Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL Network

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Summary

Mantle cell lymphoma (MCL) is a distinct lymphoma subtype with a particularly poor clinical outcome. The clinical relevance of the morphological characteristics of these tumours remains uncertain. The European MCL Network reviewed 304 cases of MCL to determine the prognostic significance of histopathological characteristics. Cytomorphological subtypes, growth pattern and markers of proliferation (mitotic and Ki-67 indices) were analysed. In addition to the known cytological subtypes, classical (87.5%), small cell (3.6%), pleomorphic (5.9%) and blastic (2.6%), we identified new pleomorphic subgroups with mixtures of cells (classical + pleomorphic type); 6.5%) or transitions (classical/pleomorphic type; 1.6%), which, however, did not differ significantly in overall survival time. Exactly 80.5% of cases displayed a diffuse growth pattern, whereas 19.5% of cases had a nodular growth pattern, which was associated with a slightly more favourable prognosis. A high proliferation rate (mitotic or Ki-67 indices) was associated with shorter overall survival. Cut-off levels were defined that allowed three subgroups with different proliferation rates to be discriminated, which showed significantly different clinical outcomes (P < 0.0001). Based on this large clinicopathological study of prospective clinical trials, multivariate analysis confirmed the central prognostic role of cell proliferation and its superiority to all other histomorphological and clinical criteria.

Keywords: mantle cell lymphoma, cytology, growth pattern, Ki-67 index, mitotic index.
Based on cytomorphological and histopathological observations, mantle cell lymphoma (MCL) was a well-accepted lymphoma entity long before the characteristic chromosomal translocation t(11;14) was described (Van Den Berghe et al., 1979; Medeiros et al., 1990; Williams et al., 1991). In addition to the classical cytomorphology of MCL with small to medium-sized cells containing medium-sized indented nuclei, three cytological variants have been described: small cell (B-cell chronic lymphocytic leukaemia (B-CLL)-like), pleomorphic and blastic (Lardelli et al., 1990; Zoldan et al., 1996; Campo et al., 1999). Although knowledge of these cytological variants is useful for the diagnosis of MCL, their prognostic significance is still uncertain (Lardelli et al., 1990; Fisher et al., 1995; Argatoff et al., 1997; Decaudin et al., 1997). The term MCL derives from the growth pattern of this lymphoma in the early stages of the disease, when tumour cells surround residual reactive germinal centres and replace the normal follicle mantle (mantle zone pattern) (Weisenburger et al., 1982; Campo et al., 1999). Alternatively, MCL may show a nodular or diffuse growth pattern (Zucca et al., 1994; Weisenburger & Armitage, 1996; Campo et al., 1999); however, there is still disagreement as to the frequency and clinical relevance of these three different growth patterns (Weisenburger et al., 1981; Lardelli et al., 1990).

Generally, MCL is characterised by an aggressive clinical course, a continuous relapse pattern after conventional chemotherapy and the poorest long-term outcome of all lymphoma subtypes (Lenz et al., 2004a). Recently, novel treatment options, such as combined immuno-chemotherapy and myeloablative consolidation, have improved response rates and progression-free survival, however, so far no survival plateau indicating long-term remissions or potential cure has been observed (Dreyling et al., 2004; Lenz et al., 2004b). In contrast, a small subgroup of MCL patients shows a very indolent course with an overall survival of up to 5–10 years (Campo et al., 1999). Thus, reliable and routinely applicable prognostic markers are urgently needed to identify the different prognostic patient subgroups and allow risk-adjusted therapeutic approaches in MCL (Lenz et al., 2004b).

Several lines of evidence link a high cell proliferation rate in MCL to an unfavourable course of the disease. Cell proliferation, assessed by counting of mitotic figures or expression of Ki-67, has been previously proposed as a marker of poor clinical outcome (Swedlow et al., 1983; Argatoff et al., 1997; Bosch et al., 1998; Raty et al., 2002; Schrader et al., 2004). Additionally, gene expression profiling recently revealed that the increased expression of the 'proliferation signature' representing an integrator of oncogenic events leading to the higher expression of genes associated with cell proliferation and cell cycle progression is a strong indicator of outcome in MCL (Rosenwald et al., 2003). Some of the genes identified by gene expression profiling, such as topoisomerase IIa (Rosenwald et al., 2003), have been confirmed in immunohistochemical studies (Schrader et al., 2004). The clinical relevance of the most widely used marker of cell proliferation, Ki-67, is still being debated (Lardelli et al., 1990; Velders et al., 1996; Raty et al., 2002).

