Pol J Pathol 2011; 4: 189-205

Prognostic and predictive markers in hematologic neoplasms. A review*

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*Presented at the 18th Congress of the Polish Society of Pathologists in Międzyzdroje, September 2011.

Introduction

Clinical behaviour, response to treatment and prognosis vary greatly not only between different hematopoietic neoplasms, but also within individual disorders. Establishing the proper diagnosis is just the first step in patients' management. The decision about the initiation of therapy and type of treatment is often dictated by many additional parameters, which may vary from standard laboratory values to complex prognostic indices to new sophisticated molecular tests. With the improved treatment modalities many prior parameters lose their prognostic or predictive values. The development of targeted therapy dictates the need for constant update of the prognostic parameters and better definition of predictive markers, more and more at the molecular level.

The prognostic and predictive parameters can be broadly divided into standard and novel markers. The former include clinical data, laboratory values, morphologic and immunophenotypic features and the latter include chromosomal and molecular markers. In the first part of this review, individual parameters will be briefly described and the second part will summarize the prognostic and predictive values in most common hematologic neoplasms.

Clinical and laboratory parameters

Age

Age is an important prognostic marker in both indolent and aggressive hematologic tumours. Generally, the older the patient the worse the prognosis. This can be explained, at least partially, by decreased tolerance of older patients to toxic therapies and by comorbidities common in this age group. Patients with a history of chemotherapy (e.g. for solid tumours) have an inferior response to treatment of newly diagnosed hematologic malignancy. Older patients have also a "better" chance of suffering from preceding disorders, which often influence treatment options and prognosis, e.g. pre-existing myelodysplasia in elderly patients with acute myeloid leukaemia (AML). Considering age and prognosis, one has to remember that patient's "fitness"/performance status and tolerance for treatment are more important than the actual age.

Patients with AML above 60 years of age have a poorer prognosis (older patients have a higher frequency of leukemic cells with multidrug resistance and unfavourable karyotypes). Paediatric patients with AML have slightly better results than adults with AML, and are currently reported to have disease-free survival (DFS) rates in the 40% to 66% range (the group below 2 years has a slightly worse prognosis). Among adult patients with acute lymphoblastic leukaemia (ALL), those above 35 years old have a worse prognosis and among children, the best prognosis is reported between the age of 1 and 10 (infants below 1 year old and children above 10 years old have a worse prognosis). As age increases, the percentage of ALL patients with unfavourable karyotype such as t(9;22) rises. The survival for ALL patients above 75 years old is 3% [1]. In Burkitt lymphoma (BL), age $>$ 10 years is a poor prognostic factor [2]. In splenic marginal zone lymphoma, age and haemoglobin levels, not chromosomal changes appear to influence the prognosis [3, 4]. In Hodgkin lymphoma (HL) and multiple myeloma (MM), patients above 45 and 65 years of age have poorer outcomes, respectively. The median overall survival among patients with MM aged $<$ 65 years is 42 months, and $>$ 65 years is 18 months [5].

Location

Among primary cutaneous lymphomas, those located in the head and neck area have better prognoses than those located in lower extremities (diffuse large B-cell lymphoma, leg type). Nodal follicular lymphoma

(FL) has a worse prognosis than primary FL of the gastrointestinal tract, but nodal diffuse large B-cell lymphoma (DLBCL) has a better prognosis than primary DLBCL of the testis or central nervous system (CNS). Extranodal marginal zone lymphoma (MZL; MALT type) have a more favourable prognosis than primary nodal MZL.

Stage/prognostic indices

Stage of the disease is one of the most important prognostic factors. It is often incorporated into prognostic indices, separate for specific disease, such as the International Prognostic Index (IPI) for lymphoma, Follicular Lymphoma IPI, International Prognostic Scoring System (IPSS) for myelodysplastic syndromes (MDS), Rai and Binet staging systems for chronic lymphocytic leukaemia (CLL) and modified Ann Arbor staging system for Hodgkin lymphoma.

IPI is widely used as a predictive model in DLBCL patients of all ages and stages. The IPI combines patient age with easily measured clinical parameters, which can serve as surrogate markers of tumour burden: performance status, stage, extranodal involvement, and serum LDH levels. The subdivision of patients according to the number of prognostic factors into low risk (none or one factor), low-intermediate (two factors), high-intermediate (3 factors), or high risk (four or five factors) with predicted 5-year overall survival values of 73%, 51%, 43%, and 26%, rapidly became the most widely used and accepted prognostic model for intermediate-grade lymphoma.

IPSS based on the percent of blasts in the BM ($< 5\%$, 5-10%, 11-19% and 20-30%), type of cytogenetic abnormalities [good (normal karyotype, -Y, del(5q) and del(20q); poor (≥ 3 abnormalities or chromosome 7 anomalies); intermediate (all other)] and number of cytopenias [defined as haemoglobin (Hgb) < 10 g/dl, neutrophils $< 1.8 \times 10^9$/l and platelets $< 100 \times 10^9$/l] is being used to predict survival and risk of progression into AML in MDS patients.

The prognosis of patients with mycosis fungoides (MF) is dependent on stage, and in particular the type and extent of skin lesions and the presence of extracutaneous disease [6, 7]. Patients with limited patch/plaque-stage MF have similar life expectancy to an age-, sex- and race-matched control population, whereas patients with cutaneous tumours or generalized erythroderma have a median survival of 3 and 4.5 years, respectively.

Serological markers

The levels of Hgb and several serum markers, such as β_2-microglobulin and lactate dehydrogenase (LDH) have been identified as prognostic markers in malignant lymphomas and plasma cell myeloma. Their levels correspond to tumour burden, Hgb being low and both β_2-microglobulin and LDH being high in the advanced disease.

