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Trisomy 12 and lymphoplasmacytoid lymphocytes in chronic leukemic B-cell disorders

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Abstract

Background and Objective. **Although the finding of trisomy 12 in B-cell malignancies has been extensively documented especially in B-CLL, little is known about the clonal involvement of different tissues and there are few sequential studies documenting the development of trisomy 12 during the course of the disease. The aim of this study was, therefore, to: 1) ascertain the prevalence of trisomy 12 by FISH; 2) correlate the findings of trisomy 12 with hematologic and clinical features; 3) study the trisomy 12 positive clone during the course of the disease, and 4) compare findings of trisomy 12 in different tissues.**

Design and Methods. **This is a study of an unselected population of 118 patients with CLL or other B-cell disorders in leukemic phase from a defined geographic area. Trisomy 12 was detected by FISH.**

Results. **Trisomy 12 was found in 18 patients (15%). The aberration was significantly more common in morphologically atypical CLL (aCLL) (24%) and CLL/PL (67%) compared to typical CLL (2%) ($p<0.001$). aCLL cases had predominantly lymphocytes with lymphoplasmacytoid features. Sequential studies of peripheral blood showed an increase in the proportion of trisomic cells during the observation time, mostly associated with disease progression. None of the initially trisomy 12 negative patients acquired the aberration during follow-up. The percentage of lymphocytes exhibiting trisomy 12 was significantly ($p<0.05$) higher in the bone marrow than in peripheral blood.**

Interpretation and Conclusions. **Trisomy 12 might define a distinct disease entity with atypical lymphocytes in chronic leukemic B-cell disorders.**
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Key words: chronic leukemic B-cell disorders, CLL, fluorescence *in situ* hybridization, trisomy 12, atypical lymphocytes

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The chronic B-cell malignancies can be divided into primary B-cell chronic lymphocytic leukemia (CLL) and indolent B-cell lymphomas of which a substantial number have clonal B-lymphocytes in peripheral blood.[1] The clinical course is variable and therefore reliable predictors of prognosis are needed. The most commonly used prognostic factors are patient's age, stage at diagnosis and serum level of lactate dehydrogenase (S-LDH).[2] Advances in immunology and cytogenetics have lead to the recognition of specific disease entities such as mantle cell lymphomas with a specific immunophenotype and a characteristic chromosomal aberration, t(11;14)(q13;q32).[3,4] In follicle center cell lymphomas the translocation, t(14;18) (q32;q21) is an important diagnostic marker.[5,6]

In most chronic B-cell malignancies, standard cytogenetic methods are hampered by the fact that the tumour cells have a low spontaneous mitotic rate and are difficult to stimulate *in vitro*. These problems may be overcome by using polyclonal B-cell activators (PBA) that induce the division of malignant B-cells so that metaphases with chromosomal aberrations can be detected[7,8] or by using fluorescence *in situ* hybridization (FISH) which also allows analysis of interphase cells. In CLL the incidence of trisomy 12 and deletion of the short arm of chromosome 13, del 13(q12-14) detected by FISH is significantly higher than that by metaphase cytogenetics.[9-13]

In CLL trisomy 12 is more common in patients with a clinically aggressive disease than in newly diagnosed asymptomatic patients.[13,14] Some reports have shown that trisomy 12 is uncommon in low-stage disease and in CLL with typical morphology.[14,15] Other studies have suggested that trisomy 12 and del 13q may relate to different clinical behaviors of the disease, i.e. CLL with trisomy 12 has a more unfavourable prognosis than CLL with a normal karyotype, while CLL with del 13q has a better prognosis.[14,16-19] Trisomy 12 has also been described in other chronic B-cell lymphoproliferative disorders such as mantle zone lymphomas[6] and Waldenström's macroglobulinemia,[20,21] but the incidence of

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this abnormality in these subtypes of lymphomas is not known.

Although the finding of trisomy 12 has been extensively documented, little is known about the clonal involvement of different tissues in the same patient. Moreover, there are only few sequential studies documenting trisomy 12 during the course of the disease.[22,23] The aim of this study was, therefore, to: 1) ascertain the prevalence of trisomy 12 as detected by FISH in CLL and other B-cell disorders in leukemic phase in an unselected population of patients from a defined geographic area; 2) correlate the findings of trisomy 12 with hematological and clinical features; 3) study the trisomy 12 positive clone during the course of the disease, and 4) compare the findings of trisomy 12 in different tissues.

