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Blood Clonal B Cell Excess (CBE) at Diagnosis in Patients with Non-Hodgkin Lymphoma (NHL). Relation to Clinical Stage, Histopathology and Response to Treatment

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Abstract—*Untreated non-leukemic (lymphocytes* $\leq 4.0 \times 10^9/l$*) patients with non-Hodgkin lymphoma (NHL) of B cell type often show an excess of B cells in peripheral blood bearing the same light chain isotype as the lymph node tumor cells which may indicate a leukemic spread of the disease. The ratio between* κ- *and* λ*-bearing lymphocytes (normal range* 1.0–3.3*) was studied to evaluate the prognostic significance of clonal B cell excess (CBE) at diagnosis in* 110 *NHL patients. In total* 43% *had a CBE in peripheral blood. Fifty-two per cent of the patients in clincially advanced stages had CBE and* 30% *of the patients in stages I and II. CBE was detected in* 49% *of all patients with low-grade malignant lymphoma and in* 32% *with high-grade malignancy.*

Patients with a normal κ : λ *ratio at diagnosis entered complete remission more often than those with CBE* ($P < 0.01$). *In patients with high-grade but not with low-grade malignant lymphomas remission duration was longer for those with normal* κ : λ *distribution than for the patients with an abnormal ratio* ($P < 0.01$). *Survival was not statistically significantly influenced.*

INTRODUCTION

NON-HODGKIN lymphomas (NHL) constitute a group of tumors which is heterogeneous with respect to histopathology and prognosis. Predictors of prognosis, including histopathology and stage, are not sufficiently reliable which is why the search for other factors to indentify high-risk patients is warranted.

In the majority (85%) of NHL patients the tumor cells express phenotypic characteristics of B lymphocytes. Nine per cent of NHL are T-cell lymphomas and 6% are considered to be of non-T/non-B origin [1].

B-lymphocytes express one immunoglobulin light chain type on their surface, either κ or λ. The ratio between κ-bearing and λ-bearing lymphocytes in peripheral blood from normal donors varies between 1.0 and 3.3 [2]. A ratio outside this range may indicate a clonal B-cell excess (CBE).

In B-lymphoproliferative disorders an abnormal blood κ : λ ratio has been interpreted as a dissemination of tumor cells into the circulation [3]. In NHL patients with a normal lymphocyte count the presence of blood CBE (monoclonal B-lymphocytes) is a common finding [4–8]. The light chain isotype of the CBE in peripheral blood has been shown to be the same as on the lymph node tumor cells [9]. Thus, a large number of patients with otherwise no suspicion of blood involvement might have disseminated spread of the disease. In a preliminary report, the presence of blood monoclonal B-lymphocytes seemed to be associated with poor prognosis [9].

In the present extended material of untreated patients with NHL the analyses have been focused on the possibility of CBE being a factor of prognostic importance.

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Table 1. Patients with clonal B-cell excess in relation to histopathology and clinical stage

Histopathology	Clinical stage I	II	III	IV	Total
CLL	0/0	0/0	0/0	3/3	3/3 (100%)
IC	3/4	0/1	1/4	13/16	17/25 (68%)
CB/CC	3/11	0/3	3/10	5/11	11/35 (31%)
CC	1/3	0/0	1/1	1/2	3/6 (50%)
CBL	3/9	1/4	0/3	2/4	6/20 (30%)
LBL	1/1	0/0	1/1	0/2	2/4 (50%)
IBL	0/4	0/0	0/0	2/4	2/8 (25%)
NOS	1/2	0/2	0/2	2/3	3/9 (33%)
Total	12/34 (35%)	1/10 (10%)	6/21 (29%)	28/45 (62%)	47/110 (43%)

CLL, IC, CB/CC and CC are included in the group of low-grade malignant lymphomas according to the Kiel classification.
CBL, LBL, IBL and NOS are included in the group of high-grade malignant lymphomas according to the Kiel classification.

MATERIALS AND METHODS

Patients

The study comprises 110 previously untreated non-leukemic (WBC $\leq 9.0 \times 10^9$/l and lymphocytes $\leq 4.0 \times 10^9$/l) NHL patients. There were 57 men and 53 women with a median age of 62 years (20–87 years). The initial evaluation of the patients included a detailed history, physical examination, routine blood analysis, bone marrow and lymph node biopsies, chest radiograms and computerized tomography of the abdomen. Staging laparatomy was not routinely performed.

The Kiel classification [1] was used for histopathological diagnosis and the Ann Arbor system for clinical staging [10] (Table 1).

Clinical stage I and II patients with low-grade lymphomas identified as CLL, IC, CB/CC and CC and clinical stage I high-grade lymphomas identified as CBL, IBL, LBL and NOS were primary-treated with irradiation. The target-absorbed doses were 40–45 Gy fractionated during 4 weeks administered by ortho-volume and megavoltage techniques. Patients with low-grade histopathology stages III and IV were treated with chlorambucil and prednisone or multiple chemotherapy, mostly of the COP combination, according to protocols [11]. High-grade malignant lymphomas in clinical stages II–IV were treated by CHOP [12] or CHOP/methotrexate [13].

