Cutaneous B-cell lymphoma : classification, prognostic factors and management recommendations

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Chapter 8.

Summary and Discussion

In the last decade there has been an ongoing debate whether the EORTC or the WHO classification should be used for classification of primary cutaneous B-cell lymphomas (CBCL). The discrepant points of view were not only a matter of academic dispute, but also had major therapeutic consequences. With the publication of the WHO-EORTC classification in 2005, this controversy may have come to an end. However, a number of outstanding issues remained, that needed to be addressed.

The main issues concerned (1) the clinical usefulness of the WHO-EORTC classification in daily practice, (2) the validity of prognostic parameters reported in previous studies and identified in patient groups classified according to previously used classification schemes and (3) optimal management for the different types of CBCL as defined in the WHO-EORTC classification. In this concluding chapter, these three issues will be discussed based on the data described in this thesis and data from the literature.

The WHO-EORTC classification for CBCL in daily practice

In **Chapter 2**, the clinical significance of the WHO-EORTC classification was assessed on 300 CBCL cases, present in the database of the Dutch Cutaneous Lymphoma Group (DCLG), and compared to the previously used EORTC and WHO classification schemes. Furthermore, we aimed to more clearly define which cases should be assigned to the group of primary cutaneous diffuse large B-cell lymphoma, other (PCLBCL, other).

Using the criteria of the WHO- EORTC classification, primary cutaneous marginal zone B-cell lymphoma (PCMZL) and primary cutaneous follicle centre lymphoma (PCFCL) showed a 5-year disease-specific survival (DSS) of 98% and 95%, respectively, whereas primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT) had a 5-year DSS of only 50%. These results are in agreement with the results of recent studies and confirm that the new WHO-EORTC classification adequately distinguishes between CBCL with an indolent and CBCL with a more aggressive clinical behaviour.[1-3] Most patients with PCMZL presented with multifocal skin lesions mainly on the trunk and extremities. Although the frequency of skin relapses was high and extracutaneous dissemination was observed in 9% of patients, only one patient died due to lymphoma. In the group of PCFCL, most patients characteristically presented with skin lesions on the head or trunk and only a small minority (6%) presented with lesions on the leg(s). Expression of the so called, activated B-cell (ABC) markers, Bcl-2, MUM-1 and FOXP1, was found in only 11%, 10% and 4% of cases respectively. Extracutaneous progression was seen in 11% of patients, while ultimately 5% of patients died due to their lymphoma. Patients with PCLBCL, LT most often presented with lesions on the leg(s) and only 12% of patients had skin lesions restricted to another skin site. The neoplastic B-cells strongly expressed Bcl-2, MUM-1 and FOXP1 in the majority of cases (90%, 90% and 81% respectively), both in patients with lesions on the legs and elsewhere. This group showed a high rate of extracutaneous progression (47%) and 45% of patients died of lymphoma.

In contrast to the EORTC classification, in which differentiation between primary cutaneous follicle centre cell lymphoma (PCFCCL) and primary cutaneous large B-cell lymphoma of the leg (PCLBCL-leg) was based on site (non-leg or leg), in the WHO-EORTC differentiation between PCFCL and PCLBCL,LT is based primarily on cell morphology, i.e. cleaved cells (centrocytes) versus noncleaved or round cells (centroblasts and immunoblasts). Differentiation on the basis of morphology is known to be associated with a considerable inter-observer variation.[4;5] While cases with a clear (partly) follicular growth pattern will be easily classified as PCFCL, in cases with a diffuse growth pattern this classification might be more difficult. In such difficult cases, the presence of a considerable proportion of admixed T cells, the presence of a stromal reaction as well as demonstration of (remnants of) follicular dendritic cell networks by staining with appropriate antibodies (CD35 or CD21) may serve as useful additional criteria suggesting a diagnosis of PCFCL.[6] Moreover, since it was found that most PCLBCL, LT cases expressed Bcl-2, MUM-1 and FOXP1, this phenotypic profile might also be a useful adjunct, supporting a diagnosis of PCLBCL, LT.[1;2;6] However, since Bcl-2, MUM-1, and to a lesser extent FOXP1 are also expressed by a small minority of PCFCL and a small minority of PCLBCL, LT do not express these markers, they cannot be used as a golden standard to differentiate between both conditions.

