v. 2 or more).

A simple model, the IPI risk score, was constructed, based on the number of risk factors present in the individual patient ranging from 0 to 5. This risk score proved to be highly predictive for response and survival in patients with aggressive lymphoma treated with standard CHOP-like chemotherapy (Table 3A).

Table 3A. Outcome of aggressive lymphoma according to the International Prognostic Index. All patients (n=2031), 5 factors score.

<table>
<thead>
<tr>
<th>Risk group</th>
<th>No. factors</th>
<th>% patients</th>
<th>% CR</th>
<th>% 5-yr RFS</th>
<th>% 5 yr OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0-1</td>
<td>35</td>
<td>87</td>
<td>70</td>
<td>73</td>
</tr>
<tr>
<td>Low-intermediate</td>
<td>2</td>
<td>27</td>
<td>67</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>High-intermediate</td>
<td>3</td>
<td>22</td>
<td>55</td>
<td>49</td>
<td>43</td>
</tr>
<tr>
<td>High</td>
<td>4-5</td>
<td>16</td>
<td>44</td>
<td>40</td>
<td>26</td>
</tr>
</tbody>
</table>

CR = complete remission; 5-yr = 5-year; RFS = relapse-free survival; OS = overall survival

Table 3B. Outcome of aggressive lymphoma according to the International Prognostic Index. Age-adjusted IPI, patients younger than 61 years (n=1271), 3 factors score.

<table>
<thead>
<tr>
<th>Risk group</th>
<th>No. factors</th>
<th>% patients</th>
<th>% CR</th>
<th>% 5-yr RFS</th>
<th>% 5 yr OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0</td>
<td>22</td>
<td>92</td>
<td>86</td>
<td>83%</td>
</tr>
<tr>
<td>Low-intermediate</td>
<td>1</td>
<td>32</td>
<td>78</td>
<td>66</td>
<td>69%</td>
</tr>
<tr>
<td>High-intermediate</td>
<td>2</td>
<td>32</td>
<td>57</td>
<td>53</td>
<td>46%</td>
</tr>
<tr>
<td>High</td>
<td>3</td>
<td>14</td>
<td>46</td>
<td>58</td>
<td>32%</td>
</tr>
</tbody>
</table>

CR = complete remission; 5-yr = 5-year; RFS = relapse-free survival; OS = overall survival

Age in itself plays an important role in treatment decisions, e.g. the inclusion in intensive treatment protocols. Therefore, an age-adjusted IPI was constructed and validated for patients younger than 61 years consisting of only 3 independent factors: stage, performance status and serum LDH (Table 3B). The number of extra-nodal sites, which proved to be the weakest factor in the IPI, no longer remained an independent risk factor in young patients. Because the majority of patients (>80%) in the IPI project had diffuse large B-cell lymphoma, the prognostic value of the IPI risk classification is mainly based on this type of lymphoma.
1 General introduction

Because of its robust performance and general acceptance, treatment for previously untreated diffuse large B-cell lymphoma, at least in clinical trials, is currently stratified according to IPI risk profiles.

In addition, the IPI may also have prognostic relevance in patients with relapsed aggressive lymphoma. The age-adjusted IPI score at relapse, the so-called secondary age-adjusted IPI, is highly predictive for outcome of second-line treatment followed by autologous stem cell transplantation in aggressive lymphoma9 (chapter 5.1 this thesis).

