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Predictive factors for blastoid transformation in the common variant of mantle cell lymphoma

R. Räty[a], K. Franssila[b], S.-E. Jansson[c], H. Joensuu[d], U. Wartiovaara-Kautto[c], E. Elonen[a,*]

[a]*Department of Medicine, Helsinki University Central Hospital, PO Box 340, 00029 Huch, Helsinki, Finland*
[b]*Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland*
[c]*Department of Clinical Chemistry, Helsinki University Central Hospital, Helsinki, Finland*
[d]*Department of Oncology, Helsinki University Central Hospital, Helsinki, Finland*

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Abstract

Approximately 20% of the mantle cell lymphoma (MCL) patients present with the blastoid variant at diagnosis. Blastoid changes may occur also during the course of the disease, but factors related to blastoid transformation are poorly understood. In the present study, the incidence and predictive factors for blastoid transformation were analysed among 52 patients who primarily had the common variant of MCL and one or more biopsies taken at the time of disease progression. Blastoid transformation occurred in 18 (35%) patients. The minimum estimated risk of transformation was 42% at 5 years of follow-up. At the time of transformation, all except two patients had systemic lymphoma with lymphatic blasts in the blood. The median survival time after blastoid transformation was 3.8 months compared with 26 months in patients without transformation ($P<0.001$). The respective survival times as calculated from the initial diagnosis of MCL were 31 and 60 months. Leucocytosis, an elevated serum lactate dehyrdogenase (LDH) level, and a high proliferative activity at diagnosis as assessed by the mitotic count and Ki-67 staining were associated with an increased risk of blastoid transformation, and elevated serum LDH and blood leucocytosis with a short time interval to transformation. We conclude that blastoid transformation is not uncommon during the course of MCL, and is associated with a poor outcome. An elevated serum LDH level, a high cell proliferation rate, and leucocytosis are predictive for a high risk of blastoid transformation in MCL.

Keywords: Mantle cell lymphoma; Blastoid variant; Non-Hodgkin's lymphoma; Histopathology; Prognosis; Transformation

1. Introduction

Mantle cell lymphoma (MCL) has been characterised as a distinct type of B-cell lymphoma by morphological, immunohistochemical and cytogenetic studies. MCL is typically composed of monotonous proliferation of small to intermediate sized lymphocytes with scant cytoplasm and small more or less irregular nuclei, and with a low to moderate mitotic frequency [1–4]. Approximately 20% of patients present with a cytomorphologically more aggressive variant of MCL called the blastoid variant [5–7]. It is characterised by lymphoid cells with medium to large-sized nuclei and usually a high proliferative activity [2]. No cure can be achieved in MCL with conventional treatments, and the long-term prognosis is poor with the median survival time ranging from three to four years [5,8–16]. An even more aggressive clinical course has been reported in patients who present with the blastoid variant of MCL [5,7,9,13,16,17].

Histological progression from the common to the blastoid variant of MCL during the course of the disease has been previously reported to occur in 22 to 29% of the patients [5,16,18], and in one series the blastoid variant was present in as many as 14 (70%) of the 20 patients who died of the disease and were examined at

* Corresponding author. Tel.: +358-9-471-61369; fax: +358-9-471-72351.
E-mail address: erkki.elonen@huch.fi (E. Elonen).

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autopsy [5]. Hence, blastoid transformation is not an uncommon phenomenon, and may partly explain the poor long-term prognosis of patients with MCL. However, there is only limited information available in the literature concerning the clinical features and outcome related to blastoid transformation. To our knowledge, factors that predict the blastoid transformation have not been characterised. For that purpose, we re-examined the histopathological material of 127 MCL patients diagnosed and treated in a single institute during a 20-year time period. The frequency of blastoid transformation was analysed in sequential biopsies in patients who originally presented with the common variant of MCL. The clinicopathological features related to blastoid transformation and the predictive factors for transformation were assessed.

2. Patients and methods

2.1. Patients

Patients originally diagnosed with lymphoma of the diffuse centrocytic type (according to the Kiel classification), or the mantle cell type [according to the Revised European American Lymphoma (REAL) classification] from November 1980 to September 1999 were collected from the database of the Department of Pathology, Helsinki University Central Hospital. The histological sections of all patients were re-examined by one of the authors. A total of 127 cases fulfilled the morphological and immunohistochemical criteria of MCL according to the REAL/World Health Organization (WHO) classification [2,19], and were classified at the time of the diagnosis either as the common or the blastoid variant of MCL as previously described in Ref. [19]. 107 (84%) patients presented with the common variant of MCL and 20 (16%) with the MCL blastoid variant. 52 of the patients presenting with the common variant histology had sequential biopsies taken at the time of disease progression, and they form the basis of the present study (Table 1). The 20 patients presenting with the blastoid variant were used as a comparator group in the analysis of overall survival (OS). The clinical features at presentation were roughly similar between the 52 patients who had a subsequent biopsy taken and the 55 patients who had no rebiopsies taken at the time of disease progression or recurrence, and who were therefore excluded from the present study (Table 2). The median age of the patients with a repeat biopsy was 62 years (range 44–79 years). The median follow-up time of the surviving patients was 65 months (range 24–153 months). All 20 patients who had the blastoid variant of MCL at the time of diagnosis have died, and their median OS time was 11 months (range 0.5–57 months).