Comparing the three classification schemes demonstrated no important differences in the classification of PCMZL and PCFCL with a follicular or follicular and diffuse growth pattern. However, 65% of cases classified as diffuse large B-cell lymphoma (DLBCL) using the WHO classification, were reclassified as PCFCL using the WHO-EORTC scheme, which implies that these patients can be managed by local therapies instead of more aggressive systemic therapies. This illustrates that the WHO-EORTC classification is a major step forward as compared to the WHO classification and that it contributes to a more appropriate treatment in patients with CBCL. The reclassification of 10% of cases formerly classified by the EORTC classification, concerned seven PCFCCL patients with a predominant round-cell morphology, that were now classified as PCLBCL, LT and nine PCLBCL-leg patients with a cleaved-cell morphology that were now included as PCFCL. For the seven PCFCCL patients with round-cell morphology this reclassification proved clinically relevant since they showed the same intermediate prognosis as other cases with the same morphology presenting on the leg. However, the reclassification of the nine cleaved-cell cases presenting on the leg as PCFCL proved less fortunate, since they showed a significantly worse prognosis as compared to PCFCL cases presenting with skin lesions on other body regions.

Recent studies used the term PCLBCL, other in different ways. Some authors assigned all cases with a predominance of large round cells without Bcl-2 expression to the group of PCLBCL, other[2;7], while we and others classified such cases as PCLBCL, LT, irrespective of the location of skin lesions and Bcl-2 expression.[3;6] In our study, comparison of Bcl-2–positive and Bcl-2–negative PCLBCL, LT showed no significant difference in 5-

year DSS or 5-year overall survival (OS), which is consistent with the results of Kodama et al.[2] Similarly in both studies, no significant differences in 5-year OS and DSS were found between PCLBCL, LT with or without expression of MUM-1 or FOXP1. These data indicate that distinction between patients with or without expression of Bcl-2, MUM-1, or FOXP1 is not useful, and that categorization of Bcl-2–negative patients as PCLBCL, other, as suggested previously[2;7], is not justified. This term should be reserved for rare morphological variants of diffuse CBCL, that do not fit the criteria of either PCFCL or PCLBCL, LT.

Prognostic factors for the newly defined CBCL groups

Prognostic factors for CBCL described in previous studies, are currently not useful anymore or need to be confirmed, since they were identified on patient groups defined with older classification systems or by studying mixed diagnostic populations. Prognostic factors were studied in three chapters of this thesis.

Clinical, histological and immunophenotypical markers: a multivariate analysis

In **Chapter 2** we aimed to define prognostic parameters within the redefined categories PCFCL and PCLBCL, LT. We analyzed various clinical, histological and immunophenotypical markers using univariate and multivariate analyses.

Both in the total group of PCFCL and in the group of PCFCL with a diffuse infiltrate of large cleaved cells, patients presenting with skin lesions on the leg(s), had a significantly worse prognosis than PCFCL presenting at other sites. This finding is consistent with other recent studies and implies that within this entity further distinction should be made based on the site of presentation.[2;4;7] However, since these results are based on a relatively small number of PCFCL patients with lesions on the leg, this finding needs further validation.

Expression of Bcl-2, MUM-1 and FOXP1 was not associated with prognosis in PCFCL cases with a diffuse growth pattern. However, in the total group of PCFCL, both weak and strong expression of FOXP1 was associated with an inferior prognosis as compared to patients without expression of FOXP1. While in CBCL this marker has been reported to be associated with a round cell morphology, i.e. a diagnosis of PCLBCL, LT, prognostic significance within one of the CBCL entities has not been described so far. In systemic DLBCL, it is a matter of debate whether FOXP1 is associated with a bad prognosis or not.[8-10] These contradictory results might be explained by recently reported findings, describing that the JC12 monoclonal antibody, used to determine FOXP1 protein expression, does not distinguish between the full-length FOXP1 protein and smaller isoforms, whilst it appears that these smaller FOXP1 isoforms, rather than the full-length protein, have a potentially oncogenic role in B-cell non-Hodgkin lymphomas.[11] The exact role and prognostic significance of this marker in PCFCL needs further clarification. Other

parameters, such as age, extent of skin lesions or growth pattern did not have independent prognostic significance.

In the PCLBCL, LT group localization of skin lesions or presence or absence of Bcl-2, MUM-1 or FOXP1 was not associated with prognosis. While in univariate analysis of OS both age and extent of skin lesions were associated with a poor prognosis, in multivariate analysis no independent prognostic markers could be identified.