FDG-PET

Conventional diagnostic methods for lymphoma staging include at least a bone marrow biopsy, CT scanning of neck, thorax and abdomen. After treatment, residual masses are often present on CT scan but their predictive value for progression or recurrence of lymphoma is ill defined because this imaging technique cannot differentiate between viable tumor or scar tissue. This has hampered the meaningful use of response criteria, based on CT criteria, as primary endpoint of treatment efficacy in clinical trials. As a consequence, time dependent endpoints implying longer follow-up, such as time to treatment failure or time to progression are being used as primary efficacy endpoints in virtually all clinical studies. The use of 18-fluorodeoxyglucose positron emission tomography (FDG-PET) as imaging technique for staging and response evaluation in lymphoma might change this paradigm. The high performance of FDG-PET to distinguish between viable tumor cells or scar tissue at sites with residual mass on CT scan in aggressive lymphoma will become an important efficacy endpoint in future studies.10,11

FDG-PET scanning may also be used for mid treatment evaluation to distinguish patients responding to treatment from those who are not or less well responding12-17 (chapter 5.2 this thesis). Moreover, its use in detection of possible sites of lymphoma involvement not readily visualized by routine CT or MRI scanning, e.g. extranodal localizations, may be extremely helpful to guide further clinical decisions, e.g. localization of sites most appropriate for diagnostic biopsy (chapter 4.2 this thesis).

Gene- and protein expression

Although not (yet) integrated in the WHO classification, gene-expression profiling of lymphomas may add clinically relevant information. For instance, distinct gene expression signatures can be recognized within lymphoma subgroups, e.g. diffuse large B-cell lymphoma that are associated with different prognosis.18,19 Furthermore, this technique is increasingly used to further define and classify rare lymphoma entities, e.g. primary mediatinal B-cell lymphoma,20 or to elucidate diagnostic difficulties, for instance those encountered in Burkitt lymphoma in adults.21,22
However, gene expression profiling requires frozen tumor tissue and expensive and sophisticated techniques not readily available in routine diagnostic laboratories. Determining the appropriate cellular proteins corresponding to the discriminatory genes found in gene expression arrays and the subsequent development of an appropriate immunohistochemical technique for their detection, which can be used in paraffin embedded tissue in routine practice, is therefore of great importance. Ultimately, immunohistochemical algorithms may be developed enabling the pathologist to extract comparable – if not the same – information from paraffin embedded tissue as provided by gene expression profiling (chapter 3.3 this thesis).

1.4 Treatment

The choice as well as timing of treatment in malignant lymphoma, mainly depends on its pathological classification and clinical presentation. Treatment may vary from watch-full waiting in a patient with follicular lymphoma, antibiotics for *Helicobacter pylori* eradication in localized gastric malt lymphoma without t(11;18) translocation, tapering of immunosuppression in post transplantation lymphoma, up to brief high-intensity aggressive chemotherapy with central nervous system prophylaxis in a patient with Burkitt lymphoma. Given the scope of this thesis, this section will mainly focus on treatment of advanced diffuse large B-cell lymphoma, Burkitt lymphoma and post transplant lymphoma.

**Treatment of diffuse large B-cell lymphoma**

**Caveats**

Differences in mix of lymphoma subtypes included in trials, use of different risk factor classification systems, and the additional use of radiotherapy in some trials make comparison of results of clinical studies difficult.

Before the REAL/WHO classification became in full usage, most clinical studies have included other lymphoma subtypes with aggressive clinical behavior together with diffuse large B-cell lymphoma, e.g. mature T-cell lymphomas, follicular lymphoma grade 3, and even mantle cell lymphomas. Although most of these studies contain 80% or more diffuse large B-cell lymphoma cases, the case mix of these different histologies may have biased the results, given the independent prognostic influence of histology on outcome. For instance, 5 year overall survival rates for ALK protein positive anaplastic large cell lymphoma, diffuse large B-cell lymphoma and mature T/NK-cell lymphoma were 64%, 53% and 35% respectively in a large retrospective study of over 1800 patients uniformly treated according to the LNH87 protocol by the GELA (Groupe d’Etudes de Lymphomes
Agressives. The prognostic impact of histology was independent from IPI risk in multivariate analysis.

Apart from histology, differences in the mix of IPI risk factors of patients included in studies, designed before the IPI was in common use, may also have influenced outcome. Most study groups had their own risk classification to stratify patients for prospective studies.