Table 1
Selection of the patients

	n
All patients diagnosed with MCL	127
Excluded	
Blastoid variant at diagnosis	20
No rebiopsy material	55
Evaluable patients	52

MCL, mantle cell lymphoma.

2.2. Morphological features in common MCLs at diagnosis

The diagnostic material was obtained from a lymph node ($n=34$), pharyngeal tonsil ($n=7$), gastrointestinal tract ($n=2$), bone marrow ($n=4$), or from another extranodal site (n = 5). Immunophenotyping by immunohistochemistry (paraffin-embedded and/or frozen tissue sections) and/or by flow cytometry was performed in all cases. The lymphomas of all 52 patients were CD20- and/or CD19-positive. All 35 examined lymphomas were IgM-positive, and 29 out of 32 IgD- and 42 out of 47 CD5-positive, respectively. Immunohistochemical staining for cyclin D1 expression performed with the antibody cyclin D1-GM (Novocastra Laboratories Ltd., Newcastle, UK, dilution 1:25) was positive in 45 out of the 48 (94%) studied cases on deparaffinised tissue sections. In all four cases where a bone marrow biopsy (and an aspirate) was the only diagnostic material available, cyclin D1 expression was present in the marrow biopsy sample.

2.2.1. Architectural pattern of lymphoma

The architectural pattern of the lymphoma was defined as the mantle zone pattern when more than one half of the section area consisted of broad mantle zones of lymphoma cells surrounding reactive germinal centres. In the nodular pattern more than one half of the section area consisted of tumour cells organised into nodules. The remaining MCLs were classified as diffuse. The growth pattern was not evaluated in samples obtained from the bone marrow.

2.2.2. Proliferative activity and p53 protein

The cell proliferation rate was assessed by mitotic counting and by Ki-67 immunostaining (polyclonal Ki-67 antigen DAKO, Glostrup, Denmark, dilution 1:200). Mitoses were counted from 10 high-power fields (HPFs, ocular 10×, not wide field, objective 40×, the surface area of a HPF was 0.2 mm^2). The level of Ki-67 expression was assessed using a point-counting ocular grid. At least 200 grid cross-sections falling over the lymphoma cells were evaluated in each case, and the number of cells expressing Ki-67 and located at the grid cross-sections was counted. In patients who had both a

Table 2
Clinical characteristics of 107 patients with the common variant of MCL at the time of diagnosis. 52 patients with a rebiopsy taken at the time of disease progression (rebiopsy +) were included in the present study

Parameter	Included patients (Rebiopsy +) *n* (%)	Excluded patients (Rebiopsy −) *n* (%)	*P* value
Total	52	55	
Age >60 years	33 (63%)	42 (76%)	0.145
Male sex	39 (75%)	30 (55%)	0.027
Stage III-IV	41 (79%)	45 (82%)	0.699
B symptoms	13 (25%)	20 (36%)	0.203
Performance status (WHO) ⩽1 ($n=105$)	45 (88%)	42 (78%)	0.155
Largest tumour ⩾10 cm	8 (15%)	11 (20%)	0.503
Extranodal involvement			
>1 site ($n=101$)	24 (47%)	20 (40%)	0.474
Bone marrow ($n=98$)	33 (66%)	24 (50%)	0.108
Blood	6 (12%)	6 (11%)	0.944
Splenomegaly	21 (40%)	19 (35%)	0.527
International Prognostic Index ($n=94$)			
Low	9 (19%)	7 (15%)	
Low-intermediate	15 (32%)	15 (32%)	
High-intermediate	13 (28%)	17 (15%)	
High	10 (21%)	8 (17%)	0.680[a]
Haemoglobin ⩽125 g/l ($n=100$)	22 (45%)	23 (45%)	0.984
Leucocyte count $>10\times10^9$/l ($n=100$)	9 (18%)	18 (35%)	0.057
Lymphocyte count $>4.8\times10^9$/l ($n=94$)	6 (13%)	8 (17%)	0.506
Platelet count $<140\times10^9$/l ($n=99$)	14 (29%)	14 (28%)	0.224
ESR <20 mm/h ($n=98$)	24 (50%)	33 (66%)	0.108
C-reactive protein >10 mg/l ($n=77$)	16 (43%)	25 (63%)	0.091
LDH ⩾450 U/l ($n=96$)	19 (40%)	22 (45%)	0.658
Thymidine kinase ⩾5.0 U/l ($n=43$)	15 (75%)	18 (78%)	0.488