The pathology panel of the European MCL Network analysed MCL cases enrolled in eight clinical studies from six different countries to determine the relevance of histopathological parameters and cell proliferation to disease outcome. To our knowledge, this is the largest clinicopathological study of MCLs described so far.

## Patients and methods

### Patients

The study included biopsy specimens from 351 MCL patients (92 females and 259 males; median age 64 years, range 27–86) enrolled in three multi-centre prospective therapy trials and five retrospective studies in the period between 1972 and 1994 (Tables I and II). Only cases with appropriate sample size and quality were included after the histological diagnosis of MCL had been confirmed by one of the participating pathologists according to the criteria of the European Lymphoma Task Force (ELTF) (Zucca et al., 1994) and the current World Health Organisation (WHO) classification (Jaffe et al., 2001). If the available material was insufficient, the case was classified as not evaluable.

### Review process

The review process was performed by two interactive panels of five and six haematopathologists respectively. After all cases were examined independently by each pathologist, a consensus was considered to have been achieved if the subclassification parameters assessed by at least four of five or five of six pathologists matched (P < 0.05). Cases without consensus were re-evaluated by all 11 pathologists on a multihead microscope and the diagnosis made by the majority was defined as the consensus diagnosis. On the basis of this

<table>
<thead>
<tr>
<th>Country or centre</th>
<th>Number of patients</th>
<th>Slides available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany (trial 1988–1995)</td>
<td>90</td>
<td>56</td>
</tr>
<tr>
<td>Swiss (Locarno)</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>France (Lyon-Sud)</td>
<td>48</td>
<td>27</td>
</tr>
<tr>
<td>Great Britain (London)</td>
<td>66</td>
<td>64</td>
</tr>
<tr>
<td>Netherlands (Leiden)</td>
<td>41</td>
<td>39</td>
</tr>
<tr>
<td>Spain (Barcelona)</td>
<td>34</td>
<td>26</td>
</tr>
<tr>
<td>Germany (trial 1975–1980)</td>
<td>87</td>
<td>71</td>
</tr>
<tr>
<td>(Brittenger et al., 1984)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany (trial 1983–1988)</td>
<td>84</td>
<td>48</td>
</tr>
<tr>
<td>(Meurers et al., 1989)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>485</td>
<td>351</td>
</tr>
</tbody>
</table>
consensus diagnosis the diagnosis of MCL was confirmed or the case was excluded.

Cytology

The cytology was classified as classical, small cell (B-CLL-like), pleomorphic or blastic (Weisenburger & Armitage, 1996; Campo et al, 1999) (Table III, Fig 1). In initial control experiments, 20 cases with the confirmed diagnosis of MCL were subclassified independently by each of the 11 panel pathologists and a consensus cytology was defined. Subsequently, a second separate review was performed and the results of the cytological diagnosis were compared. In this blinded double evaluation, the cytological subtype (classical MCL/small cell type versus blastoid/pleomorphic variant) of the second review matched the consensus diagnosis in 85% of cases (17 of 20) according to the above-mentioned criteria ($\alpha < 0.05$).

Growth pattern

The growth pattern of the tumour was classified as nodular with germinal centres (mantle zone pattern), predominantly nodular (>50% nodular growth), or predominantly diffuse (<50% nodular growth) according to Argotoff et al (1997) and Zucca et al (1994).

Mitotic index

The mitotic index was analysed in 272 cases by counting the number of mitotic figures/mm$^2$. Hot spots of mitosis were analysed separately.

Ki-67 index

The immunohistochemical staining for Ki-67 (Ki-S5 or MiB-1) was performed in the laboratories of 11 centres by either the avidin–biotin complex (ABC) technique (Hsu et al, 1981) or the alkaline phosphatase anti-alkaline phosphatase (APAAP) method (Cordell et al, 1984), followed by counterstaining with Mayer’s haematoxylin. The Ki-67 index was assessed in 187 cases by staining with specific monoclonal antibodies against Ki-67 (Ki-S5 or MIB1) (Gerdes et al, 1984b; Kreipe et al, 1993). Neoplastic cells that were positive for Ki-67 were counted by one pathologist in each centre in 10 consecutive high power fields (400-fold magnification) using a haematological cell counter. By applying this method, a minimum of 500 neoplastic cells was assessed and the number of positive cells was expressed as percentage of all tumour cells counted (Ki-67 index). Proliferation hot spots were analysed separately.