The place of radiotherapy in combination with chemotherapy or with chemoinmunotherapy for the initial treatment of localized aggressive lymphoma or consolidation of bulky disease is in debate. Truly localized disease, i.e. stage I (E) disease, is rare in diffuse large B-cell lymphoma and a full discussion of this topic is beyond the scope of this thesis. However, radiotherapy has also been used by several study groups in advanced stages for consolidation of initial bulky disease, and/or sites of partial remission after chemo or chemo-immunotherapy. The German study group has routinely incorporated radiotherapy as consolidation in the treatment of patients with bulky disease in most of their randomized studies for aggressive lymphoma.

Thus, differences in mix of lymphoma subtypes, classification of risk and the additional use of radiotherapy, make direct comparison of treatment results from different studies often difficult, if not impossible. A fortiori, extrapolation of treatment results to other patient groups should only be done with extreme caution.

**CHOP chemotherapy**

Until the recent introduction of rituximab, a monoclonal anti-B-cell antibody directed against the CD20 antigen present on virtually all mature B-cells and exhibiting amazing efficacy when combined with chemotherapy in the treatment of B-cell lymphoma, CHOP chemotherapy administered three weekly has been the standard treatment for advanced aggressive lymphoma since 1976.

Attempts to improve upon CHOP by incorporating other drugs in the classical CHOP scheme have not been very successful. No difference in outcome was observed in a large North American Intergroup study, comparing 8 three-weekly cycles of CHOP (CHOP-21) with three other regimens (ProMACE-CYTABOM, MACOP-B and M-BACOD), which all had shown promising efficacy in phase II studies. Because cyclophosphamide and doxorubicin are probably the most effective cytostatic drugs in the treatment of aggressive lymphoma, the negative results of this trial might in part be explained by the lower doses of cyclophosphamide and/or doxorubicin in the third generation regimens, in order to accommodate for the incorporation of other cytostatic drugs (chapter 2.2 this thesis). Nevertheless, from its publication in 1993, the North American Intergroup study more or less proclaimed 8 cycles of CHOP-21 to be standard treatment for advanced aggressive lymphoma, against which future experimental treatments would have to be compared in the years to come.
A serious contender of CHOP-21 as the proclaimed golden standard in the treatment of aggressive lymphoma, at least in younger patients, might be the ACVBP regimen (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone). With this regimen, used by the GELA in successive large cohorts of patients since 1984, impressive, if not better results than with CHOP-21 have been reported. ACVBP is given for only 3-4 cycles, followed by consolidation with high-dose methotrexate, ifosfamide, etoposide, asparaginase, and cytarabine. Interestingly, the initial dose intensity of doxorubicin and cyclophosphamide in the ACVBP regimen (70 mg/m² and 1200 mg/m² per cycle) is higher than in CHOP-21 (50 mg/m² and 750 mg/m² per cycle). Although probably more toxic than CHOP-21, the increased early dose intensity of ACVBP might theoretically result in better outcome. However, the contribution of the intensive consolidation after 3-4 ACVBP cycles will without doubt also play an important role in treatment outcome. Unfortunately the ACVBP regimen has never been compared directly with CHOP in a randomized controlled trial in poor-risk young patients. In young patients with low-risk aggressive lymphoma no difference was observed between M-BACOD (methotrexate, bleomycin, adriamycin, cyclophosphamide, vincristin, dexamethason) and ACVBP. In elderly patients (61-69 years) with intermediate and high IPI risk scores, ACVBP appeared to be more toxic, but had superior event free and overall survival compared to CHOP.