ESR, erythrocyte sedimentation rate; LDH, serum lactate dehydrogenase; WHO, World Health Organization.
[a] Low and low-intermediate vs. high-intermediate and high risk groups.

lymph node and a bone marrow biopsy taken at the same time, mitoses were seen in the lymph node specimen, but usually not in the lymphoma infiltrates in the bone marrow. Therefore, the proliferative activity was not evaluated from the bone marrow biopsies.

Expression of p53 protein was studied immunohistochemically (monoclonal DO-7 antigen DAKO, Glostrup, Denmark, dilution 1:50). The specimen was classified as positive for p53 expression when 5% or more of the lymphoma cell nuclei stained positively. The standard streptavidin-biotin peroxidase technique with 15 min of microwave pretreatment in 10 mmol/l citrate buffer (pH 6.0) was used in the immunostainings. Formalin-fixed deparaffinised 4 μm tissue sections were incubated with the primary antibody overnight at 4 °C, and the sections were developed using 3-amino-9-ethylcarbazole (AEC) reagent. Sections of breast carcinoma with known expression for Ki-67 and p53 protein were used in each experiment as positive controls.

2.3. Rebiopsies

At the time of disease recurrence or progression one or more histological rebiopsies were taken of 40 patients (lymph node, $n=21$; tonsil, $n=3$; gastrointestinal tract, $n=5$; spleen, $n=3$; bone marrow, $n=28$; or from another extranodal site, $n=6$). The cytological variant and the growth pattern were assessed from each biopsy, and the mitotic score was counted (except for the bone marrow biopsies). In an additional 12 patients, cytological samples (peripheral blood smear and bone marrow aspirates) had been taken at the time of disease progression. Morphological analysis of these samples was performed from May–Grünwald–Giemsa stained smears.

2.3.1. Definition of blastoid transformation

Blastoid transformation was diagnosed when any of the rebiopsies taken from the patient showed the blastoid morphology. The blastoid variant was defined in rebiopsies as at the time of the primary diagnosis according to the WHO criteria [19]. The proportion of cells with the blastoid appearance was counted in the lymphoid infiltrates of the bone marrow. A large-sized lymphoid cell was considered as blastoid, provided that it had a small to moderate amount of pale or slightly basophilic cytoplasm and a fine or only minimally condensed nuclear chromatin. The specimen was defined as blastoid MCL if more than 30% of the lymphoid cells had the blastoid morphology. One thousand cells in each specimen were evaluated.

2.4. Treatment

All patients received active treatment. One patient with stage I disease was primarily treated with local radiotherapy only. Chlorambucil (with or without prednisone), or CVP (cyclophosphamide, vincristine, prednisone) were given as first-line treatment to 10 patients. The rest of the patients received more intensive, usually anthracycline-based combination chemotherapy [CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone with or without bleomycin) or CNOP (mitoxantrone instead of doxorubicin, $n=18$), M-BACOD (high-dose methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone, $n=16$), ESHAP (etoposide, methylprednisolone, cytarabine, cisplatin, $n=5$), or MEA (etoposide, cytarabine, mitoxantrone, $n=2$)]. Patients without a satisfactory response to the first-line therapy were given various chemotherapy regimes and/or radiotherapy. Therapies given after blastoid transformation of the disease are shown in Table 3.

2.5. Assessment of response and statistical methods

Response to treatment was evaluated as previously described by Miller and colleagues in Ref. [20]. The time intervals between the diagnosis and the date of rebiopsy, and survival after rebiopsy were calculated. Overall survival (OS) was defined as the time interval between the date of diagnosis and death. The patients who were still alive at the time of the last follow-up were censored when survival-times were calculated. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical program 9.0 for Windows. The chi-square test and Fisher's exact test were used to analyse contingency tables. Mann–Whitney U-test was used to compare non-normal distributions between two groups. The time from the diagnosis to the blastoid transformation, survival after the blastoid transformation, and OS were estimated using the method of Kaplan and Meier. The univariate survival analyses were performed using the Mantle–Cox and Wilcoxon tests. All P values are two-tailed.