Response to treatment

The response to treatment is one of the best prognostic parameters; its only disadvantage is the fact that it is not available at the time of diagnosis. AML patients with complete response (CR) after induction therapy have a much better prognosis than those without CR (even patients with partial CR, i.e. those with blasts below 5% and low blood CBC values are more likely to relapse than patients with CR). Most patients with AML do relapse, indicating that they have residual disease even in CR. Thus, evaluation of minimal residual disease (MRD) has become an important factor in management of patients with acute leukaemia. The MRD in AML can be assessed by flow cytometry immunophenotyping (identification of aberrant phenotype of blasts; leukaemia-associated phenotype or LAP), chromosomal (metaphase cytogenetic or FISH) and molecular tests (e.g. *RUNX1-CBFA2T1*, *CBFB-MYH11*, or *PML-RARA*).

In ALL patients, treatment response is being increasingly evaluated with MRD assays, either by PCR (clonal rearrangement of Ig and TCR) or flow cytometry. Both methods allow for detection of one ALL cell among 10,000 to 100,000 normal cells (MRD positivity being defined by the presence of 0.01% or more ALL cells). The risk of relapse is proportional to the level of MRD, especially if measured during or at the end of remission-induction therapy. Patients who attain MRD $< 0.01\%$ early during therapy have high odds to remain in continuous CR with contemporary post-remission therapy.

Morphological parameters

Grade

The histological grade correlates with a prognosis in FL, with grade 1-2 cases being indolent and not usually curable, except for the infrequent patients with localized disease. The majority of published studies show a significantly more aggressive clinical course for FL classified as grade 3 (or composed of large cells). Cases of grade 3 FL with diffuse areas $> 25\%$ (now recognized as areas of DLBCL) have a worse prognosis than purely follicular cases [8, 9].

In plasma cell myeloma, high grade tumours with anaplastic or plasmablastic features are associated with a poor prognosis. Although mantle cell lymphomas (MCL) are not graded, blastoid and pleomorphic variants are associated with an aggressive clinical course. An increased number of mitoses in MCL is associated with more aggressive clinical behaviour; high mitotic rate ($>$ 10-37.5/15 hpf, $> 50/mm^2$) is the most con-

sistently reported adverse histopathological prognostic parameter [10, 11].

High grade and clinically aggressive B-cell lymphomas include Burkitt lymphoma, B-cell lymphoma, unclassifiable with features intermediate between BL and DLBCL, and B-cell lymphomas with plasmablastic features.

Transformation

Transformation of CLL to more aggressive disease occurs in 5-10% of patients. Clinical and laboratory features observed in patients with transformation include generalized adenopathy, systemic symptoms (fever, weight loss, and worsened performance status), cytopenia (anaemia and/or thrombocytopenia), increased LDH level, hypercalcemia, and monoclonal gammopathy [12-16]. Morphological progression of CLL/SLL is represented by prolymphocytic transformation [17, 18], paraimmunoblastic variant [19], HL [20-24], DLBCL [13, 15, 25-28], and rarely precursor B-lymphoblastic lymphoma/ leukaemia (B-ALL) [29]. CLL can also get complicated by the development of the neoplasm that morphologically and immunophenotypically resembles classical HL and may be considered a Hodgkin variant of Richter's syndrome (Hodgkin transformation of CLL/SLL) [15, 16, 30, 31]. In a series reported by Tsimberidou *et al.*, 0.4% patients with CLL developed Hodgkin transformation with the median time from CLL diagnosis to transformation of 4.6 years [30]. EBV virus plays an important role in the pathogenesis of Hodgkin transformation of CLL [30, 32]. Two types of Hodgkin transformation of CLL/SLL have been described, one with large tumour cells (Reed-Sternberg cells and their variants) in the background of CLL cells (type 1) and the other with polymorphous lymphohistiocytic infiltrate separate from CLL cells (type 2) [21-23, 33-35]. It is likely that type 1 transformation represents histologic progression of the underlying CLL, and type 2 transformation represents 2 different, albeit related diseases. A clonal relationship between CLL cells and Hodgkin/Reed-Sternberg cells was demonstrated in 3 of 4 patients who had Hodgkin transformation of CLL by using single-cell PCR analysis and DNA sequencing [20]. In contrast to a generally favourable outcome of patients with *de novo* HL, only 34-47% of patients with Richter's syndrome respond to multiagent chemotherapy and the median overall survival is 8 months [16, 30]. Even patients responding to multiagent chemotherapy, such as ABVD eventually develop recurrent disease after a short period of time.

Follicular lymphoma often undergoes transformation to more aggressive or high grade lymphomas. Morphologically, progression of FL is characterized by an increased number of centroblasts (progression from FL grade 1 into FL grade 2 and/or 3), diffuse large B-cell lymphoma (DLBCL) [36-39], or less often blastic transformation in the form of BL or precursor B-lymphoblastic lymphoma/leukaemia with or without TdT expression [40-43].

In nodal MZL, presence of sheets of large cells and/or more than 50% of large cells should raise the possibility of progression into DLBCL (DLBCL and nodal MZL). Kojima *et al.* recently reported a series of 65 cases of nodal MZL, of which 20 cases had more than 50% of large cells or sheets of large cells and were classified as nodal MZL and DLBCL [44]. Those cases had a significantly worse outcome. The overall criteria for progression of nodal MZL into DLBCL are not well established, and there is no cutoff for proliferation (Ki67) to aid in this distinction.

Mycosis fungoides is usually classified as transformed if biopsy shows large cells (≥ 4 times the size of a small lymphocyte) in more than 25% of the infiltrate or if they formed microscopic nodules [45-47]. Transformation has been associated with an expression of CD30 in approximately 40% of cases [48]. The transformation is more common in patients with tumours and with a more advanced clinical stage of the disease; however a small number of patients can be histologically identified before clinical progression. Some studies showed the presence of usually small, but variable numbers of $CD30^+$ cells in non-transformed MF [49]. The increased number of $CD30^+$ cells in the epidermis is typical of pagetoid reticulosis. The survival in patients with transformed cutaneous lymphoma is significantly shorter than in patients without transformation; survival after diagnosis of transformation is short [45, 46, 50]. Patients with transformation have a relatively poor survival, especially if transformation occurs early (within 2 years) in the course of disease or if they are at a higher stage. Patients with extracutaneous transformation had a shorter median survival after transformation than those with transformation limited to skin; extracutaneous transformation apparently indicates a poorer prognosis than cutaneous transformation [46]. In univariate analysis by Vergier *et al.* only extracutaneous progression was associated with a worse prognosis (5-year actuarial survival: 7.8% vs. 32%) [47].