**Dose intensification of CHOP**

Before the availability of hematopoietic growth factors, further dose escalation of cyclophosphamide and doxorubicin without stem cell support was not feasible due to hematological toxicity. For instance, in a randomized study performed by the NCIC (National Cancer Institute of Canada), comparing doxorubicin-escalated M-BACOD (80 mg/m² doxorubicin per cycle) with standard M-BACOD (doxorubicin 50 mg/m²), the escalated regimen did not improve response and survival, but increased toxicity. However, the actual received dose intensity of doxorubicin in the escalated arm was only 70% of the projected (intended) dose as a result of dose-attenuation because of hematological toxicity. The introduction of growth factors, e.g. granulocyte colony stimulating factor (G-CSF, Filgastrim, PEG-Filgastrim), which can improve neutrophil recovery after chemotherapy and mobilize stem cells, has enabled studies on dose intensification of CHOP in aggressive lymphoma.

The German study group, DSHNHL (Deutsche Studiengruppe für Hochgradige NHL) has addressed this issue by shortening the dose interval of CHOP supported by G-CSF in patients with advanced aggressive lymphoma. They reported the results of two large randomized trials comparing six cycles of CHOP or CHOEP (CHOP with the addition of etoposide) administered at three-weekly intervals, CHO(E)P-21, with the same regimens at two-weekly intervals,
CHO(E)P-14, supported by G-CSF. Additional radiotherapy after chemotherapy was given to patients with initial bulky disease (>10 cm). Inclusion criteria for young patients were good-risk disease according to the DSHNHL, i.e. they had normal serum LDH. Retrospectively, the majority had low-, or low-intermediate age-adjusted IPI risk. For elderly patients no risk classification was used and, retrospectively, all IPI risks were included. In both young and elderly patients the two-weekly regimen supported by G-CSF was superior in terms of event-free and overall survival compared with the three weekly regimen. A significant difference in 5 year overall survival was seen for CHOP-14 compared with CHOP-21 (53% v. 41%) in the elderly group, and both for CHOP-14 and CHOEP-14 compared with CHOP-21 in the young patients group. Although the addition of etoposide showed fractionally better results in young patients, the improved response and time to treatment progression of CHOEP-14 compared with CHOP-14 was nullified by the increased toxicity of etoposide in the CHOEP regimen in elderly patients. Importantly, with the use of growth factor support, the observed neutropenia and thrombocytopenia, even in elderly patients, were not different between the CHOP-14 and the CHOP-21 regimen.

The results of these large multicenter studies indicate that shortening of treatment interval supported by G-CSF is feasible and may improve outcome in both young low and low-intermediate IPI risk patients, as well as in elderly patients with aggressive lymphoma.

At the same time, the Dutch-Belgian study group HOVON conducted a randomized trial in which the hypothesis of improved efficacy by dose intensification of CHOP supported by G-CSF was tested even further. Patients below 66 years with intermediate-risk aggressive lymphoma according to HOVON criteria, corresponding to low, or low-intermediate, age-adjusted IPI risk, were randomized to compare eight cycles of CHOP-21 with six cycles of intensified CHOP-14. In this study, both the dose of cyclophosphamide and doxorubicin were increased and interval between cycles was shortened, resulting in a projected dose intensity of cyclophosphamide and doxorubicin in the intensified CHOP-14 arm that was twice that of CHOP-21. The results of this study and a further discussion of dose intensification of CHOP supported by G-CSF in young intermediate-risk patients are presented in chapter 2.2 of this thesis.

Autologous stem cell transplantation as first-line treatment

With autologous stem cell support even further dose escalation is feasible. However, this treatment strategy is commonly reserved for young patients, due to the toxicity of myeloablative therapy and autologous stem cell transplantation (ASCT) in the elderly. Based on the successful experience with ASCT in treatment of young patients with relapsed aggressive lymphoma, many subsequent studies have been conducted exploring ASCT as first-line treatment. After a sub-
stantial number of randomized trials with controversial results, the place of ASCT in first-line treatment of aggressive lymphoma is still in debate.\textsuperscript{40,41} Selection of patients as well as differences in induction treatment and timing of ASCT may explain part of the controversies. Two consecutive studies of ASCT as first-line treatment in patients with poor-risk disease were conducted by HOVON. The results of these studies, HOVON-27 and -40, and a further discussion of ASCT as first-line treatment in aggressive lymphoma can be found in \textbf{chapter 2.1} of this thesis.