3. Results

3.1. Histopathological features of the common variant MCLs at diagnosis

The architectural pattern of lymphoma at the time of diagnosis could not be defined reliably in 6 of the 52 cases due to a lack of tissue (a bone marrow biopsy only was available, $n=4$; an insufficient size of biopsy, $n=2$). In the remaining 46 cases, the mantle zone growth pattern was present in 1 (2%), the nodular pattern in 10 (22%), and the diffuse pattern in 35 (76%). The median mitotic score was 4 (range 0–22), and the median level of Ki-67 expression 19% (range 4–42%). p53 overexpression was present in 3 (8%) of the 36 examined lymphomas.

3.2. Histopathological changes during the course of the disease

Cytological progression from the common to the blastoid morphology occurred in 18 (35%) out of the 52 patients primarily with the common variant of MCL (Tables 3 and 4). The minimum estimated risk of blastoid transformation was 24% at three years and 42% at five years of follow-up (Fig. 1).

Changes in the growth pattern of lymphoma and in the mitotic score could be evaluated in 28 patients (including two patients showing blastoid transformation) from the longitudinally taken histological biopsies. The growth pattern remained unchanged in 24 (86%) of the patients (diffuse, $n=23$; nodular, $n=1$). In three lymphomas, the growth pattern changed from the nodular to the diffuse type, and in one patient from the diffuse to the nodular type, respectively. The median mitotic score was higher at the time of disease progression (median 6, range 0–27) than at the time of the diagnosis (median 4, range 0–22).

3.3. Characteristics related to the blastoid transformation

The clinical features, treatment, and outcome of the 18 patients whose lymphoma showed blastoid transformation during the course of the disease are presented in Tables 3 and 4. At diagnosis, most of these patients had an advanced stage of the disease, and 6 patients had lymphoma cells present also in the peripheral blood. However, the performance status remained good (WHO 0 or 1) in all but 2 patients, and only 6 patients presented with B symptoms. The growth pattern of the lymphoma was diffuse in 12, nodular in two, and not evaluable in four cases. 16 of the patients responded well (achieving a CR or a PR) to the primary treatment given at the time of diagnosis of MCL.

Blastoid transformation took place in 10 patients at the time of the first lymphoma relapse, in 1 patient at first progression of refractory disease, and in 7 patients during later progression of the disease. At the time of transformation, all patients had advanced disease with bone marrow involvement, and lymphatic cells with the blastoid morphology were detected in the peripheral blood in all except 2 patients. In six cases (nos. 1, 2, 4, 6, 8 and 11 in Tables 3 and 4) no histological biopsy had been taken at the time of disease progression, and the diagnosis of transformation was based solely on morphological findings in a bone marrow aspirate and in

Table 3
The related clinical features and outcome in 18 patients showing blastoid transformation of MCL during the course of the disease

No.	Status and sites of disease at the time of transformation			Site of rebiopsy[a]	Time from diagnosis to transformation (months)	Treatment after transformation and its results		Survival after transformation (months)
1	1st	Progression	GL, BM, blood	Blood	33	CHOP×1	PD	1.4
2	1st	Relapse	GL, BM, spleen, blood	BM aspirate, blood	19	Symptomatic	PD	3.2
3	1st	Progression	GL, BM, blood	BM aspirate and biopsy, spleen	15	CNOP×2, vincristine + doxorubicin	NR	3.8
4	1st	Progression	GL, BM, blood	blood	30	Symptomatic	PD	0.5
5	1st	Progression	LN, BM, spleen, blood	BM aspirate and biopsy, LN, spleen	10	Ifosfamide, HD-Mtx, dexamethasone + allogeneic BMT	CR	9.6
6	2nd	Progression	GL, BM, blood	BM aspirate, blood	21	TAD×1	PD	0.7
7	2nd	Progression	GL, BM, blood	BM aspirate and biopsy, blood	48	ALL-86×4, ESHAP×2, CEP×3	NR→PD	10.3
8	1st	Relapse	GL, BM, blood	BM aspirate	53	CEP×3 + chlorambucil	PR	23.6
9	3rd	Progression	GL, Waldeyer, BM, blood	BM aspirate[c], blood	70	Chlorambucil + COP×1	PD	3.8
10	1st	Progression	GL, BM, spleen, blood	BM biopsy, blood	23	Symptomatic	PD	2.5
11	1st	Relapse	BM, blood	BM aspirate, blood	33	CNOP×5, PMP×2	NR→PD	7.3
12	3rd	Progression	Waldeyer, subcutis[b]	Lymph node	61	Local RT + chlorambucil, carmofur 200 mg/day	PD	6.6
13	2nd	Progression	GL, BM, blood	BM aspirate[c]	53	CEP×1, chlorambucil + prednisone	PD	2.4
14	1st	Progression	GL, BM, spleen, blood	BM aspirate and biopsy, blood	13	Chlorambucil	PD	3.1
15	1st	Relapse	BM, blood	BM aspirate and biopsy	19	Cyclophosphamide	PD	0.4
16	2nd	Progression	BM, spleen	BM aspirate and biopsy	46	Fludarabine 50 mg×3, dexamethasone	NR→PD	7.3
17	2nd	Progression	GL, BM, blood	BM aspirate and biopsy	48	Symptomatic	PD	3.8
18	1st	Relapse	GL, BM, blood	BM aspirate[c]	26	BFM×1	PD	0.6