A transformation of essential thrombocythemia (ET) to AML, MDS or myelofibrosis is a relatively rare event and occurs in 1-5% of all patients [51-57]. The risk of leukemic or any myeloid disease transformation is low in the first 10 years (1.4% and 9.1%, respectively) but increases substantially in the second (8.1% and 28.3%, respectively) and third (24.0% and 58.5%, respectively) decades of the disease [51]. Cytogenetic changes that may be associated with the transformation to AML are common and include: t(2;3), der(1;7)(q10;p10), t(2;17), del(17p), trisomy 8, trisomy 21 and chromosome 7 and 13q abnormalities [58-64].

Mutations of the *TP53* gene are commonly detected in the blast phase of ET (chronic phase usually lacks the alterations involving the *TP53*, *NRAS*, *KRAS*, and *MDM2* genes) [65].

In PV, the risk of disease transformation into marrow fibrosis or myelodysplasia/acute myeloid leukaemia (MDS/AML) increases over time and ranges from 5% to 15% after 10 years of disease [66-70]. ET and PV patients who transformed to acute leukaemia had all been previously exposed to cytotoxic therapy; there were no ET or PV patients in the study who transformed to acute leukaemia exposed solely to anagrelide [71]. Exposure to P32, busulphan, and pipobroman, but not to hydroxyurea, has an independent role in producing an excess risk for progression to AML/MDS compared with treatment with phlebotomy or interferon [72]. Most of the patients die from thrombosis or haemorrhage, but up to 20% succumb to MDS or AML.

MDS can be divided into three risk groups based on the duration of survival and risk of progression into AML: low, intermediate and high risk. Refractory cytopenia with unilineage dysplasia (RCUD) and refractory anaemia with ringed sideroblasts (RARS) belong to low risk. Refractory cytopenia with multilineage dysplasia (RCMD) and refractory anaemia with excess blasts-1 (RAEB-1) belong to intermediate risk and RAEB-2 comprises a high risk group. MDS with del(5q) has the rate of progression into AML from 17% to 25% (with or without lenalidomide treatment) [73, 74]. In low risk MDS, the median survival is approximately 66 months, and for patients above 70 years old is similar to non-affected population. The rate of progression into AML is approximately 2% at 5 years. For RCMD, the overall survival is approximately 30 months and the risk of progression into AML is 10% at 2 years. Approximately 25% of patients with RAEB-1 and 33% of patients with RAEB-2 progress into AML and the remaining patients succumb to complications of BM failure. The median survival is approximately 16 months for RAEB-1 and 9 months for RAEB-2.

Background elements

Klapper *et al.* reported the association between the presence of sclerosis within the follicular lymphoma (FL) and poor overall survival (independently from the grade or FLIPI) [75]. In FL, an increased number of T-cells is associated with a better prognosis, whereas increased monocytes/macrophages has a poor prognosis [76]. A high number of epithelioid histiocytes may be associated with a slightly better prognosis in diffuse large B-cell lymphoma and T-cell lymphomas. High content of histiocytes (expressing either CD68 or 163; > 20%) is significantly correlated with a poor prognosis in Hodgkin lymphoma (HL) patients [77-79]. A higher ratio of CD163-positive to CD68-positive histiocytes (macrophages) in angioimmunoblastic T-cell lymphoma (AITL) correlates with worse overall survival.

BM involvement

The influence of bone marrow biopsy histology on the prognosis and management of FL remains controversial. Extensive bone marrow involvement, however, is a significant predictor of poor survival of patients with grade 1 and 2 follicular lymphoma [80].

In CLL, the pattern and extent of bone marrow involvement (diffuse vs. non-diffuse) have prognostic implications [81], although it is not independent of the staging and the latter appears to be a better predictive factor of survival probability in CLL patients [82]. Nodular and interstitial patterns are associated with early disease and better prognosis, while a diffuse marrow infiltrate is associated with a worse prognosis and advanced disease, although not all studies support this [81, 83-85]. Patients with > 70% marrow involvement before therapy have a significantly shorter time to progression [86]. The extent of bone marrow involvement after chemotherapy does not correlate with the interval between the treatment and relapse [86]. A comparison between the infiltration pattern and *IGVH* mutation status revealed that the samples with a diffuse pattern were *IGVH*-unmutated and the non-diffuse CLL samples were *IGVH*-mutated [87]. The same report showed that the expression of ZAP-70 was related to the infiltration type: in all samples with a diffuse infiltration pattern, the leukemic cells showed ZAP-70 staining, whereas leukemic cells in a nodular infiltration pattern were negative (the mixed-pattern type showed a variable ZAP-70 expression) [87].

Marginal zone lymphoma (MZL) with both bone marrow and nodal involvement are associated with shorter overall survival [88]. Extensive bone marrow involvement by multiple myeloma (50%) is associated with a worse prognosis.

The clinical stage of HL (Ann Arbor classification updated at Cotswold) determines the treatment regimen and the prognosis. Tumour burden is the most important prognostic factor in HL [89, 90]. In a series reported by Specht *et al.*, both lymphocytopenia and bone marrow involvement had an independent prognostic significance and these two factors emerged as the most important prognostic factors in disseminated Hodgkin disease, and both appeared to be related to the patient's total tumour burden [89, 90].

Blood involvement

Plasma cell leukaemia is defined by circulating clonal plasma cells ($> 2 \times 10^9$/l or ≥ 20% of leukocyte differential count). Detection of myeloma cells in blood (cir-

culating myeloma cells) by flow cytometry in patients with plasma cell myeloma indicates active disease. Of 246 patients undergoing autologous stem-cell transplantation (ASCT) analyzed by Dingli *et al.*, 95 had myeloma cells in blood [91]. Complete response (CR) rates after transplantation were 32% and 36% for patients with and without circulating myeloma cells, respectively, and an overall survival was 33.2 and 58.6 months, respectively. On multivariate analysis, circulating myeloma cells remained independent of cytogenetics and disease status at the time of transplantation [91]. Taking into account both cytogenetics and presence of myeloma cells in blood, patients with neither, one, or both parameters had a median overall survival of 55, 48, and 21.5 months and a median time to progression of 22, 15.4, and 6.5 months, respectively [91].