Controls

Blood lymphocytes from 74 healthy persons were used as controls. The median age was 44.7 years (range 20–74 years). None had signs of infectious or inflammatory disease or was on medication.

Blood lymphocyte counts

The total number of white blood cells were counted in Türk's solution. The percentage of lymphocytes was determined by differential count on blood smear stained by May–Grünewald and Giemsa. The total lymphocyte count was determined.

Blood lymphocyte preparation

Lymphocytes were purified from defibrinated or heparinized venous blood. The leucocyte-rich supernatant obtained after sedimentation of blood in gelatin was treated with iron-powder. Phagocytic cells were removed by a magnet. The lymphocytes were further purified by centrifugation on a Ficoll–Isopaque gradient to remove remaining red blood cells [14]. The lymphocyte suspension contained $\geq$ 98% lymphocytes.

$SmIg^+$ lymphocytes (B-lymphocytes)

$SmIg^+$ lymphocytes were identified by direct IFL with $F(ab')_2$ fragments of a polyspecific fluorescein-conjugated rabbit anti-human-Ig serum (Kallestad Lab., Houston, Texas, U.S.A.) and rabbit antisera against κ and λ light chains (Dakopatt AS, Copenhagen, Denmark). Before staining the lymphocytes were incubated in serum-free medium for 30 min at 37°C and washed 3 times at 37°C to remove absorbed IgG [15]. Membrane fluorescence was examined in a Leitz Dialuxe 20 with epi-illumination in transmitted u.v. light and ordinary light $\times$ 1000 magnification. Four hundred lymphocytes were counted. CBE was defined as a $\kappa : \lambda$ ratio outside the normal range (1.0–3.3).

Preparation of tissue specimens and demonstration of immunological markers on lymph node tissue

Unfixed biopsy material was divided into 3 parts. One part was fixed in B5 fixative for conventional histological examination and PAP-staining [16, 17]. Another part was quick-frozen in streaming CO_2 and stored at $-70°$ C. The frozen biopsies were sectioned at about 5 μm and air-dried at room

temperature. Unfixed cryosections were used for direct IFL. For indirect IFL, dried sections were fixed in cool acetone for 1 min. The third part was minced to produce a cell suspension for single-cell studies by IFL as described above. In addition, immunohistochemical staining by the PAP method was used for detection of immunoglobulins in paraffin-embedded material (antisera and PAP complexes, DAKO, Dakopatt AS, Copenhagen, Denmark) [17].

Statistical methods

In order to test the hypothesis of 2 means being equal against the 2-sided alternative, Student's *t*-test was used.

Survival curves were generated according to Peto *et al.* [18] and differences in survival were analyzed by the log-rank test taking censored data into account.

The Cox proportional hazard linear model was used to determine the relative importance of main prognostic factors [19].

RESULTS

Forty-seven out of 110 (43%) untreated NHL patients had CBE (Table 1). In low-grade malignant lymphomas the total lymphocyte counts (log No./CMM ± S.D., 3.22 ± 0.21) was not different from that of controls (log No./CMM 3.23 ± 0.20). In high-grade lymphoma it was slightly reduced (log No./CMM 3.09 ± 0.21). In all cases where immunophenotyping on lymph node tissues was performed ($n = 43$) the CBE had the same light chain isotype as that found on the tumor lymph node cells (data not shown). In low-grade malignant lymphomas 49% (34/69) of the patients exhibited a blood monoclonal B-cell fraction while the corresponding figure for high-grade malignant lymphomas was 32% (13/41; Table 1). A CBE was found in 52% (34/66) of the patients in advanced stages (III and IV) and in 30% (13/44) of the patients in low stages (I or II) (Table 1).

In total 46% (51/110) of the patients achieved a complete remission (CR). Patients with CBE entered CR less often than those with a normal κ : λ ratio (Table 2). In low-grade malignant lymphomas 62% (21/34) with a normal κ : λ ratio entered CR while only 26% (9/35) with CBE achieved CR. In high-grade lymphomas 62% (8/13) of the patients with no evidence of CBE achieved CR while 46% (13/28) with CBE obtained CR.

The median duration time for first CR for all patient was 18 months (1–83 months). The duration of CR was related to the presence of CBE at diagnosis. In high-grade NHL the CR duration was significantly shorter for patients with CBE than for those with a normal κ : λ ratio ($P < 0.01$) (Fig. 1). In low-grade malignant lymphomas no significant association to CR duration time was noted.

The median survival for patients with low-grade malignant lymphomas was 68 months (1–92 months) and for patients with high-grade malignant lymphomas 27 months (1–84 months). CBE was not associated with survival time either in the group of low-grade or high-grade lymphomas. However, patients with low-grade histopathology and advanced clinical stage with signs of CBE had a median survival of 39 months compared to 60 months for those without CBE but the difference was not statistically significant. In the group of high grade lymphoma patients with advanced clinical stage the corresponding survival time was 8 months for patients with, and 14 months for patients without, signs of CBE. This difference too, was not statistically significant.

