Primary diffuse large B-cell lymphoma of bone

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Chapter 7

Summary, general discussion and future perspectives

Since Oberling first mentioned primary lymphoma of bone in 1928 as a distinct entity, knowledge on lymphoma in general has increased substantially. The improvements in diagnosis and treatment of primary diffuse large B lymphoma of bone in over eighty years has changed treatment from amputation of an extremity to combined chemo- and radiotherapy. The earlier treatment of amputation usually resulted in complete remission, but with a high treatment related morbidity.

Primary diffuse large B cell lymphoma of bone however has remained somewhat of an outcast in lymphoma literature and in clinical practice. The incidence is low, although with the establishment of immunohistochemical markers for monoclonal B-cells the entity has become easier to recognize. Currently there is the suggestion that the frequency of occurrence might have been underestimated in the past. While the diagnostic improvements have helped the patient, ironically it has had an adverse effect on research, since less tumor material is sent to referral hospitals or registries such as the Netherlands Committee on Bone Tumors for advice. In general hospitals extranodal lymphoma of any particular site is not always documented as such, which makes it more difficult to find adequate patient numbers for research purposes. Moreover, there are often technical difficulties in handling the osseous tumor material, which hampers scientific research as well. This most likely explains the few studies on primary diffuse large B cell lymphoma of bone in literature, with often small patient numbers and poorly compatible cohorts. By definition, primary lymphoma of bone excludes secondary bone localization of nodal lymphoma, but when a patient presents with stage IV primary lymphoma of bone at different skeletal sites, it can be difficult to exclude primary nodal disease. For this reason, stage IV patients are often excluded in primary lymphoma of bone studies, causing an incomplete representation of the clinical spectrum. Although clinical stage is defined as a negative prognostic factor, the stage four cases often do reach complete remission. It was therefore important to us to include all tumor stages in our study cohorts.

This thesis describes the first extensive multidisciplinary study on primary diffuse large B cell lymphoma of bone with large patient numbers, including our cohort of 60 cases in **chapter two**. When studied in large numbers, it is noticeable how homogeneous the clinico-pathological presentation of primary diffuse large B cell lymphoma of bone in fact is. The tumor presents in either the femur or the humerus in over 50% of the cases, the stage of the disease is most often stage I with pain usually as the presenting symptom. The clinical outcome following therapy including chemotherapy and irradiation is complete remission in most cases, with a low incidence of recurrent disease. Morphologically, the tumor most frequently consists of large centroblastic cells with either multilobated features or mono-/polymorphic features, although some cases with an immunoblastic tumor cell phenotype are also encountered. The treatment schedules varied in this cohort, from surgery and radiotherapy to CHOP multi-agent chemotherapy and radiotherapy. The 5 year overall survival in our series for the whole cohort was 61%. No significant difference was found between the different treatment schedules, as long as a combination of radiotherapy and chemotherapy was given. We demonstrated that the IPI risk factors age at presentation and stage of disease have a negative influence on prognosis

in primary lymphoma of bone. We did not find a significant impact on prognosis of tumor localization, which is opposite earlier reported findings. We found a trend towards worse survival for the immunoblastic tumor subtype, as it was called at that time, as compared to the centroblastic mono-/polymorphic or multilobated tumor subtype according to the updated Kiel classification.

Of note, as we describe in **chapter three**, the clinico-pathological homogeneity is in contradiction with the radiological characteristics of the disease. We discovered that the MRI image of primary diffuse large B cell lymphoma of bone can be rather deceptive. We studied the MRI characteristics of 29 bone lymphoma patients. We found that the MRI features are not uniform at all. The majority of the patients displayed a combination of definite cortical abnormalities and extension to the soft tissue, but up to 31% of the patients showed MRI features that looked radiologically non-aggressive or even benign. The study stresses the need for accurate core biopsy to establish a definite diagnosis of primary diffuse large B cell lymphoma of bone, following adequate local imaging including MRI.