Circulating blasts (above normal control, i.e. ≥ 1%) in patients with MDS is associated with lower survival and higher proportion of progression into AML, in both low and high grade MDS [92, 93].

Basophils

In chronic myeloid leukaemia (CML), presence of less than 5% of basophils is one of the criterion for complete hematologic response. The combination of spleen size and number of basophils is a predictor for complete cytogenetic response in CML treated with Gleevec. In MDS, bone marrow basophilia (> 1%) is associated with a poorer prognosis [94].

In situ lymphoma

Lymphomas *in situ* include follicular lymphoma (FL) *in situ*, mantle cell lymphoma (MCL) *in situ*, γ-herpesvirus associated B-cell lymphoproliferative disorders, plasmablastic microlymphoma and germinotropic lymphoproliferative disorder. FL *in situ* (or "intrafollicular neoplasia"; WHO classification) is often discovered incidentally and comprises isolated scattered follicles colonized by monoclonal t(14;18)+ B-cells over-expressing BCL2 and CD10 within an otherwise uninvolved lymph node [95-97]. Further evaluation reveals evidence of FL at another site in about half of the patients, but the risk of progression to clinically significant lymphoma is not yet fully known for these focal lesions. FL *in situ* without overt lymphoma has an indolent clinical course and requires follow-up without treatment. FL *in situ* with synchronous FL is treated as typical (overt) FL. FL *in situ* may precede FL or other lymphomas (e.g. DLBCL, MCL, SMZL and classical HL) by years.

Phenotypic markers

ALK 1

The prognosis of ALK^+ anaplastic large cell lymphoma (ALCL) is favourable, except for cases with blood involvement, which are aggressive. Patients with ALK^- ALCL have a poor prognosis, similar to only slightly better than peripheral T-cell lymphoma (PTCL). Supervised analysis with microarray gene-expression profiling has shown that ALK^+ ALCL and ALK^- ALCL have different gene-expression profiles, further confirming that they are different entities [98].

Large B-cell lymphoma expressing the ALK kinase is a rare variant of large B-cell lymphoma with immunoblastic or plasmablastic morphology and cytoplasmic expression of ALK [99-101]. In most cases it lacks t(2;5) [*ALK-NPM*] translocation characteristic of ALCL. Most patients present with advanced disease. The clinical course is aggressive despite polychemotherapy [101].

CD5

De novo $CD5^+$ DLBCL is known to have phenotypically and genotypically different characteristics than $CD5^-$ DLBCL. $CD5^+$ DLBCL is characterized by a survival curve that is significantly inferior to that for patients without CD5 expression [102-105]. $CD5^+$ DLBCL shows a higher incidence of bone marrow and spleen involvement [104-106]. Among various phenotypic markers, optimal cut points predicting overall survival of patients treated in the rituximab era could only be determined for CD5 and Ki67, whereas such cut points for BCL2, BCL6, HLA-DR, and MUM1 could only be defined in patients not receiving rituximab [107]. Among 347 patients with DLBCL treated with R-CHOP, Salles *et al.* demonstrated that only CD5 retained a prognostic significance (CD10, BCL6, and MUM1 expression did not provide prognostic information in the rituximab era) [107].

CD13/CD33

A subset of B-lymphoblastic leukaemias (B-ALL) expresses CD33 or less often CD13; co-expression of both antigens is rare [108-111]. The expression of myeloid antigens is associated with a worse prognosis [109]. Pan-myeloid antigen expression may be associated with Philadelphia chromosome, balanced t(12;17) or extra *ETV6/RUNX1*$^+$ fusion signals [108, 112]. Mylotarg (gemtuzumab; anti-CD33 antibody) is no longer available for treatment of patients with AML.

CD19

The expression of CD19 by multiple myeloma cells is associated with a worse prognosis [113].

CD20

The expression of CD20 predicts the response to monoclonal therapy with anti-CD20 antibody, rituximab (Rituxan).

CD30

Peripheral T-cell lymphoma, not otherwise specified (PTCL) with CD30 expression is associated with a worse prognosis. Transformation of mycosis fungoides (MF) into more aggressive large T-cell lymphoma has been associated with an expression of CD30 in approximately 40% of cases [48]. New promising therapies targeting CD30 are being developed.

CD38

Among B-cells, CD38 is expressed at high levels by B lineage progenitors in BM and by B-lymphocytes in germinal centre, in activated tonsils, and by terminally differentiated plasma cells. In CLL, CD38 expression identifies two subgroups of patients with different clinical outcomes, including overall survival, time to first treatment, number of leukemic cells with abnormal morphology, extent of adenopathy, absolute lymphocyte count, and LDH and β_2-microglobulin levels. In the majority of studies, the threshold is considered as ≥ 30# $CD38^+$ clonal cells [114-119]. At the molecular level, the $CD38^+$ and $CD38^-$ clones differ in the level of expression of activation markers CD69 and HLA-DR, Ki67 fraction, ZAP70 expression, IGVH mutational status, telomere lengths and telomerase levels and in high-risk genomic abnormalities [114, 120-122].

CD52

Tumour expressing CD52 responds to treatment with humanized anti-CD52 antibody alemtuzumab (Campath). In T-cell prolymphocytic leukaemia (T-PLL), the overall response rate was reported at 76% with 60% complete remission (CR) and 16% partial remission [123]. Patients with serous effusion or hepatic or CNS involvement were more resistant to alemtuzumab treatment. Treatment with alemtuzumab is a reasonable option for patients with progressive and symptomatic CLL that is refractory to both alkylator-based and fludarabine-based regimens.