Statistics

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 11.
Results

Cytomorphology

Lymph node biopsy specimens from 351 MCL patients in eight clinical trials were evaluated (Table I). Exactly 304 cases (87%) were confirmed as MCL, 26 cases (7%) were classified as not evaluable due to insufficient samples and in 21 cases (6%) a different diagnosis was made. The cytological classification revealed a classical cytology (Fig 1A) in 87.5% (n = 266), a small cell type (Fig 1B) in 3.6% of cases (n = 11), a pleomorphic type (Fig 1C) in 5.9% (n = 18) and a blastic type (Fig 1D) in 2.6% (n = 8). In one case no cytological classification was performed.

In 10 of 18 cases of the pleomorphic type a mixture of classical and pleomorphic areas was present. Based on these observations we defined two new cytomorphological subtypes of MCL: the classical + pleomorphic type (Fig 1E, n = 5; 1.6%) consisted of a mixture of two cell populations, one with classical (C, left side) and the other with pleomorphic (P, right side) cytology. H&E, ×400. (F) The classical/pleomorphic type consists of cells that display cytology resembling a continuous transition between the classical and pleomorphic type. H&E, ×630.

Overall survival time was calculated from the date of diagnosis until death or loss to follow-up. Survival curves were estimated according to the Kaplan–Meier method, and statistical comparisons were performed by log-rank test. P < 0.05 was considered statistically significant. Univariate Cox regression analysis was performed for all prognostic factors with respect to overall survival. For 149 patients Ki-67 index, mitotic index, age, performance status, lactate dehydrogenase (LDH), stage and sex were available. They were used to compute a Cox regression with likelihood ratio excluding stepwise parameters with P > 0.1.
Growth pattern

The architecture of the lymphoma infiltrates in the examined lymph nodes displayed a predominantly nodular pattern in 18.1% of cases (n = 38) and a nodular growth pattern with prominent residual germinal centres (mantle zone pattern) in 1.4% (n = 3). Diffuse growth of the lymphoma cells was observed in 80.5% (n = 169) of cases. 94 cases could not be evaluated, e.g. because of the small size of the biopsy specimen. Cases showing nodular growth (nodular or mantle zone pattern) with a diffuse component of <50% (n = 41) had a more favourable prognosis (median overall survival: 43 months) than cases that contained a diffusely growing lymphoma component of more than 50% (median: 29 months, P = 0.0074, Fig 2B). No statistically significant correlation was found between the cytological subtype and the growth pattern (data not shown).

Proliferation

The mitotic index was assessed in 273 cases and the Ki-67 index was assessed in 187 cases. The mitotic index ranged from 0 to 112 mitotic figures/mm² with a mean of 15.4 and a median of 11.0. The Ki-67 index ranged from 1% to 70% with a mean of 16.8% and a median of 15.0%. As expected, we found a strong correlation between the mitotic index and the Ki-67 index (P < 0.0001, Fig 3A). Cases with classical or small cell cytology showed a lower mitotic index (mean: 13.6%) and Ki-67 index (mean: 15.3%) than cases with pleomorphic and blastoid cytology (28.9% and 28.8% respectively; P < 0.0001 for Ki-67, Fig 3B). No correlation was found between the growth pattern of the lymphoma and either the mitotic index (P = 0.8194) or the Ki-67 index (P = 0.1107, Fig 3C).

To evaluate the mitotic index and the Ki-67 index as prognostic markers in MCL, we performed a quartile-based analysis of the study population. Significant differences between the four quartile subgroups were found for both the mitotic index (P < 0.0001) and the Ki-67 index (P = 0.0292). We defined cut-off levels of 25–50 mitotic figures/mm² for the mitotic index and 10%/40% for the Ki-67 index. The lower cut-off level was chosen on the basis of values reported in the literature (Velders et al, 1996). The higher cut-off level was...
chosen on the basis of the data published by Gerdes et al. (1984a), who demonstrated that high-grade lymphomas showed Ki-67 indices of more than 40%. The survival curves of the resulting three subgroups were compared by log-rank test (Table IV). As shown in Fig 4(A), cases with a high mitotic index of more than 50 mitotic figures/mm² had a significantly worse clinical outcome (median overall survival: 17 months, \( n = 14 \)) than cases with a moderate mitotic index between 25 and 50 mitotic figures/mm² (21 months, \( n = 34 \)) or cases with a low mitotic index below 25/mm² (38 months, \( n = 225 \), \( P = 0.0019 \)). Similarly a high Ki-67 index of more than 40% \( (n = 7) \) resulted in significantly shorter overall survival (median: 15 months, \( n = 48 \), \( P = 0.0002 \)). In the second analysis of 53 patients the two proliferation markers were analysed in relation to the IPI. The IPI also had no clinical impact in this smaller group and only the mitotic index was significant \( (P = 0.0066) \). In the third multivariate analysis of 149 patients, Ki-67 index, mitotic index, age, performance status, LDH, stage and sex were available and were used to compute a Cox regression with likelihood ratio and a stepwise conditional approach excluded parameters with \( P > 0.1 \). Only the parameters Ki-67 index, mitotic index and age were of significant influence in this model (Table V).