We have used various techniques to identify parameters for risk stratification in primary diffuse large B cell lymphoma of bone patients. In **chapter four**, we determined the prognostic significance of several immunohistochemical markers: BCL-6, CD10, MUM1, BCL-2, p53, CD30 and CD44. We also investigated the possible germinal center derivation of primary diffuse large B cell lymphoma of bone. Applying the Hans' algorithm, which is an algorithm using immunohistochemical staining results for BCL-6, CD10 and MUM1 to determine the germinal center B-cell subtype of diffuse large B-cell lymphoma versus the non-germinal center B-cell subtype, we concluded that 19 out of a cohort of 36 cases displayed a germinal center-like phenotype. Eight of 36 cases demonstrated a non-germinal center-like phenotype, whereas nine of 36 cases were of indeterminate phenotype. No significant difference in survival was found between the different tumor phenotypes, nor for the tested immunohistochemical markers individually. We did find again a statistically significant influence on prognosis of the IPI risk factors age at presentation and stage of disease. This confirmed our findings from our earlier study (chapter two), although this might be partially explained by the fact that some patients were included in both study cohorts.

In **chapter five**, we described the first array-CGH study of primary diffuse large B cell lymphoma of bone, in which we investigated genomic alterations in nine such cases. We found several recurrent genomic aberrations, but none had statistically significant prognostic influence. The most frequent finding was five cases with gain of 1q (five out of nine cases) and 2p16.1 amplification (four out of nine cases). The amplificated region 2p16.1 encodes for the proto-oncogene REL, a transcription factor of the NF-κB family.

In **chapter six** we described the first study on NF-κB pathway activation in primary diffuse large B cell lymphoma of bone. This signaling pathway is known to have a role in tumorigenesis in lymphoma; recent research has focused on elucidating its working mechanisms through the classical pathway and the alternative pathway and on its possible role in targeted therapy. We investigated 50 cases for involvement of aberrant NF-κB activation by performing

immunohistochemical stainings for different NF-κB family members and evaluated its possible prognostic influence. In a minority (19%) of cases, we found substantial nuclear staining of p50, as an indication of aberrant activation of the classical pathway of NF-κB activation, while alternative activation did not appear to be significantly involved. The nuclear expression of p50 was not preferentially detected in non-germinal center or germinal center type cases, nor related to an inferior prognosis.

The knowledge of this rare type of extranodal non-Hodgkin lymphoma has increased by describing the complete clinicopathological spectrum of primary diffuse large B cell lymphoma of bone, including clinical characteristics, radiological aspects and molecular genetic factors. When we studied this disease in a large number of patients it became clear that primary diffuse large B cell lymphoma of bone is a homogeneous entity with a favorable outcome, contrary to what was earlier believed for extranodal lymphoma in general and primary diffuse large B cell lymphoma of bone in particular. We have demonstrated that primary diffuse large B cell lymphoma of bone is often of germinal center cell derivation, but the associated favorable outcome was also seen in cases with characteristics of the non-germinal center phenotype. Interestingly, in our first study we did find a worse outcome for the so-called immunoblastic tumor subtype, which largely corresponds to the non-germinal center phenotype. In later studies we could not confirm this trend. We could not identify any statistically significant molecular-biological prognostic parameters. We think this is explained partly by the favorable survival of our cohort, with very similar morphology, phenotype and clinical course for the majority of the cases. This positive and homogeneous clinical course results in few statistical events.

The pathogenesis of primary diffuse large B cell lymphoma of bone is unknown. Most cases probably arise de novo without a recognizable initial noxe; more specifically there is no relationship with a history of osteomyelitis or fracture. Of note, of all the prognostic parameters that we studied, the clinical parameters, especially the IPI risk factors age at presentation and stage of disease, have the strongest influence on prognosis.

We hope that this thesis on primary diffuse large B cell lymphoma of bone, a rare and fascinating subtype of extranodal lymphoma, will ultimately help to improve the treatment of diffuse large B cell lymphoma patients.