

Short communication

Significance of chromosomal markers in the diagnosis of mantle cell lymphoma (MCL)

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Abstract. According to the REAL/WHO classification, the diagnosis of mantle cell lymphoma (MCL) should be based on clinical, histopathological, immunological and cytogenetic or molecular data. This study is based on 13 cases, which were initially diagnosed as MCL with the use of conventional cytogenetic method and fluorescent *in situ* hybridization (FISH). MCL is associated with a specific cytogenetic aberration t(11;14)(q13;q32). The chromosomal analyses confirmed the MCL diagnosis in four cases. A neartetraploid cell line and two copies of t(11;14) were observed in three cases. These results correspond with a blastoid variant of MCL, accompanied by aggressive course and poor prognosis. The presence of karyotype with t(11;14) as the sole anomaly predicts an intermediate clinical outcome. Six patients had normal karyotypes, which is characteristic for the typical form of MCL, associated with a better prognosis. In this study we show that detection of chromosomal abnormalities is useful in diagnosis of MCL and has some prognostic significance.

Key words: cytogenetics, mantle cell lymphoma, tetraploidy, t(11;14).

Mantle cell lymphoma (MCL) is a rare, specific subtype of lymphoma and accounts for about 5 to 10% of non-Hodgkin lymphomas. According to the REAL/WHO classification, the diagnosis of mantle cell lymphoma should be based on clinical, histopathological, immunological and cytogenetic or molecular data (ARGATOFF et al. 1997). MCL is most commonly observed in the male popu-

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lation ranging from 50 to 70 years of age and is characterized by disseminated disease, usually involving lymph nodes, bone marrow and spleen. Frequently, there is extranodal involvement including the gastrointestinal tract. Cytologically, MCL usually consists of a monotonous population of atypical, small to medium-sized lymphoid cells with irregular and indented nuclei, moderately coarse chromatin, inconspicuous nuclei and scanty cytoplasm. The immunohistological features reveal a characteristic phenotype with expression of a variety of pan-B-cell antigens (CD19, 20, 22) and the HLADR antigen. The MCL cells usually are negative for CD10 and CD23 antigens. The phenotype of MCL is remarkably similar to that of small lymphocytic lymphoma, except for more intense sIg and CD 20 staining and lack of CD23 expression.

The majority of MCLs can be diagnosed by conventional examination. However, in cases when cytometric tests do not confirm a preceding histopathological diagnosis, the material undergoes for cytogenetic analysis. MCL is associated with a specific cytogenetic aberration t(11;14)(q13;q32), present in 60-75% of cases (CUNEO 2000). This translocation results in movement of the BCL-1 locus to the immunoglobulin heavy chain gene enhancer region located at 14q32, leading to cyclin D1 over expression. Cyclin D1 over expression in MCL is thought to play a major role in lymphomagenesis, although the precise mechanism of tumor formation and progression is not fully understood. In the majority of mantle cell lymphomas the t(11;14) is accompanied by multiple chromosomal aberrations (MONNI et al. 1998, PIEŃKOWSKA-GRELA 2002). The most frequent recurrent secondary changes are the derivative chromosomes: 1, 2, 3, 9, 13, 17, and very often unidentified markers. In MCLs also tetraploidy with increased number of chromosome imbalances can occur. This phenomenon is characteristic for blastoid variants of MCL.

Normal karyotype or karyotype with the sole anomaly t(11;14) are good prognostic factors, contrary to complex or neartetraploid karyotypes. The degree of karyotype complexity has a strong impact on prognosis in this neoplasia.

The presence of cytogenetic abnormalities can be important for final diagnosis in MCL. The detailed karyotypic analysis is a useful method for defining subtypes of MCL; moreover it has some prognostic significance.

All diagnoses of MCL were based on paraffin sections (hematoxylin and eosin stained H-P), immunohistochemical reactions (an avidin-biotin technique using antibodies against) and flow cytometry. Immunophenotypic analysis was performed on FACSscan (Becton-Dickinson Immunocytometry system) by using lymph node or peripheral blood cells stained with control antibodies IgG1 (FITC) AND IGG2 (PE) .

Cytogenetic analysis was carried out on lymphoid cells obtained from lymph node biopsies or peripheral blood after unstimulated short-term cultures or cultures stimulated by TPA (Phorbol 12-miristate 13 acetate) or LF (*Phaseolus vulgaris* extract). Chromosome analyses were done according to standard G-banding (GTG) methods; the fluorescent *in situ* hybridisation (FISH) technique

was also used (PINKEL et al. 1986). FISH studies were performed by using a centromeric probe (Oncor, D12Z3) for interphases and painting probe (Oncor, coatosome 3 P5207-DG.5) for metaphases. Chromosome abnormalities were classified according to the International System for Chromosome Nomenclature (ISCN 1995).