CD56

CD56 is positive in 11% to 20% of acute promyelocytic leukaemia (APL) [124, 125]. It is more common in the hypogranular variant. $CD56^+$ APL is associated with high white blood cell counts; low albumin levels; BCR3 isoform; and the co-expression of CD2, CD34, CD7, HLA-DR, CD15, and CD117 antigens [124]. The expression of CD56 antigen in APL blasts has been associated with short remission duration and extramedullary relapse [124, 126-128]. In a series reported by Montesinos *et al.* for $CD56^+$ APL, the 5-year relapse rate was 22%, compared with a 10% relapse rate for $CD56^-$ APL, and $CD56^+$ APL also showed a greater risk of extramedullary relapse [124].

In anaplastic large cell lymphoma, CD56+ cases show a higher incidence of BM involvement and a significantly poorer prognosis than $CD56^-$ tumours (in both ALK^+ and ALK^- groups) [129].

Ki67 (MIB1)

In mantle cell lymphoma (MCL) a high proportion of Ki67 positive cells (> 40-60%) is an adverse prognostic indicator [10, 130]. The 3 MCL groups with different Ki67 index of less than 10%, 10% to less than 30%, and 30% or more show significantly different overall survival in patients treated with CHOP as well as in patients treated with CHOP in combination with anti-CD20 therapy (R-CHOP) [131, 132]. High Ki67 index is associated with a poor prognosis in peripheral T-cell lymphomas.

The Ki67 staining is often high in DLBCL. In a series published by Salles *et al.*, Ki67 of 0-25% of tumour cells was noted in 3%, 26-50% in 13%, 51-75% in 30% and above 75% in the remaining 54% of cases. Ki67 overexpression (> 75% of tumour cells) appears to confer a poor prognosis in DLBCL patients treated with R-CHOP, which may be of importance for patients with intermediate IPI scores [107].

ZAP-70

ZAP-70 (70-kD zeta-associated protein), a tyrosine kinase, is a key signalling molecule for T-lymphocytes and natural killer cells. Although it does not normally function in B-cells, it is anomalously expressed in CLL cells with unmutated *IGVH* genes and may enhance the signalling process when the B-cell receptor is engaged [133-139]. The expression of ZAP-70 can be effectively measured by flow cytometry and immunohistochemistry. Aggressive CLL is characterized by unmutated *IGVH* and expression of ZAP-70, whereas in indolent disease, has mutated *IGVH* and lacks expression of ZAP-70. Recent comparison between a flow cytometry assay for ZAP-70 and the mutational status of *IGVH* genes showed a strong association between the expression of ZAP-70 in CLL cells (ZAP-70 level above a defined threshold of 20%) and unmutated *IGVH* genes. However, ~20% CLLs show discordant results (mutated *IGVH* and positive ZAP-70 or unmutated *IGVH* and negative ZAP-70).

Chromosomal and molecular markers

Chromosomal and molecular markers are described with specific neoplasms (see below).

The overview of prognostic markers in some of the most common malignancies

Chronic lymphocytic leukaemia

The clinical course of CLL is variable and depends on a number of factors including age, gender, Binet/Rai stage, performance status, laboratory parameters (lymphocyte count, thymidine kinase, soluble CD23, β_2-microglobulin, LDH), atypical cytologic features, pattern and extent of bone marrow infiltration, 17p deletion, *TP53* mutation/loss, deletion of chromosome 11q23, *ATM* status, *IGVH* mutational status and CD38 and ZAP-70 expression [117, 118, 140, 141]. Although CLL remains incurable with standard treatments with resistance to therapy developing in the majority of patients, the significant improvements in remission rates are achieved with newer therapeutic approaches, such as the purine analogues (e.g. fludarabine) in combination with monoclonal antibodies and stem cell transplantation [142-144]. The combination of rituximab and fludarabine or fludarabine-containing regimens has yielded overall response rates of 95%, with complete response rates up to 66% in previously untreated CLL patients [145]. Some patients have aggressive disease and require therapy within a relatively short time after diagnosis, whereas others have indolent, asymptomatic disease and are not likely to benefit from palliative chemotherapy. The median survival of patients with CLL is 10 years. Patients with poor prognostic factors have median survival of approximately 3 years.

The expression of CD38 (> 30%) or ZAP-70 (> 20%) is associated with worse prognosis and often correlates with unmutated *IGVH* status. Nuclear p53 expression by immunohistochemistry is strongly associated with hemizygous *TP53* deletion [146]. Pattern and extent of bone marrow involvement (diffuse vs. non-diffuse) have prognostic implications [81], although it is not independent of the staging and the latter appears to be a better predictive factor of survival probability in CLL patients [82]. Nodular and interstitial patterns are associated with early disease and better prognosis, while a diffuse marrow infiltrate is associated with a worse prognosis and advanced disease, although not all studies support this [81, 83-85]. Patients with > 70% marrow involvement before therapy have a significantly shorter time to progression [86].

The extent of bone marrow involvement after chemotherapy does not correlate with the interval between the treatment and relapse [86]. A comparison between the infiltration pattern and *IGVH* mutation status revealed that the samples with a diffuse pattern were *IGVH*-unmutated and non-diffuse CLL samples were *IGVH*-mutated [87]. The same report showed that the expression of ZAP-70 was related to the infiltration type: in all samples with a diffuse infiltration pattern, the leukemic cells showed ZAP-70 staining, whereas leukemic cells in a nodular infiltration pattern were negative (the mixed-pattern type showed a variable ZAP-70 expression) [87].

Patients with normal karyotype or deletion of 13q14 as the sole genetic abnormality have a better prognosis than those with a complex karyotype or deletion of 11q23 or 17p13. Response rate to chemotherapy is significantly higher in patients with normal karyotypes than in those with abnormal karyotypes, especially with complex changes. In series by Dohner *et al.*, the median survival times for patients with 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as the sole abnormality were 32, 79, 114, 111, and 133 months, respectively [147]. Patients with 17p deletions had the shortest median treatment-free interval (9 months), and those with 13q deletions had the longest (92 months) [147]. The response to rituximab was noted to vary by cytogenetic group: del(17)(p13.1), 0%; del(11)(q22.3), 66%; del(13)(q14.3), 86%; and +12, 25% [148]. Alemtuzumab (Campath) may be an effective initial therapy for patients with *p53* mutations or del(17q)(p13.1) or both, as opposed to fludarabine, chlorambucil, or rituximab [149].