\textbf{Anti-CD20 monoclonal antibodies (Rituximab)}

The introduction of rituximab, a chimeric monoclonal antibody against the CD20 antigen, has substantially improved treatment results in B-cell lymphoma. CD20, a membrane antigen with a yet unknown function, is present on most mature B-cells, except plasma cells. The major mechanisms of action of rituximab on lymphoma cells are: cell lysis as a result of complement activation, antigen dependent cellular toxicity as a result of the humanized Fc-part of the antibody, and direct triggering of apoptosis of B-cells by signaling through CD20.\textsuperscript{42}

Response rates of 33\% were observed in patients with relapsed or progressive aggressive B-cell lymphoma treated with eight weekly rituximab infusions.\textsuperscript{43} No difference in response was observed between a rituximab dose of 500 mg/m\textsuperscript{2} or 375 mg/m\textsuperscript{2}. These doses were based on phase I tolerability\textsuperscript{44} and efficacy studies in follicular lymphoma,\textsuperscript{45} respectively. Although the optimal timing and dose of rituximab is still in debate, the dose of 375 mg/m\textsuperscript{2} has subsequently been used in most lymphoma trials. After the feasibility of combining rituximab with CHOP chemotherapy in the treatment of diffuse large B-cell lymphoma had been established,\textsuperscript{46} several randomized studies have been conducted comparing rituximab with chemotherapy versus chemotherapy alone in the treatment of B-cell lymphoma, all showing superior results of the chemo-immunotherapy combination.\textsuperscript{28,47-52} In addition, a 26\% improvement in 3 year survival was observed after changing the standard CHOP-21 treatment guideline to R-CHOP-21 in a registry based cohort study in British Columbia.\textsuperscript{53}

Based on these data and the virtual absence of severe toxicity of rituximab, the combination of chemotherapy with rituximab has become standard treatment for diffuse large B-cell lymphoma, although the addition of rituximab to chemotherapy has not been studied formally in a randomized controlled trial in young patients with intermediate or high-risk disease. Ongoing studies will have to elucidate whether results of treatment may be improved by combination(s) of rituximab, or other (radio-)immunotherapy, with dose-intensified CHOP or ASCT in subgroups of patients with diffuse large B-cell lymphoma.
1 General introduction

Treatment of relapse

Young patients with aggressive lymphoma relapsing after first-line treatment may still be cured by myeloablative chemo- and or radiotherapy followed by autologous stem cell transplantation, provided they still have chemosensitive disease, i.e. respond to second-line chemotherapy. Current practice is largely based on the evidence from the PARMA study, a randomized multicenter trial in 215 patients with aggressive lymphoma who relapsed after first-line doxorubicin containing chemotherapy. After two courses of conventional second-line chemotherapy, responding patients were randomly assigned to receive either four additional courses of chemotherapy plus radiotherapy, or radiotherapy plus intensive chemotherapy followed by autologous bone marrow transplantation. Patients with resistant disease, i.e. those with less than partial response after the 2nd chemotherapy course, went off protocol treatment. At five years the survival of patients in the transplantation arm was significantly superior to those in the chemotherapy arm: 53% versus 32%. However, only half of the patients responded to second-line chemotherapy. Patients who had relapsed early, i.e. during first-line chemotherapy, had a much lower response rate to second-line chemotherapy than patients with late relapse 21% versus 64%. Patients not responding to second-line treatment had dismal survival. Time to relapse after first-line chemotherapy, and serum-LDH at relapse were independent prognostic factors for response to second-line therapy.