This is based on 13 cases of lymphoma, preliminarily considered as MCL or suspected of being MCL. The cytogenetic analysis confirmed the preliminary MCL diagnosis in four of the 13 patients (Table 1). In each of them the typical (for MCL) translocation $t(11;14)(q13;q32)$ was found. The hypotetraploid karyotypes with two copies of the specific MCL marker $t(11;14)$ were observed in two of them (#1 and #2) (Figure 1), while the third sample (#3), with a neardiploid modal line, showed the presence of one copy of the $t(11;14)$ marker (Figure 2). In the latter case, a tetraploid side line with two copies of $t(11;14)$ and additional chromosomal abnormalities was observed. These results can indicate a blastoid subtype of MCL, associated with a more aggressive course and poor prognosis. Two of the patients with the blastic form of MCL were chemotherapy resistant. The fourth patient (#4) had a neardiploid karyotype with a few unidentified markers.

Six of the 13 patients had normal karyotypes, of which four showed the MCL phenotype by flow cytometry (#5, #6, #7, #9). These results could suggest a typical form of MCL in consideration of the preliminary histopathological diagnosis. This variant of MCL is associated with better prognosis. Diagnosis of one patient (#10) was difficult to establish, as flow cytometry indicated the marginal zone lymphoma, while the clinical and histopathological features were characteristic for MCL. Cytogenetic study has not resolved the doubt yet. In case #8, lymphocytes in peripheral blood showed a normal immunophenotypic profile.

In one case the conventional cytogenetic analysis revealed a hyperdiploid karyotype with an additional unidentified marker (#11). This patient was chemotherapy resistant.

In the last two cases the final diagnosis turned from MCL to chronic lymphocytic leukemia (CLL). In one sample (#12) the detection of trisomy 12 was useful in defining the final diagnosis of CLL; the three copies of chromosome 12 are in fact one cytogenetic parameter predictive of poor prognosis in CLL and MCL. The result of cytogenetic analysis in case #13 was not informative.

MCL is a lymphoproliferative disorder derived from a subset of naive pregerminal centre cells and associated with a specific cytogenetic alteration $t(11;14)(q13;q32)$, which results in a rearrangement and deregulation of BCL-1, leading to over expression of cyclin D1. This translocation is present in 50-75% of MCL cases and may be detected by conventional and FISH cytogenetic analysis. There is evidence that genetic lesions may help to identify different groups of NHL. The detailed karyotypic investigations performed with conventional cytogenetic analysis and fluorescence *in situ* hybridization appeared to be a useful

Table 1. Clinical and cytogenetic findings in patients with a preliminary diagnosis of mantle cell lymphoma

Case no.	Clinical diagnosis	Flow cytometry diagnosis	Cytogenetic analysis	Final diagnosis	Clinical context
1	Mantle cell lymphoma	CD22+, CD20+, CD5+, CD23- CD10+/-, CD11C- KAPPA+ =>MCL, high cell proliferation	80-85, XXY, del(1p), del(2p), der(3), der(3), -6, der(7), -8, -9, t(11;14)(q13;q32)x 2, -13, -14, der(17)	Mantle cell lymphoma	grade IV A E typical course Chth resistance survival: 17+ months
2	Malignant lymphoma	CD20+, CD5+ CD22+, CD10+/- CD23-, CD11C-, KAPPA+ =>MCL, high cell proliferation	Tetraploid with t(11:14) x 2	Blastoid variant of mantle cell lymphoma	grade IV A typical course Rituximab treatment remission survival: 7+ months
3	Blastoid variant of mantle cell lymph. v/s high grade B-cell lymphoma	CD20+ bright, CD22+, CD23- CD5+, CD10-, CD11C-, KAPPA+ =>MCL	44-46, XY, del(1)(p13-p22), der(3)(3:?) (p25:?), t(11;14)(q13;q32), -13, -14, +mar3, +mar4 / neartetraploid line 46, XY, add(3)(p25). ish add(3)(p25)(wpc 3+)	Blastoid variant of mantle cell lymphoma	grade I A E typical course Chth resistance-fatal outcome survival: 15 months
4	Follicular lymphoma/Chronic lymphocytic leukaemia/Mantle cell lymphoma	CD45+, CD19+, CD20+, CD5+, CD22+C D23- CD11-, CD10- =>MCL	44-46, XX, der(1), -5, t(11;14)(q13;q32), -13, -22, +mar1, +mar2, +mar3	Mantle cell lymphoma	grade IV Chth treatment for FL survival 6 years+
5	Low grade lymphoma	CD5+, CD22+, CD20+ bright CD23-/+ , CD11C-/+ =>MCL	46, XX	Mantle cell lymphoma	grade IV A typical course short time remissions survival: 19 + months