Diffuse large B-cell lymphoma

In DLBCL, poor prognostic indicators include age > 60 years old, high serum LDH and/or β_2-macroglobulin levels, poor performance status, advanced stage, extranodal involvement (≥2 sites), immunoblastic morphology, 17p loss, p53 expression/TP53 mutation, 18q amplification and CD5 expression. The International Prognostic Index (IPI; see above) subdivides patients according to the number of prognostic factors into low risk (none or one factor), low-intermediate (two factors), high-intermediate (3 factors), or high risk (four or five factors) with predicted 5-year overall survival values of 73%, 51%, 43%, and 26%, respectively.

Evaluation of DLBCL gene expression profiles by cDNA microarray techniques and oligonucleotide microarrays has identified molecularly distinct forms of DLBCL: germinal centre (GC) B-cell-like DLBCL (GCB), characterized by the expression of genes normally expressed by GC B-cells, non-germinal centre like DLBCL consisting of activated B-cells (ABC-like DLBCL) and "type 3" [150, 151]. The activated B-cell-like DLBCL (ABC) is characterized by the expression of genes involved in activation of B-cells as well as some genes normally expressed by plasma cells, thus suggesting their post-GC origin [151, 152]. Type 3 is a heterogeneous DLBCL subtype that does not express high levels of either the GC or ABC set of genes. The GCB group is characterized by a better prognosis in comparison with ABC or type 3 groups. The GCB group shows t(14;18) translocation, CD10 expression, c-*REL*

amplification and evidence of ongoing somatic hypermutation in the immunoglobulin variable heavy genes (*IGVH*) and is characterized by a better overall survival [150, 153, 154]. GCB and ABC subtypes are associated with different prognosis (76% vs. 31% for 5-year progression free survival and 80% vs. 45% for overall survival). Bortezomib enhances the activity of chemotherapy in ABC, but not GCB DLBCL.

In patients treated with R-CHOP, among various phenotypic markers only CD5 retains a prognostic significance; none of the immunostaining profiles based on the expression of CD10, BCL2, BCL6 and MUM1 retains the prognostic impact of the GCB versus non-GCB profiles [155].

Patients with bone marrow (BM) involvement that consists of ≥ 50% of large cells or BM involvement of ≥ 70% have a poorer overall survival [156, 157]. Nodal and extranodal DLBCL differ in clinical presentation, behaviour and prognosis [158]. For stage I patients, extranodal DLBCL was independently associated with poor survival. In contrast, among stage IV patients those with extranodal DLBCL survived longer [158].

The Ki67 staining is often high in DLBCL. Ki67 overexpression (> 75% of tumour cells) appears to confer a poor prognosis in DLBCL patients treated with R-CHOP, which may be of importance for patients with intermediate IPI scores [107]. Patients with tumour cells that overexpressed BCL2 (> 75%) presented significantly more frequently with a higher IPI, whereas those that were either CD10 or HLA-DR positive had a significantly lower IPI at diagnosis [107]. Salles *et al.* showed that low-risk patients were separated by BCL2 and the low-intermediate/high-intermediate patients were separated by Ki67. The most favourable group comprised patients with a low IPI and BCL2 of ≤ 75% and had an expected 4-year overall survival of 94%. Patients with low IPI and BCL2 > 75% as well as low-intermediate/high-intermediate IPI with Ki67 positivity in ≤ 75% of cells had 4-year overall survival of 81%. Patients with low-intermediate/high-intermediate IPI and Ki67 > 75% had 4-year survival at 62%, and patients with a high IPI had 4-year overall survival of 45% [107].

Approximately 5-17% of diffuse large B-cell lymphomas (DLBCLs) harbour an *MYC* oncogene rearrangement [159]. The 5-year progression-free survival (66% vs. 31%) and overall survival (72% vs. 33%) is inferior in cases of DLBCL treated with R-CHOP that harboured an *MYC* rearrangement [160]. Treatment regimens similar to those used in Burkitt lymphoma may be more appropriate in this patient population.

Follicular lymphoma

Follicular lymphoma is a heterogeneous group of tumours with a variable course, but the majority of cases have an indolent and slowly progressive clinical course with relatively long median survival, good response to initial treatment and a continuous pattern of relapses, sometimes followed by histologic transformation into high grade lymphoma [161-167]. Prognosis is closely related to the extent of the disease at the time of diagnosis. The International Prognostic Index for FL (FLIPI) is a strong predictor of outcome [167]. Approximately 65% of patients are in stage III or IV at the time of diagnosis [39].

Adverse prognostic factors in FL include age >60 years, Ann Arbor stage III-IV, haemoglobin level < 120 g/l, B symptoms, hepatosplenomegaly, bulky disease, number of nodal areas > 4, high number of extranodal involvement sites, high serum β_2-microglobulin level, poor performance status, high erythrocyte sedimentation rate, and high serum LDH level [167-172]. The peripheral blood absolute lymphocyte count (ALC) at the time of diagnosis of FL was identified as a predictor for overall survival: ALC ≥ 1.0×10^9/l predicted a longer overall survival versus ALC < 1.0×10^9/l (175 vs. 73 months, respectively) [173].

Histological grade correlates with prognosis in FL, with grade 1-2 cases being indolent and not usually curable, except for the infrequent localized cases. The majority of published studies show a significantly more aggressive clinical course for FL classified as grade 3 or large cell. Klapper *et al.* reported the association between the presence of sclerosis within the lymphoma and poor overall survival (independently from grade or FLIPI) [75].

The presence of more than 6 chromosomal breaks and a complex karyotype has been shown to be associated with a poor outcome; in addition, del 6q23-26, del17p and mutations in TP53 as well as –1p, –12, +18p, +Xp confer a worse prognosis and a shorter time to transformation [174, 175].

The survival rate is higher in extranodal FL than in nodal FL. Patients with primary cutaneous follicular lymphoma have a more favourable long-term prognosis than those with equivalent nodal disease [176]. Follicular lymphomas in non-cutaneous extranodal sites have similarly favourable outcome [177]. In follicular lymphoma of the gastrointestinal tract, the estimated 5-year disease-free survival is 62%, and the median disease-free survival is 69 months [178].