The TNM classification system for primary cutaneous lymphomas other than mycosis fungoides (MF) and Sézary syndrome (SS)

In 2007 a new clinical staging system was proposed for primary cutaneous lymphoma other than MF and SS.[12] This TNM based system was meant to document extent of disease in a consistent matter, thereby facilitating comparison between different research centres and study populations. At the time of publication it was unknown if the system had prognostic significance in CBCL. In **Chapter 4** we applied this system to the 300 CBCL that had been reclassified according to the WHO-EORTC classification, described in **Chapter 2**. The results of **Chapter 4** show that the TNM system can easily be applied to the three main groups of CBCL. With regard to prognostic significance, it was found that increasing T-score was associated with decreased survival in the group of PCLBCL, LT. Although the association was not statistically significant, this is in accordance with two other recent reports on PCLBCL, LT. These studies report a better prognosis for PCLBCL, LT patients presenting with a solitary tumour as compared to patients presenting with multiple tumours on one or both leg(s).[3;4] These results are also in line with the findings described in **Chapter 2**. Here we found extent of skin lesions to be associated with an unfavourable prognosis only in univariate analysis. For both indolent groups of CBCL, PCMZL and PCFCL, the TNM classification did not provide prognostic significance.

In addition, we compared the proposed TNM classification system with the scoring of the DCLG, which has been in use for many years and was used to report disease extent in the study described in **Chapter 2**. There was a discrepancy in the classification of 20 cases, all indolent types of CBCL. This concerned cases with multiple skin lesions at two contiguous body sites. As there is no anatomical or biological relation between such separate lesions, in the DCLG system such lesions are classified as multifocal or generalized disease. However, as the T3 category in the TNM system is defined as involvement of two non-contiguous body regions or three or more body regions, such cases are classified as T2, which denotes regional skin involvement. In both indolent types of lymphoma, classification as T2 is of minor clinical importance, since they can be treated with non-aggressive therapies, irrespective of disease extent. However, in other, more aggressive cutaneous lymphomas this discrepant classification of multiple distant skin lesions restricted to two adjacent skin sites as T2 might have important therapeutic consequences. Since in our study the staging of PCLBCL, LT did not show any discrepancies between both systems, the applicability of the definitions for T2 and T3

should be evaluated in other cohorts of (more aggressive) cutaneous lymphomas, in order to avoid undesirable therapeutic consequences.

Inactivation of CDKN2a as a prognostic marker in PCLBCL, LT

A recent study suggested inactivation of CDKN2A as a prognostic marker in the PCLBCL, LT group.[13] Since this was only investigated in a small number of cases, we sought to confirm these findings on a larger patient group. For that purpose, we needed a technique that could be applied on DNA derived from formalin-fixed paraffin-embedded (FFPE) material, since this is more readily available. We tested a recently described technique, called Multiplex Ligation-dependent Probe Amplification (MLPA), that was reported to be less sensitive to DNA degradation and shows reliable results when applied to DNA derived from FFPE material.[14-17] Indeed, we found that comparison between fresh-frozen and FFPE material obtained from the same tumour in two patients, showed identical results. In an EORTC Cutaneous Lymphoma Group (CLG) multicentre study, described in **Chapter 3**, we analyzed tumour DNA of 64 PCLBCL, LT patients, which is the largest group described so far. We were able to confirm inactivation of CDKN2A in a large part (75%) of PCLBCL, LT patients, which was correlated with reduced survival (5-year DSS for patients with versus without inactivation of CDKN2A: 43% versus 70%, respectively). However, our results were not mutually exclusive, in the sense that the study group contained patients with inactivation of CDKN2A that had a good prognosis, but more importantly, it contained five patients (8% of the total study group) that did not show aberrations in CDKN2A but nonetheless died of their lymphoma. The latter patients run the risk of being undertreated when management would be solely based on CDKN2A status. So, while inactivation of this gene is associated with a worse prognosis, caution is warranted before these results are incorporated into clinical decision making. Regardless of these results, MLPA has proven to be a valuable technique providing new possibilities for molecular studies on larger patient groups of rare diseases, using the candidate gene approach.