6	Low grade lymphoma +breast cancer	CD20+,CD23-,CD22+,CD11C- CD5+, CD10+/- KAPPA+/++ =>MCL	46,XX	Mantle cell lymphoma	grade IVA not typical course Tamoxifen treatment good condition survival: 14+ months
7	Mantle cell lymph v/s follicular lymphoma	CD22+,CD20+, CD5+, CD23-, CD11C-, CD10-,CD8-/+ =>MCL, high cell proliferation Normal blood profile	46,XY	Mantle cell lymphoma	grade IA remission survival:10+ months
8	Mantle cell lymphoma	Normal blood profile	46,XY	Mantle cell lymphoma	grade IA no treatment remission, survival: 11+ months
9	Mantle cell lymphoma	CD45+,CD19+,CD20+,CD5+, CD22+CD25+ CD23-/+ CD11C-,CD10- =>MCL	46,XX	Mantle cell lymphoma	grade IVB Chth treatment good condition survival 24+ months
10	Diffuse large B cell lymphoma/Mantle cell lymphoma	CD45+,CD19+,CD20+,CD22+,CD23+/- ,CD5-,CD25-,CD11C-,CD10-,CD103- =>MZL	46,XY	Marginal zone lymphoma/Mantle cell lymphoma	Therapy in another hospital
11	Mantle cell lymphoma + leukemia	CD5+,CD23-, CD20+,CD11C- CD22+,CD10-, CD25+/- =>MCL	47,XY,+mar	Mantle cell lymphoma	grade II AE Rituximab treatment Chth resistance survival:11+ months
12	Lymphoma + leukemia	CD20-, CD5+, CD22+,CD11C+, CD23-, KAPPA+ =>MCL v/s CLL	47,XX,+12/48,XX,+12,+16	Chronic lymphocytic leukemia	grade IV AE typical course remission, survival:7+ months
13	Mantle cell lymphoma	CD20+,CD5+,CD23+,CD22+/- CD11C+/- CLL	No trisomy 12 nuc ish 12cent(D12Z3x2)	Chronic lymphocytic leukemia	grade IV A, no treatment good condition

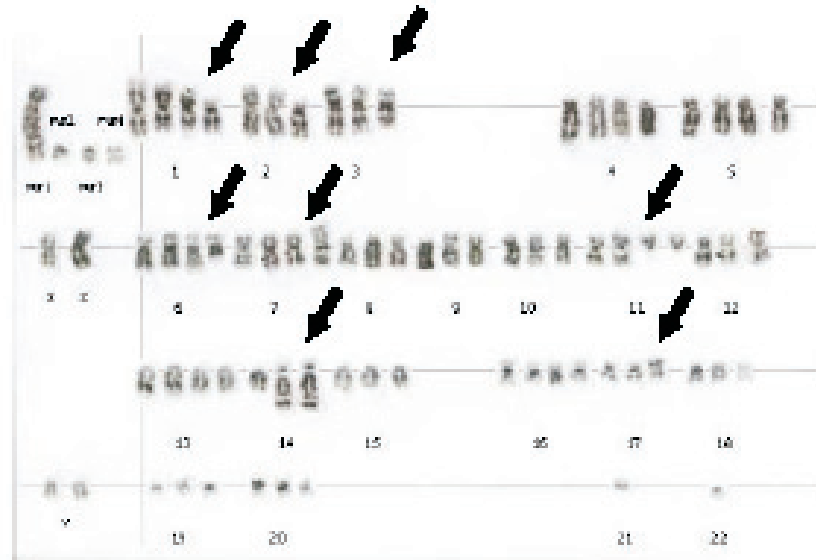


Figure 1. Complex karyotype of blastoid variant mantle cell lymphoma. Near-tetraploid cell with two copies of characteristic t(11;14) marker. Arrows indicate aberrations affected 1, 2, 6, 7, 11, 14 and 17 chromosomes.

78,XXYY,del(1)(p13),-2,del(2)(p11),-3,der(3),del(6p),add(7p),-8,-9,-10,2xt(11;14)(q13;q32),-12,-14,-15,-17, der(17),-18,-19,-20,-21,-21,-21,-22,-22,-22,-22,+4mar