GL, generalised lymphadenopathy; BM, bone marrow; LN, lymph node; CHOP, prednisone, cyclophosphamide, doxorubicin, vincristine; CNOP, prednisone, cyclophosphamide, mitoxantrone, vincristine; HD-Mtx, high-dose methotrexate; BMT, bone marrow transplantation; TAD, cytarabine, tioguanine, daunorubicin; ALL-86 chemotherapy cycles used in treatment of acute lymphatic leukaemia including mitoxantrone, etoposide, cytarabine, daunorubicin, dexamethasone, teniposide, vincristine, methotrexate; ESHAP, etoposide, methylprednisolone, cytarabine, cisplatin; CEP, lomustine, etoposide, prednimustine; COP, cyclophosphamide, vincristine, prednisone; PMP, prednisone, methotrexate, mercaptopurine; RT, radiotherapy; BFM, vincristine, high-dose methotrexate, ifosfamide, dexamethasone, teniposide, cytarabine; PD, progressive disease; NR, no response; CR, complete remission; PR, partial remission.

[a] Detected transformation.

[b] Infiltration of MCL detected at autopsy also in gastrointestinal tract, kidneys, left lung, lymph nodes in abdominal and thoracic cavity.

[c] Less than 20% of lymphatic cells showed blastoid cytology in the corresponding bone marrow biopsy.

Table 4
Histological and clinical features at diagnosis of the 18 patients with the transformation from the common to the blastoid variant of MCL during the course of the disease

No.	Clinical features at diagnosis						Growth pattern at diagnosis	Mitotic score[a] at diagnosis	Primary treatment and it's result (duration in months)		OS (months)
	Age (years)/ sex	Stage and BM/PB involvement		PS (WHO)	IPI	LDH					
1	77/M	IVB	BM+, PB+	1	N.D.	N.D.	Diffuse	4	CHOP×7	PR (8)	35
2	74/F	IVB	BM+, PB+	2	4	305	Diffuse	2	CHOP×8	CR (10)	22
3	80/F	IVA	BM+, PB+	1	4	600	N.D.	N.D.	Chlorambucil + Prednisolone	PR (12)	19
4	58/M	IVA	BM+, PB+	1	2	1520	N.D.	N.D.	MEA×3 + CHOP×6	PR (14)	31
5	51/M	IVA	BM+, PB+	0	2	351	N.D.	N.D.	Fludarabine×3	NR	20
6	46/M	IIIA	BM−, PB−	0	N.D.	N.D.	Diffuse	7	CHOP×4	NR	21
7	45/M	IIA	BM−, PB−	0	1	342	Diffuse	13	M-BACOD×6	CR (29)	58
8	61/M	IVA	BM+, PB−	0	3	727	Nodular	8	M-BACOD×9	CR (31)	77
9	68/F	IVA	BM+, PB−	0	2	393	Diffuse	5	CHOP×10	CR (13)	74
10	60/M	IVA	BM+, PB−	1	2	643	Nodular	12	CNOP×4	PR (12)	26
11	72/F	IVA	BM+, PB+	0	3	455	N.D.	N.D.	ESHAP×5 + CHOP×4	CR (14)	40
12	46/M	IVA	BM+, PB−	1	3	565	Diffuse	14	M-BACOD×10	CR (6)	67
13	57/M	IVB	BM+, PB−	1	2	1914	Diffuse	15	CHOP×7	PR (42)	56
14	75/F	IVB	BM+, PB−	1	4	667	Diffuse	5	ESHAP×6	PR (8)	18
15	63/M	IVA	BM+, PB−	1	3	714	Diffuse	11	CHOP×2 + CVAD/MTX-AraC×2	CR (6)	19
16	63/M	IVB	BM+, PB−	1	3	780	Diffuse	2	CHOP×9	CR (22)	53
17	60/F	IVB	BM−, PB−	1	2	329	Diffuse	5	M-BACOD×10 + radiotherapy	CR (13)	52
18	61/F	IVA	BM+, PB−	2	4	714	Diffuse	0	ESHAP×6 + CHOP×3	CR (14)	27

BM, bone marrow; PB, peripheral blood; PS, performance status; IPI, International Prognostic Index; OS, overall survival; N.D., not definable; M, male; F, female; CHOP, prednisone, cyclophosphamide, doxorubicin, vincristine; MEA, etoposide, cytarabine, mitoxantrone; M-BACOD, high-dose methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone; CNOP, prednisone, cyclophosphamide, mitoxantrone, vincristine; ESHAP, etoposide, methylprednisolone, cytarabine, cisplatin; CVAD/MTX-AraC, cyclophosphamide, doxorubicin, vincristine, dexamethasone, methotrexate, cytarabine; PR, partial response; CR, complete response; NR, no response.