In summary, the result of Chapters 2, 3 and 4 have not provided an independent prognostic marker for PCLBCL, LT which is useful at the time of diagnosis. It seems worthwhile to further investigate other possible prognostic markers for this rare group, by exploring newer genetic and epigenetic parameters. For instance, new promising research in the field of cancer prognostication is formed by studying microRNAs. It has been suggested that microRNA expression can distinguish between the germinal centre B-cell (GCB)-like and the activated B-cell (ABC)-like subtypes of DLBCL.[18] More recently it was shown that elevated levels of tumour-associated microRNAs can be detected in the serum of patients with DLBCL and that specific microRNAs may have prognostic relevance.[19] Since microRNAs proof to be relatively resistant to RNase degradation and can be successfully isolated from FFPE tissues[18], they might form an ideal target for studies in CBCL samples.

Analysis of microRNAs in patient serum may provide new, non-invasive possibilities for finding relevant prognostic factors.

Management and treatment of patients with a (primary) cutaneous B-cell lymphoma.

Staging

A diagnosis of primary cutaneous lymphoma can only be made after adequate staging investigations have been conducted. However, whether or not bone marrow biopsies should be performed routinely in patients with skin lesions that show histological features consistent with an indolent lymphoma has recently been debated.[12] Since there are no studies that have addressed this question for this particular group of lymphomas, in the study described in **Chapter 5** we evaluated the results of bone marrow examinations in a large group of patients who presented with skin tumours, histologically suggestive of an indolent B-cell lymphoma, either marginal zone lymphoma (MZL) or follicle centre lymphoma (FCL).

In the total group of 275 patients, 24 patients (9%) showed a positive bone marrow histology, while in 10 patients (4%; one MZL and nine FCL) this was the only evidence of extracutaneous disease. If bone marrow examination had not been performed, these 10 patients would have been wrongfully classified as CBCL. For patients with a histology of MZL the clinical consequences of this "misdiagnosis" are expected to be limited, since treatment of MZL with skin lesions and isolated bone marrow involvement will not be different from that of MZL presenting with only skin involvement (i.e. PCMZL). However, the nine patients with the histologic features of a FCL and isolated bone marrow involvement had a significantly worse prognosis as compared to genuine PCFCL (5-year DSS 63% versus 95% respectively), which may have therapeutic consequences. In summary, the results of the study showed that bone marrow investigation is an essential component of the staging procedure in patients with an FCL first presenting in the skin and that bone marrow examination appears to have limited value in patients with MZL presenting in the skin.

These conclusions are in line with other staging recommendations such as the most recent National Comprehensive Cancer Network (NCCN) clinical practice guidelines (version 2.2008). In these guidelines, bone marrow investigation is considered essential in indolent follicular lymphomas (FL), while in extranodal MZL (including PCMZL) it is only considered useful in selected cases. Interestingly, in previous versions of the NCCN guidelines (versions 2.2006, 1.2007 and 3.2007), also bone marrow examination in indolent FL was only considered useful in selected cases. In line with these previous NCCN guidelines, but not substantiated by any published data, some cutaneous lymphoma centres in the United States still argue that bone marrow examination in FCL patients should be considered optional and should only be performed in selected cases, e.g. patients with other positive staging assessments. Since survival of these patients is significantly different and

they may thus require a different management strategy, this outstanding question needs further clarification.

Treatment

In **Chapters 6 and 7** we evaluated the results of radiotherapy in the newly defined groups of CBCL and subsequently aimed to provide consensus recommendations for the management of these diseases.

Local radiotherapy (RT) is a well known and effective treatment modality in the field of CBCL. It is widely given to patients with indolent CBCL with curative intent. Since it was expected that a number of patients would be assigned to a different prognostic category, using the criteria of the new WHO-EORTC classification, we sought to evaluate the results of this treatment in the newly defined categories. The results, described in **Chapter 6**, show that the large majority of tumours is very sensitive to RT. All but two of 153 patients (99%) responded to initial RT with a complete remission (CR). The two patients that did not reach CR were both PCLBCL, LT patients. Relapse rates for PCMZL, PCFCL and PCLBCL, LT were 60%, 29% and 64% and the 5-year DSS was 95%, 97% and 59% respectively. PCFCL patients who presented with skin lesions on the leg(s) had a higher relapse rate (63%) and a much lower 5-year DSS (44%) as compared to PCFCL patients presenting with skin lesions at other sites (relapse rate 25% and 5-year DSS 99%).