method in defining subtypes of MCL. Cytogenetic findings may help to recognize biologically relevant entities, which are sometimes hard to distinguish by morphologic, cytomorphologic and immunologic criteria alone. Recent genetic studies performed with FISH and comparative genomic hybridization techniques have demonstrated other genetic alterations of MCL that may also play an important role in the development and progression of these tumors (ROSENWALD et al. 1999, ESPINET et al. 1999). MCL can have a typical or blastoid variant; blastic morphology is associated with poor prognosis and a high proliferative activity, an increased number of cytogenetic aberrations and molecular alterations in tumor suppressor genes (OTT et al. 1997, BEA et al. 1999). In three of our patients the presence of a near-tetraploid karyotype with amplification of the t(11;14) marker confirmed the diagnosis of blastoid subtype of MCL. Two of them were chemotherapy resistant and the other died. Tetraploidy with amplification of the typical t(11;14) marker and frequent chromosomal imbalances are characteristic for the blastoid variant. These aberrations can include 1p, 3q, 7p, 11q, 12q, 13q or 17p. Latest studies have shown that some chromosomal regions may potentially have prognostic significance: these deleted or rearranged chromosomal parts involve 6q15-21, 6q25, 1p21-32 and 1q21-23 regions. Attention was also drawn to the cycline-dependent kinase (CDK) inhibitor, located on chromosome

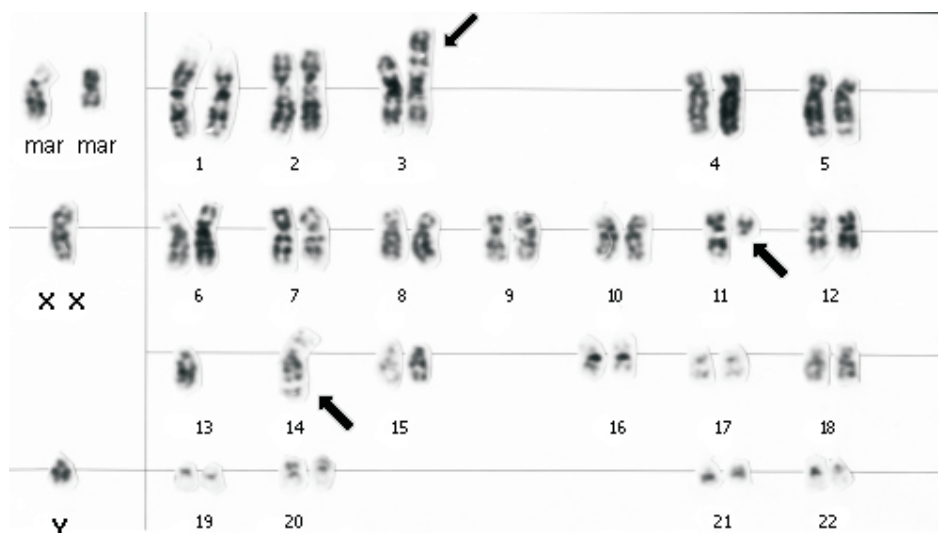


Figure 2. Diploid karyotype of mantle cell lymphoma with t(11;14) marker and additional abnormalities. Arrows indicate aberrations affected 3, 11 and 14 chromosomes

45,XY,del(1)(p13-p22),dup(3p),t(11;14)(q13;q22),-13,-14,+2mar

9p21, the deletion of which was shown to be associated with a high proliferation index. The alterations within the 1p21-23 region predicted a shorter duration of complete remission (OFFIT et al. 1991). Deletion of band 13q14 was found in a relatively high incidence in atypical CLL carrying t(11;14) and in 70% of MCLs. The loss of genetic material involving the 13q14 region may be an important step in the transformation of CD5+ B-cell neoplasias; the losses of 17p, strongly correlated with P53 gene alterations, are described by some investigators as associated with aggressive variants of MCL (BEA et al. 1999, CUNEO et al. 1999, SCHLEGERBERGER et al. 1999).

In three cases the karyotypic aberrations affected 1p, 3, 6, 9, 13 and 17 chromosomes. As it was mentioned above it is possible that these rearrangements could represent an important event in the progression of MCLs. Structural and numerical alterations on chromosome 3 were described as associated with more aggressive subtypes of non-Hodgkin lymphomas (BEA et al. 1999).

Six of our patients had normal karyotypes. As the flow cytometry and clinical diagnoses confirmed MCL, these results suggest the typical form of MCL. This variant of neoplasia is characterized by better prognosis.

In one case detection of trisomy 12 was useful in defining the final diagnosis as CLL. Gains of chromosome 12 were detected in primary mediastinal B-cell lymphomas (31%), primary gastrointestinal large-cell lymphomas (23%) and CLLs

(16%). However, CLL and gastrointestinal tumors showed preferentially trisomy 12 (BEA et al. 1999).

The importance of karyotypic examination of the MCL malignant cells should be emphasized. The overall cytogenetic profile and the chromosome abnormalities improve our knowledge on the clinicobiological significance of cytogenetics in MCL and have a prognostic meaning as well.

Further research on additional and/or multiple cytogenetic abnormalities, which may predict poor outcome, is ongoing.

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