[a] The number of mitoses per 10 high-power fields.

peripheral blood smears. In addition, in three cases (nos. 9, 13 and 18) over 50% of the lymphoid cells were regarded as blastoid cells in a bone marrow aspirate, but in the corresponding bone marrow biopsy only a small proportion of the cells fulfilled the criteria of the blastoid variant. In the only case where a bone marrow biopsy but no bone marrow aspirate was taken at the time of disease progression the lymphoma cells had a typical non-blastoid morphology in the bone marrow biopsy.

After detection of blastoid transformation, 13 of the patients received chemotherapy, but with limited success, since only 6 of these patients survived for longer than six months. 1 patient achieved a CR following allogeneic bone marrow transplantation performed two months after the diagnosis of transformation, but an aggressive leukaemic relapse occurred only six months later.

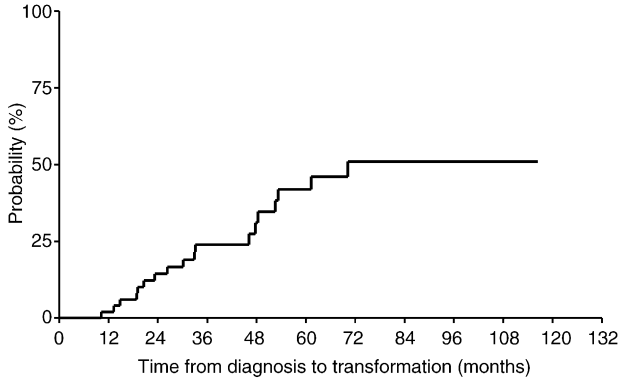

Fig. 1. The estimated risk of blastoid transformation during the course of the disease.

3.4. Factors associated with an increased risk of blastoid transformation

An elevated serum lactate dehydrogenase (LDH) level, peripheral blood leucocytosis, and a high proliferative activity of lymphoma cells assessed by the Ki-67 score at the time of the diagnosis were associated with the development of blastoid transformation during the course of the disease. Only 18% (5 out of 28) of the patients with LDH <450 U/l at presentation developed blastoid transformation compared with 58% (11 out of 19) of the patients with a higher LDH level ($P=0.004$). Similarly, 28% (11 out of 40) of the patients presenting with a leucocyte count of $\leqslant 10\times10^9$/l developed blastoid transformation compared with 67% (6 out of 9) of those with leucocytosis ($P=0.049$). The predictive value of lymphocytosis could not be reliably evaluated due to a lack of data. Blastoid transformation occurred earlier after the diagnosis in patients with a high LDH level or leuvocytosis at presentation (Figs. 2 and 3). In addition,

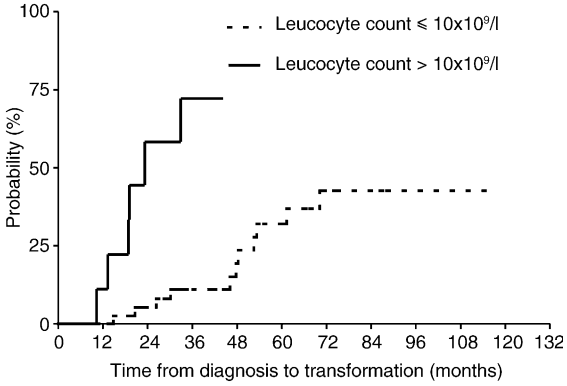

Fig. 2. 11 of the 40 patients with a leucocyte count $\leqslant 10\times10^9$/l compared with 6 of the 9 patients with a leucocyte count $>10\times10^9$/l at diagnosis developed blastoid transformation, $P=0.001$.