In summary, the results of this large retrospective study indicate that RT is a safe and effective treatment for patients with PCMZL and PCFCL with solitary or localized skin lesions. Patients with multifocal skin disease showed a tendency towards higher relapse rate (PCMZL and PCFCL) and extracutaneous dissemination (PCFCL), suggesting that other treatment modalities might be considered in such patients. Moreover, for PCFCL patients presenting with skin lesions on the leg and PCLBCL, LT patients, RT should not be the first choice of treatment.

For the paper described in **Chapter 7,** we integrated the results of **Chapters 4, 5 and 6** and performed an extensive literature study. Together with the results of discussions among a multidisciplinary group of dermatologists, haematologists/oncologists and radiation oncologists , selected from the International Society of Cutaneous Lymphoma (ISCL) and the EORTC Cutaneous Lymphoma Group (EORTC-CLG), we were able to formulate consensus recommendations for the management of the three main types of CBCL, which are summarized in Table 4 of **Chapter 7**. Major limitations in reviewing the literature on the treatment of CBCL were that (1) there was a complete lack of systematic reviews and large (randomized) controlled trials, (2) information on relapse-free survival or progression –free survival was often not included and (3) in many studies follow-up was too short to draw conclusions on long-term efficacy. Moreover, most of the reported studies so far have been based on formerly used classification schemes or heterogeneous study groups of different types of CBCL. Despite these limitations, there was consensus among

the members of the multidisciplinary expert panel that these recommendations reflect the state of the art management as currently practised in major cutaneous lymphoma centres.

In brief, the main conclusions from **Chapter 7** are, that for both types of indolent CBCL with solitary or localized disease, non-aggressive therapies such as excision or local RT can be applied. In PCMZL patients with multifocal or generalized skin lesions, a wait-and-see policy seems justified. In contrast, patients with PCLBCL, LT follow a more aggressive clinical course and should be treated accordingly.

Regarding PCFCL patients with generalized skin lesions, optimal treatment remains to be determined. Currently employed management strategies vary from a wait-and-see policy to RT of all visible skin lesions, while multi-agent chemotherapy is only considered in exceptional cases. Apart from RT, favourable results have recently been described for both intralesional and intravenous administration of rituximab in the treatment of CBCL.[20-25] Rituximab is a chimeric (human-mouse) monoclonal antibody directed against the CD20 molecule present on the surface of all mature B-cells. Binding of rituximab to the B-cells expressing CD20, results in the elimination of all B-cells in the body. Systemic use of this agent has improved outcome rates in nodal FL and DLBCL significantly. [26-30] It seems interesting to explore the long-term efficacy of this agent in generalized PCFCL. Moreover, comparison between systemic and intralesional treatment deserves further investigation, since also in patients treated intralesionally a complete disappearance of B-cells in the peripheral blood has been noted, indicating a systemic effect.[20;31] Thus, intralesional rituximab might prove to be an equally effective, but much cheaper alternative for systemic rituximab in PCFCL patients with extensive skin lesions.

Besides its use in generalized PCFCL, the addition of rituximab to the standard chemotherapy regiments in PCLBCL, LT warrant future controlled trials in order to confirm recent promising findings.[3;32] Controlled multicentre studies are also required to assess the efficacy of several other new therapies, such as intralesional interferon alpha for indolent CBCL[33-36], yttrium-90 ibritumomab tiuxetan (Zevalin®) or 131I-tositumomab (Bexxar®) radioimmunotherapy[37], pegylated liposomal doxorubicin[38](plus rituximab) and gene therapy with adenovirus-mediated transfer of IFN-gamma.[39-41]

Concluding remarks and future perspectives

In summary, the WHO-EORTC proved clinically very relevant and delineates distinct disease entities with different prognoses. It is therefore fortunate that both PCFCL and PCLBCL, LT will be incorporated as new distinct disease entities into the updated WHO classification for tumours of hematopoietic and lymphoid tissues, which will be published in 2008. PCMZL is incorporated into the broad category of extranodal marginal zone lymphomas, which is unfortunate in view of the major pathogenetic differences that exist in terms of translocations and antigenic stimuli involved in the development of marginal zone lymphomas at different sites.[42-44] When PCMZL is no longer distinguished from other

extranodal marginal zone lymphomas this may hamper elucidation of the pathogenesis of genuine PCMZL.

The recognition of PCFCL and PCLBCL, LT as distinct disease entities in the WHO classification 2008 will definitely aid in the wider recognition of CBCL by hematopathologists and hematologists/oncologists worldwide. This will hopefully result in more uniform diagnosis and more appropriate management, leading to more homogeneous patient groups available for further studies.

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