The paediatric variant of FL usually presents with localized disease and is of high histological grade. These lymphomas lack BCL2/IGH translocation and often do not express BCL2 protein. They may present at nodal or extranodal sites (testis, GI tract, Waldeyer's ring) [179]. Paediatric FLs have a good prognosis.

Mantle cell lymphoma

The clinical behaviour of mantle cell lymphoma (MCL) is aggressive with a median overall survival of around 3-4 years. The median survival is 32 to 48 months [180, 181] and 5-year survival is less than 10% [182]. In patients younger than 65 years, a dose-in-

tensive consolidation comprising high-dose radiochemotherapy, immunochemotherapy, and autologous stem cell transplantation after a CHOP-like induction results in an improved progression-free survival [183, 184]. However, relapses are still observed at a high frequency. The addition of rituximab to transplantation protocols appears to be a very promising strategy for patients with relapsed MCL after autologous stem cell transplantation [185, 186].

Mantle cell lymphoma appears to be largely resistant to complete eradication by conventional chemotherapy [187]. Majority of patients have positive MRD (*BCL1/IGH*-PCR) in bone marrow irrespective of histological involvement [188]. New treatment regimen with rituximab and combination chemotherapy may transiently clear blood or bone marrow of detectable tumour cells, but molecular remission does not translate into prolonged progression-free survival (16.5 months in MRD^+ vs. 18.8 months in MRD^-) [189]. Brugger *et al.* showed that one single course of rituximab consolidation given after autologous stem cell transplantation may help to eliminate MRD and may translate into improved event-free survival in both FL and MCL patients [186].

Parameters associated with a poor prognosis or shorter survival include poor performance status, splenomegaly, B-symptoms, leukocyte count $> 10 \times \times 10^9$/l, high LDH level, blastic variant, and high/intermediate or high risk IPI [181]. Stage IV, high/intermediate or high risk IPI, and increased LDH level are associated with a lower response rate to chemotherapy [181]. However, the value of IPI in MCL is not well established, partly because most of the patients are within high-risk categories. In a series by Raty *et al.*, high Ki67 expression, Ann Arbor stage III-IV, and age over 60 years had independent influence on survival in a multivariate analysis, whereas serum LDH, the number of extranodal sites, and performance status did not [190]. According to the MCL international prognostic index (MIPI), patients can be classified into low risk (44% of patients, median OS not reached), intermediate risk (35%, 51 months), and high risk groups (21%, 29 months), based on the 4 independent prognostic factors: age, performance status, lactate dehydrogenase (LDH), and leukocyte count [191].

The most consistently reported adverse histopathological prognostic parameter is a high mitotic rate ($>$ 10-37.5/15 hpf, $> 50/mm^2$) [10, 11]. A high proportion of Ki67 positive cells ($>$ 40-60%) is also an adverse prognostic indicator [10, 130].

Multiple myeloma

Clinical outcomes of myeloma are highly heterogeneous and range from indolent (smouldering) to aggressive disseminated disease with leukemic blood involvement with survival ranging from a few days to more than 10 years [192, 193]. The overall survivals at 1, 2, and 5 years for myeloma patients are 72%, 55% and 22%, respectively [194]. Poor risk factors include renal insufficiency, elevated serum beta-2-microglobulin, low serum albumin, elevated LDH, high C-reactive protein, increased plasma cell proliferative activity, high degree of BM replacement, plasmablastic morphology and genetic changes. Patients with abnormalities by conventional cytogenetics have significantly shorter median survival than those without them [116, 195-200]. The most important independent negative prognostic indicators include t(4;14) and the *MAF* translocations t(14;16) and t(14;20), deletion of 17p/*TP53* and increased serum β_2-microglobulin.

Chromosomal aberrations are frequently observed in myeloma patients. The del(13), t(11;14), t(4;14), hyperdiploidy, c-*MYC* translocations, and del(17p) are present in 48%, 21%, 14%, 39%, 13%, and 11% of the patients, respectively [195]. In univariate statistical analyses del(13), t(4;14), non-hyperdiploidy, and del(17p) negatively impact both the event-free survival and the overall survival, whereas t(11;14) and c-*MYC* translocations do not influence the prognosis [195]. High-risk disease is associated with t(4;14), t(14;16), t(14;20), deletion of 17q13, aneuploidy or deletion of chromosome 13 by conventional cytogenetics, or plasma cell labelling index > 3.0 [200].

Acute myeloid leukaemia

The diagnosis and prognosis is most accurately provided by pre-treatment assessment of the morphology, immunophenotype and underlying chromosomal/molecular aberrations. Patients with acute promyelocytic leukaemia (APL) are treated with an all-trans-retinoic acid (ATRA), whereas patients with other types of AML are typically given conventional chemotherapy (e.g. 3 days of anthracycline + 7 days of ara-C). Over the last decades, improvement in the diagnosis (especially identification of prognostically relevant cytogenetic groups), new treatment strategies and advances in supportive care have increased the survival rate in patients with AML [201, 202]. Despite this, however, nearly two thirds of patients diagnosed with AML will die of the disease and/or complications of treatment. The prognosis of patients aged 60 years or older with AML remains extremely poor, with 5% to 15% or less patients alive beyond 5 years [203, 204].

Cytogenetic abnormalities in AML at presentation have been identified as one of the most important prognostic factors and has been shown to be independent of age, WBC count, and type of leukaemia [205-209]. Approximately 30% of AML patients carry recurrent chromosomal abnormalities associated with different clinical features and prognosis [96, 208]. Three chromosomal abnormalities, t(8;21) [*RUNX1/RUNX1T1*], t(15;17) [*PML/RARA*] and

inv(16)/t(16;16) are regarded as favourable prognostic factors, whereas AML with abnormalities of 3q, deletion of 5q, monosomies of chromosome 5 and/or 7 (–5/–7), monosomal karyotype (≥2 monosomies or a combination of monosomy with structural abnormalities) or complex karyotype are associated with poor prognoses. Patients with normal karyotype, –Y, t(9;11), del(11q), +13, del(20q) and +21 belong to intermediate prognostic category [208, 210, 211]. Patients with normal karyotype and mutated *NPM1* and wild type *FLT3* have a favourable prognosis. AML with t(9;22)/*BCR-ABL* may occur *de novo* or represent the blast phase of CML. *BCR-ABL*$^+$ AML is a rare disease, characterized by a poor prognosis, with resistance to induction chemotherapy and frequent relapses in responsive patients.