Because only patients responding to second-line chemotherapy will be candidates for ASCT, we wondered whether the IPI at relapse, i.e. the secondary age-adjusted IPI would also have prognostic relevance in patients who actually receive ASCT, i.e. those with chemosensitive relapsed aggressive lymphoma. The results of a retrospective study investigating prognostic factors in chemosensitive patients transplanted for relapsed or primary progressive aggressive lymphoma in our hospital (UMCG) are presented in chapter 5.1 of this thesis. In the meantime, the independent prognostic significance of this secondary IPI in patients with relapsed or primary progressive diffuse large B-cell lymphoma has also been confirmed by others.

Chemosensitivity remains the most important factor for the prediction of outcome in patients failing first line chemotherapy and the early detection of these patients has important clinical consequences. Given the uncertainties of CT scanning in the prediction of response, FDG-PET might be a better predictor for true chemosensitive disease in these patients. A pilot study investigating this issue in patients with relapsed lymphoma is presented in chapter 5.2.

Treatment of Burkitt lymphoma

Sporadic Burkitt Lymphoma is a rare, highly aggressive tumor, often originating in extranodal abdominal sites and having a tendency to meningal dis-
semination. Clinical presentation and incidence may differ between children and adults, as described in chapter 3.2 of this thesis. Despite its aggressive behavior, Burkitt lymphoma is highly curable, provided the correct diagnosis is made and the appropriate treatment is rapidly instituted. The distinction between Burkitt and diffuse large B-cell lymphoma in adult patients can be very difficult. However, a swift and correct pathological diagnosis is important, because treatment of Burkitt lymphoma differs considerably from diffuse large B-cell lymphoma. In adults Burkitt lymphoma accounts for less than 5% of the lymphomas. In childhood, Burkitt lymphoma is relatively more frequent and may constitute up to 40% of B-cell lymphomas. Therefore, clinical studies of Burkitt lymphoma in adults are sparse, and treatment guidelines are mainly based on the experience gained from cohort studies in children.

Current treatment strategies for Burkitt lymphoma mainly consist of brief duration, high-intensity chemotherapy, containing aggressive central nervous system prophylaxis. With this type of approach, treatment outcome in children is excellent, with over 80% cure rate even in patients with adverse prognostic risk factors at diagnosis. Only few data on treatment outcome in adult patients have been published. High cure rates have been reported in nonrandomized cohort studies of adult patients treated with 'pediatric' type of therapy approaches. However, children still have superior outcome and tolerate treatment better than older patients.

Based on promising results of ASCT in patients with aggressive lymphoma in first remission, including Burkitt lymphoma, HOVON initiated a multi-center phase II study in December 1994 to evaluate a strategy of short intensive sequential chemotherapy, and subsequent ASCT in adult patients with Burkitt lymphoma. For the results of this study and a further discussion of treatment of Burkitt lymphoma in adult patients the reader is referred to chapter 3.1 of this thesis.

**Treatment of post-transplantation lymphoma**

Because PTLD originates in the context of depressed T-cell mediated immune surveillance, caused by immunosuppressive drugs administered to the transplant recipient to prevent graft rejection, reduction of immunosuppression is usually the first step in treatment of this lymphoma. However, reduction of immunosuppression will often not suffice. Therefore, many other strategies have been applied, such as antiviral agents, polychemotherapy and anti-B-cell immunotherapy.

Because the pathogenesis of the majority of PTLD is associated with proliferating B-cells latently infected with Epstein Barr virus, antiviral agents inhibiting active proliferation of human herpes viruses, such as acyclovir and gancy-
clovir have frequently been used, but are not very effective in the treatment of PTLD.\textsuperscript{66}

In the past, CHOP-like chemotherapy has usually been the treatment of choice in recipients of solid organ transplants with PTLD failing to respond to reduction of immunosuppression, or in those in whom reduction of immunosuppression was considered not feasible in view of the consequences of possible graft rejection. However, this type of treatment is associated with substantial mortality, mainly due to severe infectious complications in the early post transplant period. Nowadays, rituximab has become first line treatment of CD20 positive PTLD\textsuperscript{67}. Chemotherapy for PTLD in solid organ transplant recipients is reserved for patients with CD20 negative PTLD and/or in whom other treatment options have failed.