The $\hat{\beta}$ values of the Cox regression analysis (Table 3) show that CBE at diagnosis was of significant importance for CR duration time, even more so than stage and histopathology. Total survival was, however, not associated with the presence of circulating monoclonal B-cells while histopathology ($P < 0.01$) as well as stage ($P < 0.01$) were of importance, as expected (data not shown).

DISCUSSION

CBE defined as a ratio between κ-bearing and λ-bearing lymphocytes outside the normal range is a rather frequent finding in NHL of B-cell type [3, 4, 6–8, 20, 21]. The lymphocytes representing the CBE express the same light chain type as the lymph node tumor cells [6, 7]. This suggests that they belong to the malignant B-cell clone though final proof of this conclusion is lacking in the individual patient. The use of anti-idiotypic antibodies and cytogenetic markers may further support this notion [2, 22]. However, the presence of CBE may indicate a leukemic component of the disease.

In the present study 110 untreated non-leukemic NHL patients were studied. A CBE was found in 43% of the patients. This is in good agreement with observations by others [4, 6–8]. In our study, CBE was analyzed by the immunofluorescence technique. Using a more sensitive technique such as immunoflowcytometry monoclonal B-lymphocytes in blood could be detected in about a further 15% of the patients [5, 21].

An abnormal κ : λ ratio was more frequently observed in low-grade lymphomas and in advanced clinical stages. This may indicate that CBE is a sign of advanced disease. However, a substantial number of patients with stages I and II had CBE, which has also been observed by others [8, 21]. This may reflect the fact that our patients were clinically staged, why it is reasonable to assume that a greater number of patients would have achieved a more

Table 2. Complete remission rate in relation to clonal B-cell excess and histopathology

Histopathology	Clonal B cell excess Present	Absent	*P*-value*
Low grade malignant lymphoma ($n = 69$)	26% (9/35)	62% (21/34)	< 0.01
High grade malignant lymphoma ($n = 41$)	46% (13/28)	62% (8/13)	NS
Total ($n = 110$)	35% (22/63)	62% (29/47)	< 0.01

N.S. = not significant.
* Chi-square analysis.

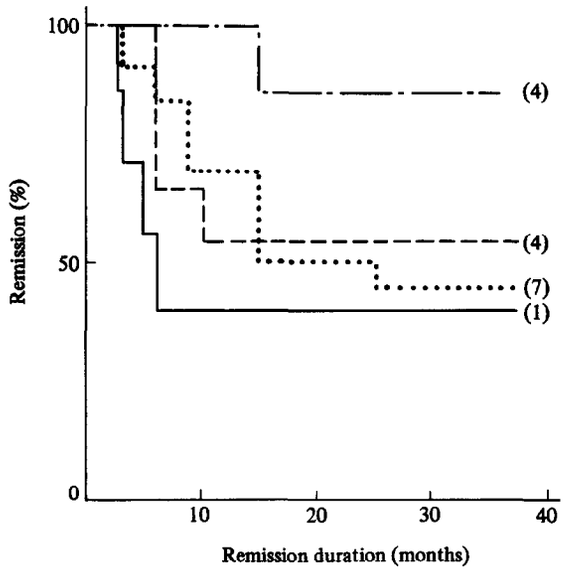

Fig. 1. Complete remission duration time. Low-grade malignant lymphoma patients without CBE at diagnosis (·····) (n = 21) *and with CBE* (-----) (n = 9) *(not significant). High-grade malignant lymphoma patients without CBE at diagnosis* (-·-·-·-·) (*n* = 8) *and with CBE* ( ) (n = 13) (P < 0.01). *Numbers within square brackets indicate patients at risk.*

advanced stage if invasive staging procedures had been used.

Table 3. Analysis of factors influencing complete remision duration time (proportional hazard general linear model; Cox)

Predictor	$\hat{\beta}$ (multiple)	*P*-value
Blood CBE	1.91	0.001
Stage	1.19	0.02
Histopathology	−1.18	0.07

Clonal B-cell excess was related to CR induction. Patients with no sign of CBE entered CR more often than those who had circulating monoclonal B-lymphocytes. In terms of CR duration the presence of CBE was also of importance. CR duration time was significantly shorter for high-grade malignant lymphoma patients with CBE at diagnosis compared to those with a normal κ : λ ratio. Using Cox analysis CBE seems to be an even better predictor for CR duration time than stage and histopathology for the whole group of NHL. The lack of prognostic information of monoclonal B-lymphocytes in low-grade malignant lymphomas with regard to CR duration in the present material may be due to the difficulty of eradicating the low-grade non-Hodgkin lymphoma tumors by treatment and to the prolonged life expectancy of these patients even if they do not achieve CR. The rather short observation time and the limited number of patients in the different subgroups may also be of importance.

Determination of CBE in non-leukemic NHL patients seems to be an important tool to predict response to therapy. We suggest that the analysis should be included in the staging procedure to obtain more detailed information of the spread of the disease. Perhaps CBE might also serve as a guideline for therapy in the future.

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