Myelodysplastic syndrome

Based on clinical symptoms, morphology, number of blasts and chromosomal changes, WHO classification recognizes several categories of MDS: refractory cytopenia with unilineage dysplasia (RCUD); including refractory anaemia (RA); refractory neutropenia (RN); and refractory thrombocytopenia (RT), refractory anaemia with ringed sideroblasts (RARS), refractory cytopenia with multilineage dysplasia (RCMD), refractory anaemia with excess blasts (RAEB), myelodysplastic syndrome, unclassified (MDS-U) and MDS associated with isolated deletion of 5q (5q$^-$ syndrome) [96]. RAEB is further subdivided into type 1 (5-9% of blasts in the BM) and type 2 (10-19% of blasts in the BM). Monocytes are less than 1×10^9/l in blood.

MDS can be divided into three risk groups based on duration of survival and risk of progression into AML: low, intermediate and high risk. RCUD and RARS belong to low risk, RCMD and RAEB-1 belong to intermediate risk and RAEB-2 comprise a high risk group. MDS with del(5q) has the rate of progression into AML from 17% to 25% (with or without lenalidomide treatment) [73, 74]. In a low risk group, the median survival is approximately 66 months, and for patients above 70 years old it is similar to non-affected population. The rate of progression into AML is approximately 2% at 5 years. For RCMD the overall survival is approximately 30 months and the risk of progression into AML is 10% at 2 years. Approximately 25% of patients with RAEB-1 and 33% of patients with RAEB-2 progress into AML and the remaining patients succumb to complications of BM failure. The median survival is approximately 16 months for RAEB-1 and 9 months for RAEB-2.

Three risk-based cytogenetic groups (good, intermediate and poor) can be distinguished. The cytogenetic subgroup with good outcome includes normal karyotype, -Y alone (monosomy Y), del(5q) alone, del(20q) alone; poor outcome includes complex karyotype (≥ 3 abnormalities) or chromosome 7 abnormalities; and intermediate outcome includes all other abnormalities [212]. Abnormalities of chromosomes 5 and 7 or complex aberrations are seen only in RCMD and RAEB. Patients with normal karyotype, del(5q) alone, or del(20q) alone have median survival > 3 years, whereas patients with high risk cytogenetic changes (complex karyotype, chromosome 3 abnormalities, or chromosome 7 abnormalities) have median survival < 12 months [213-216]. MDS patients with complex karyotype and –7/del(7q) have a greater risk of progression to AML. In patients with higher risk MDS treated with azacitidine (AZA), previous low-dose AraC, BM blasts >15% and abnormal karyotype predict a lower response rate; performance status ≥ 2, intermediate or poor cytogenetics, presence of circulating blasts and red blood cell transfusion dependency predict poorer overall survival. Presence of circulating blasts (in patients treated with AZA) is associated with a worse prognosis. Blasts with Auer rods predict poorer outcome.

Acute lymphoblastic leukaemia

As outcomes with modern therapy in paediatric ALL have improved, many factors that had previously been shown to be important for predicting prognosis have lost statistical significance. Five factors that are readily apparent at either initial diagnosis or within the first month of treatment have retained their prognostic significance and constitute the basis on which patients are stratified in most treatment protocols. These factors include: (1) age at presentation, (2) WBC at presentation, (3) specific cytogenetic abnormalities, (4) presence or absence of CNS involvement, and (5) rapidity of initial response to chemotherapy.

The age-determined risk groups are age less than 1 year (infant ALL), ages of 1.0 to 9.99 years (standard risk ALL), and age of 10 years and above (high-risk ALL). The infant group is a very high-risk subcategory of patients who usually demonstrate 11q23 abnormalities, most often the t(4;11) (q21;q23) translocation. These infants generally have high presenting WBCs, large amounts of extramedullary disease, and their leukemic blasts are usually CALLA (CD10)-negative. Good prognostic parameters in ALL include age between 1 and 9 years, white blood cell count < 50,000, DNA index > 1.16 (trisomies for chromosomes 4, 10, and 17), chromosomal translocation t(12;22) /*ETV6-RUNX1*, lack of involvement of CNS, rapid response to induction chemotherapy and negative MRD after treatment.

Precursor B-lymphoblastic leukaemia with hyperdiploidy (> 50 chromosomes) has favourable prognosis [217-221], especially when there is a concurrent trisomy of chromosome 4, 10, and/or 17 [222-224]. Near-tetraploid tumours (92-94 chromosomes) are associ-

ated with a high frequency of treatment failure and therefore differ in prognosis from other hyperdiploid ALL [225]. Hypodiploid tumours with less than 45 chromosomes, especially those with 24 to 28 chromosomes, has a significant worse prognosis despite intensive treatment [226-230].

Adult patients with early-pre-B-ALL and t(4;11) or t(9;22) have poor prognosis and the absence of both of these translocations correlates with a significantly better clinical outcome after intensive polychemotherapy treatment [231]. Based on the cytogenetic-molecular aberrations and disease-free survival (DFS), Mancini *et al.* divided adult ALL into 3 prognostic categories: (1) no genetic abnormalities or isolated del(9p)(p15-p16) predicted relatively favourable outcome (median DFS > 3 years); (2) the t(9;22)$^{BCR/ABL}$, t(4;11)$^{MLL/AF4}$ or t(1;19)$^{E2A/PBX1}$ predicted highly adverse prognosis (median DFS 7 months), and (3) 6q deletions, other miscellaneous structural aberrations and hyperdiploidy predicted an intermediate prognosis (median DFS 19 months) [232].

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