In our center more than 2000 kidney and lung transplants have been performed between 1968 and 2002. We analyzed the incidence, patient characteristics, clinical presentation and prognostic factors of importance for treatment outcome and survival in patients who developed PTLD in these cohorts during this time frame. The results are presented in chapter 4.1 of this thesis.

1.5
Outline of this thesis

This thesis contains the results of a number of studies concerning treatment, and prognostic aspects of poor-risk aggressive lymphomas, in particular Burkitt lymphoma, diffuse large B-cell lymphoma, and post-transplant lymphoma. The studies were either conducted in multicenter collaboration (HOVON), or as singlecenter study based on data from patients treated in the department of hematology at UMCG. The studies have been grouped by disease entity.

Chapter 2: Diffuse large B-cell lymphoma

In chapter 2.1 the results of two consecutive multicenter HOVON phase II studies (HOVON-27 and HOVON-40) are presented, investigating up-front high-dose therapy followed by ASCT as first-line treatment in advanced poor-risk aggressive lymphoma. The large majority (80\%) of patients accrued in these studies had diffuse large B-cell lymphoma.

In chapter 2.2 the results of a prospective randomized phase III study (HOVON-26) are presented in which we investigated the impact of doubling the dose intensity of cyclophosphamide and doxorubicin in the CHOP regimen, supported by G-CSF compared to standard CHOP treatment in patients with intermediate-risk advanced aggressive lymphoma.

Chapter 2.3 contains an analysis of the prognostic impact of immunophenotype and chromosomal breakpoints in diffuse large B-cell lymphomas included in the
HOVON-27 and -40 studies. The primary objective of this retrospective biological study was to investigate the prognostic value of germinal center B-cell type versus non germinal center B-cell type diffuse large B-cell lymphoma, as determined by immunohistochemical algorithm in a homogeneous group of poor-risk patients, treated with high-dose therapy and ASCT as first-line treatment.

Chapter 3: Burkitt lymphoma
In chapter 3.1 results are presented of a multicenter phase II study by HOVON (HOVON-27BL) investigating the feasibility and efficacy of short, intensive high-dose treatment followed by ASCT in adult Burkitt lymphoma. This study ran in parallel with a study in other advanced poor-risk aggressive lymphomas, treated according to the same regimen (HOVON-27), described in chapter 2.1 of this thesis.

In chapter 3.2 the results are presented of incidence and clinical presentation of Burkitt lymphoma in children versus adults, based on data obtained from the National Cancer Registry, the SLWNK (Stichting Leukemie Werkgroep Nederland voor Kinderen) and the HOVON-27BL study.

Chapter 4: Post-transplant lymphoma
In chapter 4.1 the salient clinical features and treatment results are described of post-transplant lymphoproliferative disease observed in a cohort of over 2000 recipients of a lung or kidney transplant between 1968 and 2002 in our hospital. In chapter 4.2 the preliminary experience with FDG-PET scanning as tool for staging and treatment evaluation in post transplantation lymphoma is described.

Chapter 5: Relapsed aggressive lymphoma
In chapter 5.1 a retrospective analysis is presented of the prognostic impact of the age-adjusted IPI score at relapse (secondary age-adjusted IPI) in young patients with chemo sensitive relapsed aggressive lymphoma who subsequently received autologous stem cell transplantation in our hospital.

In chapter 5.2 we analyzed the value of midtreatment FDG-PET scanning during second-line induction treatment to predict the subsequent outcome of autologous stem cell transplantation in patients with relapsed lymphoma.

Chapter 6: Summary and General Discussion

References
1 General introduction


1 General introduction


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