Discussion

Mantle cell lymphoma was originally recognised by Tolksdorf et al. (1980) on the basis of its cytomorphological features, which in some respect resemble those of centrocytes. Additional cytological variants, such as blastic (Ott et al., 1997, 1998;...
Table V. Univariate Cox regression analysis of all prognostic factors with respect to overall survival in 304 MCL patients. For 149 patients the Ki-67 index, mitotic index, age, performance status, LDH, stage and sex were available and were used to compute a Cox regression with likelihood ratio excluding stepwise parameters with a P-value >0.1. Only the parameters Ki-67 index, mitotic index and age were of significant influence in this model (n.i. = not included in the computation of the model because too many data were missing).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Reference level</th>
<th>P value Univariate</th>
<th>P value Multivariate (n = 149)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67 index</td>
<td>&lt;10% vs. 10–40% vs.&gt;40%</td>
<td>n = 187, P &lt; 0.0001</td>
<td>P = 0.0812</td>
</tr>
<tr>
<td>Mitotic index</td>
<td>&lt;25 vs. 25–50 vs. &gt;50</td>
<td>n = 271, P = 0.0001</td>
<td>P = 0.0009</td>
</tr>
<tr>
<td>Growth pattern</td>
<td>Mantle zone + nodular versus diffuse</td>
<td>n = 208, P = 0.0074</td>
<td>n.i.</td>
</tr>
<tr>
<td>Cytology</td>
<td>Classical + small cell versus blastic + plo + variants</td>
<td>n = 284, P = 0.1413</td>
<td>n.i.</td>
</tr>
<tr>
<td>International Prognostic Index</td>
<td>0–1 vs. ≥2</td>
<td>n = 90, P = 0.3629</td>
<td>n.i.</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;60 years vs. &gt;60 years</td>
<td>n = 304, P = 0.0020</td>
<td>P = 0.0191</td>
</tr>
<tr>
<td>Bone marrow infiltration</td>
<td>Yes versus no</td>
<td>n = 288, P = 0.011</td>
<td>n.i.</td>
</tr>
<tr>
<td>Performance status (WHO)</td>
<td>0 + 1 vs. ≥2</td>
<td>n = 276, P = 0.044</td>
<td>P = 0.8900</td>
</tr>
<tr>
<td>LDH</td>
<td>Normal versus elevated</td>
<td>n = 196, P = 0.0305</td>
<td>P = 0.1602</td>
</tr>
<tr>
<td>Stage</td>
<td>1 + 2 vs. 3 + 4</td>
<td>n = 290, P = 0.0048</td>
<td>P = 0.2347</td>
</tr>
<tr>
<td>B symptoms</td>
<td>Yes versus no</td>
<td>n = 262, P = 0.0343</td>
<td>n.i.</td>
</tr>
<tr>
<td>Sex</td>
<td>Male versus female</td>
<td>n = 304, P = 0.2749</td>
<td>P = 0.8346</td>
</tr>
<tr>
<td>Extranodal involvement</td>
<td>Yes versus no</td>
<td>n = 177, P = 0.0041</td>
<td>n.i.</td>
</tr>
</tbody>
</table>

WHO, World Health Organization; LDH, lactate dehydrogenase

Campo et al, 1999; Laszlo & Matolcsy, 1999), pleomorphic (Zucca et al, 1994; Campo et al, 1999) and small cell type (Weisenburger & Armitage, 1996; Decaudin et al, 1997; Campo et al, 1999) have been described. Several authors have not distinguished between the pleomorphic and blastic subtypes and used the term blastic to summarise both types (Jaffe et al, 2001). The current study included an exceptionally large number of cases that were all enrolled in controlled studies, and was carried out by a panel of highly experienced haematopathologists. It confirmed the existence of the cytological subtypes classical, small cell, pleomorphic and blastic. Furthermore, we described two new cytomorphological subtypes of MCL. The classical + pleomorphic type shows a mixture of cells of classical and pleomorphic cytology, whereas the classical/pleomorphic type consists of cells of intermediate differentiation between classical and pleomorphic cytology. The clinical relevance of the cytomorphological variants is still being debated. The blastic subtype has been described as having a more aggressive clinical course (Lardelli et al, 1990; Zucca et al, 1994; Fisher et al, 1995; Weisenburger & Armitage, 1996; Decaudin et al, 1997; Ott et al, 1997; Campo et al, 1999). However, in the present study there was no significant difference in overall survival between classic MCL and the small cell type versus the pleomorphic and blastic variants, respectively, although the latter subtype displayed a significantly higher mitotic and Ki-67 index. This discrepancy might be explained by the relatively low number of pleomorphic and blastic subtypes in our series, which altogether represented <10% of all cases. Similar to previous reports (Bosch et al, 1998; Raty et al, 2002), the vast majority of MCL in our series displayed a classical cytology. As our series represents a large number of multicentre studies with various inclusion criteria, the abundance of the cytological subtypes in our series might reflect a more realistic prevalence of these subtypes. Consequently, variant subtypes of MCL with small cell, pleomorphic and blastic cytomorphology should be considered to be rare and their histological classification can pose a diagnostic problem. The reproducibility of cytomorphological classifications is a problem in routine diagnostic work. Univariate and multivariate analysis did not show cytomorphological subtypes to be a prognostic marker, but knowledge of these cytomorphological variants is important for the differential diagnosis of this disease.