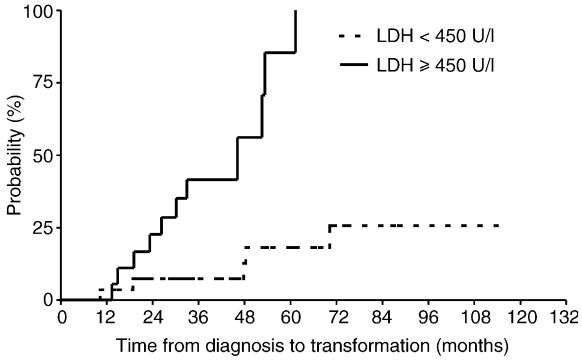

Fig. 3. 5 of the 28 patients with a serum LDH level <450 U/l at diagnosis compared with 11 of the 19 patients with a LDH level $\geqslant 450$ U/l developed blastoid transformation, $P=0.001$.

a high Ki-67 expression level (median 24% versus 17%, $P=0.023$) and a high mitotic score (median 5 versus 3, $P=0.056$) at presentation were typical of the tumours of patients who developed blastoid transformation. 2 of the 3 patients who showed positive p53 expression at the time of diagnosis developed blastoid transformation during the course of the disease.

3.5. Outcome

The median survival time after blastoid transformation was only 3.8 months [95% Confidence Interval (CI): 2.4–5.2 months] as compared with 26 months following the latest rebiopsy in patients without transformation (95% CI: 17–35 months, $P<0.001$) (Fig. 4). The respective median OS-times from the first diagnosis of MCL were 31 (95% CI: 15–47 months) and 60 months (95% CI: 27–93 months, $P<0.001$). Of note, 1 of the 18 patients with blastoid transformation developed a central nervous system lymphoma.

The OS curve of the patients presenting with the blastoid variant is shown for comparison in Fig. 5 (median OS 11 months, 95% CI: 9–14 months).

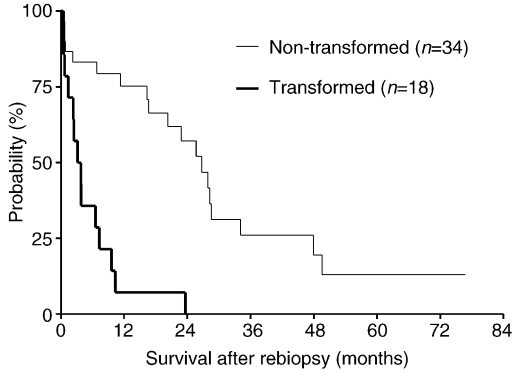

Fig. 4. Survival from the time of the rebiopsy to death of patients with blastoid transformation ("transformed") and of patients with no transformation at the time of disease progression ("non-transformed").

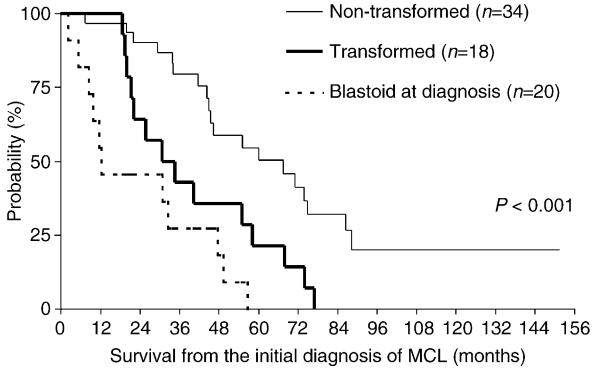

Fig. 5. Outcome of the patients presenting with the common type of MCL, but who later developed blastoid transformation ("transformed"), patients who presented with the common type that did not transform during the course of the disease ("non-transformed"), and patients who presented with the blastoid variant ("blastoid at diagnosis"). Survival was computed from the date of the MCL diagnosis to death.

4. Discussion

In the present study, we reviewed the tissue samples of 52 patients who presented with the common variant of MCL and had sequential biopsies taken during the course of the disease. Blastoid transformation at the time of disease progression was diagnosed in 18 (35%) patients, and the minimum estimated risk of transformation was 24% at three years and 42% at five years of follow-up. This cytological progression was generally associated with an aggressive clinical course with a median survival time of less than four months after the rebiopsy compared with 26 months in patients who did not have blastoid transformation at the time of the rebiopsy. The survival time as computed from the first diagnosis of MCL was also significantly shorter among patients who were later diagnosed with blastoid transformation (median 31 months) than in patients who did not have lymphoma transformation (median 60 months). Patients who present with the blastoid variant

of MCL seem to have even poorer outcome with the reported median survival times ranging from one to two years only [5,7, 9,13,16]. In accordance with these findings, of all 127 MCL patients reviewed for this study, 20 (16%) patients presented with the blastoid variant of MCL already at the time of initial diagnosis. These patients had the poorest outcome of all subgroups investigated with a median OS of only 11 months.

Because this is a retrospective study, we can not exclude the possibility that the results of the 52 patients evaluated with a rebiopsy may be a selected subgroup. However, the clinical characteristics of the 52 patients included in the study and who presented with the common MCL and had a rebiopsy taken were similar to the excluded 55 patients who did not have a rebiopsy taken. This suggests that these two groups of the patients may be biologically comparable.