Mantle cell lymphoma shows three characteristic growth patterns: mantle zone, nodular and diffuse (Weisenburger et al, 1981; Pittaluga et al, 1995; Campo et al, 1999). It has been suggested that the growth pattern represents a progression of the disease, with the lymphoma initially growing in the mantle zone and then progressing to nodular and finally diffuse growth (Swerdlow et al, 1983; Pittaluga et al, 1995; Majlis et al, 1997). The clinical relevance of the different growth patterns has not yet been determined. In some studies lymphomas with a mantle zone pattern showed a significantly better prognosis (Lardelli et al, 1990; Majlis et al, 1997), whereas other studies did not find any differences in overall survival time between the different growth patterns (Argatoff et al, 1997). As in our series a mantle zone pattern was rare (n = 3) and the number of cases was too small for a separate statistical analysis, we grouped those cases with the pure nodular cases and compared them to the cases that showed a diffuse component. Patients with a predominantly nodular growth pattern had a more favourable prognosis than patients with a growth pattern showing a diffuse component (P = 0.0074). The growth pattern thus represents a readily assessable prognostic marker.

In previous studies, Ki-67 labelling in classical MCL was low, whereas (blastic or pleomorphic) variants have been suggested...
to show high indices (Lardelli et al, 1990; Ott et al, 1994, 1997; Jares et al, 1996; Campo et al, 1999). The present, extensive study of various prospective trials confirmed these data. The majority of cases with a classical cytology had a low Ki-67 index of <10%. Nevertheless, some classical subtypes displayed a high proliferation rate of more than 40%. In contrast to previous reports, our study applied different antigen retrieval methods, but a standardised counting method (Lardelli et al, 1990; Velders et al, 1996).

Several reports have suggested that an increased mitotic index is an important prognostic parameter in MCL (Lardelli et al, 1990; Argatoff et al, 1997; Bosch et al, 1998). Recently, gene expression profiling indicated that upregulation of genes associated with proliferation and cell cycle progression can be detected in a subgroup of MCL with poor prognosis. Accordingly, in our study the subgroup of MCL with high Ki-67 or mitotic index had a significantly worse clinical outcome (median overall survival 15 months vs. 42 months in the cases with low proliferation). Interestingly, a subset of MCL with high cell proliferation but classical cytology was identified that also displayed a poor clinical outcome (Pinyol et al, 1998; Campo et al, 1999).

In the current study, we employed two well-established methods to evaluate the proliferation of malignant cells in paraffin-embedded tissue. The mitotic index and the Ki-67 index can be assessed during routine diagnostic procedures. Both proliferation indices showed a good correlation with clinical outcome. However, the mitotic index was found to be superior in the multivariate analysis (Table V). In our study no inter- or intra-observer variability was tested. However, this issue will be of importance if proliferation indices are to be assessed for use in risk-adapted therapy.

In summary, our study included an exceptionally large number of MCL cases that were analysed by a large number of highly experienced haematopathologists to generate reliable markers for histological subtyping of MCL. We conclude that highly experienced haematopathologists to generate reliable markers for histological subtyping of MCL. We conclude that high-quality pathological analysis is necessary for the establishment of a combined biological and clinical risk score for MCL that is superior to the IPI, defined for diffuse large cell lymphomas and the follicular lymphoma IPI (Solal-Celigny et al, 2004). Additionally, we need to re-evaluate whether the Ki-67 and mitotic indices remain important prognostic markers in patients treated with combined immuno-chemotherapy or myeloablative consolidation followed by autologous stem cell transplantation.

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References