In line with the present findings, blastoid transformation during the course of the disease has not been found to be uncommon in a few recent series. Norton and colleagues [5] found histological transformation from the common to the blastoid variant took place in 11 (22%) of the 50 patients who had sequential biopsies taken, and in as many as 14 (70%) of the 20 cases where an autopsy was carried out. These authors also reported that the blastoid change at the time of diagnosis or at any other time during the course of the disease was strongly associated with poor survival. In two other studies, based on sequential biopsies, blastoid transformation was reported to occur in 22 and 29% of the MCL patients [16,18]. However, unlike in the present series, blastoid transformation during the course of the disease was not found to have a significant effect on survival following the diagnosis of transformation in these studies, although in one study [16] the blastoid morphology at the time of the initial diagnosis predicted unfavourable prognosis. The authors suggested that a lead-time bias may explain the similar outcome following rebiopsy between patients with and without transformation, since rebiopsy was taken later after the initial diagnosis of patients with transformation (median 34 months) than in those without transformation (median 15 months). In the present study, the time interval between the initial biopsy and the subsequent biopsy was roughly similar in patients with and without detected blastoid transformation (32 vs. 26 months, respectively).

Other histopathological changes, such as changes in the growth pattern of lymphoma or changes in the mitotic activity in sequential biopsies, were relatively uncommon in the present series. However, there was only a limited number of the sequential histological samples available for reliable histological evaluation, since often only a bone marrow aspirate or a marrow biopsy had been taken at the time of disease progression. Histological progression from the mantle zone or the nodular growth pattern to the diffuse growth pattern has been described [5,16,21], but also oscillations between different patterns have been reported to occur during the course of the disease [5]. It is unclear whether a change in the growth pattern has any significance regarding the clinical outcome, but growth pattern progression may occur less frequently than cytological progression in MCL.

Blastoid transformation took place relatively late during the course of the disease. Most patients had had a good response to the primary treatment, and transformation occurred in 40% of the patients during the second or later progression of the disease. However, some adverse prognostic factors at presentation were found to be associated with the risk of blastoid transformation during the course of the disease. Of the clinical features leucocytosis and an elevated serum LDH level were significantly associated with the risk of blastoid transformation, and transformation also occurred earlier after the primary diagnosis of MCL if these features were present at presentation. Blastoid transformation also took place more often in patients with lymphoma showing a high cell proliferation rate at the time of the diagnosis than in patients with lymphoma with a low proliferation rate.

There may be an association between the blastoid morphology and the central nervous system (CNS) involvement in MCL. In the present study, 1 of the 18 patients with blastoid transformation developed a CNS lymphoma after the detection of transformation. In addition, 4 of the 20 patients who presented with the blastoid MCL developed CNS relapse later during the course of the disease. On the other hand, we have found earlier that only 2 of a total of 107 patients diagnosed with the common variant of MCL and who had no evidence of blastoid transformation developed CNS involvement [22]. Montserrat and colleagues [13,23] reported CNS involvement during the course of the disease in seven (12%) of 59 patients with MCL, and CNS lymphoma was related with the blastoid variant in three of these patients, findings that are in line with our data.

More secondary cytogenetic and molecular changes have been detected in aggressive variants of MCL than in the common variant. Lymphomas of the blastoid variant more often have an increased number of chromosomal imbalances and high-level DNA amplifications [24, 25] or tetraploid chromosome clones [26] than the common variant. A higher incidence of *TP53* mutations and a loss of negative cell cycle regulatory proteins, such as $p16^{INK4a}$, have also been associated with the aggressive variants rather than the common variants of MCL [26–29]. However, the molecular pathogenesis related to the development of blastoid transformation is still poorly understood. Aberrations of the *TP53* gene have been suggested as a possible mechanism for lymphoma progression. Greinen and colleagues [27] reported progression from the common to the blastoid cytology in two of the four patients with

MCL with a mutated *TP53* gene. Interestingly, in the present study, 2 of the 3 patients who had the common variant of MCL with a positive p53 expression in immunohistochemical stainings at the time of the diagnosis also developed blastoid transformation during the course of the disease. Molecular studies based on sequential biopsies are needed to establish other putative mechanisms that may be involved in blastoid transformation.

In conclusion, blastoid transformation of MCL during the course of the disease is not uncommon and is related to a very poor patient outcome. Our findings also suggest that development of CNS lymphoma in MCL is strongly related to the blastoid morphology. Leucocytosis, an elevated serum LDH level, and a high cell proliferative activity at the time of the diagnosis were associated with an increased risk of blastoid transformation during the course of the disease, but further studies are needed to confirm this. Identification of the molecular factors predictive for the blastoid transformation is of particular importance.

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