Materials and Methods

Patients

All patients with a chronic leukemic B-cell disorder followed at the Division of Hematology, Danderyd hospital between September 1994 and July 1996 were evaluated. All but one case, in complete remission after an allogenic bone marrow transplantation, were included in this study. Leukemic disease was defined as clonal B-lymphocytes $\geq 5\times10^9$/L or a relative lymphocytosis (≥ 60%) with a predominant monoclonal lymphocyte population in the peripheral blood as shown by immunoglobulin (Ig) light chain restriction. Blood, bone marrow and/or lymph node samples were collected from 118 patients. All patients were tested for trisomy 12, forty-seven at diagnosis and 71 later during the course of the disease. Forty-two of these 71 patients had received therapy prior to the test. To exclude the possibility of a selection bias the patient register at the Division of Hematology was compared with the diagnosis register (histopathology) at the Department of Pathology, Danderyd hospital, which is the only pathology department in the area. All patients from the diagnosis register at the Department of Pathology were known at the Division of Hematology. The study was approved by the Medical Ethical Committee at Karolinska hospital.

Table 1. Clinical and hematological features of 118 patients with chronic leukemic B-cell disorders.

	Male	*Female*	*Total (%)*
Number of patients	70	48	118
Tested at diagnosis	33	14	47 (40)
Age at diagnosis (y; median)	69	72	70
Asymptomatic at diagnosis	41	27	68 (58)
Required therapy during follow-up	36	21	57 (48)
Time to treatment (month; median)	4	7	5
Deceased	9	4	13 (11)
Median survival time (months)			162+
Monoclonal band	18	12	30 (25)
Hypogammaglobulinemia	18	17	35 (30)

The main clinical and hematologic features of the patients are described in Table 1. The median age at diagnosis was 70 years and the male:female ratio was 1.5:1. One quarter of the patients had a monoclonal band in serum and/or in urine. Fifty-eight percent of the patients were asymptomatic at diagnosis, while 48% required therapy within 5 months. The median actuarial survival of this series was 162 months and 105 patients were still alive at the time of follow-up.

Diagnostic criteria

Peripheral blood, bone marrow and lymph node smears and/or histological material were routinely classified, according to the updated Kiel classification[24] at the Division of Pathology, Danderyd Hospital. All slides were reviewed and reclassified according to both the REAL[1] classification and the French American British (FAB) criteria[25] by two of us (EM, CS). Peripheral blood and bone marrow aspirates were available for review in all patients, bone marrow trephines in 50 patients (42%) and lymph node aspirates or biopsies in 32 cases (27%).

According to the REAL classification, the cases were classified as follows: B-cell chronic lymphocytic leukemia (CLL; n=100), B-cell prolymphocytic leukemia (PLL; n=1), B-cell small lymphocytic lymphoma (B-LL; n=6), lymphoplasmacytoid lymphoma (LPCL; n=10) and splenic marginal zone B-cell lymphoma (equivalent to splenic lymphoma with villous lymphocytes; n=1). There were no cases with mantle zone lymphoma, follicle centre cell lymphoma or extranodal marginal zone B-cell lymphoma.

CLL was further subdivided into typical and atypical CLL (aCLL)[14] on morphology without considering the immunophenotype. aCLL includes cases with more than 15% of the peripheral blood lymphocytes showing lymphoplasmacytoid features or cleaved nuclei.[6,14,25] CLL in prolymphocytic transformation (CLL/PL) was defined as CLL with ≥10%, but less than 50% prolymphocytes in the peripheral blood.

Since the material included both CLL and leukemic lymphomas, staging according to Rai, Binet or Cotswold was not used. As an indirect measurement of stage and tumor burden, levels of hemoglobin, white blood cell counts, platelet counts and S-LDH were used. Disease progression was defined by the need for treatment.

Flow cytometry (FACS)

Immunophenotyping was performed according to standard methods[26,27] with a direct dual color immunofluorescence staining of mononuclear cells (MNC) obtained from peripheral blood (n=109), bone mar-

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row (n=22) and/or lymph nodes (n=14). The flow cytometer used was a FACScan, Becton-Dickinson, and the reagents were obtained from Becton-Dickinson or DAKO. The panel of monoclonal antibodies (MoAb) included: CD 2 (T11), CD 3 (Leu4), CD 4 (Leu3), CD 5 (Leu1), CD 8 (Leu2a), CD10 (calla), CD 19 (Leu12), CD 20 (Leu16), CD 45 (LCA) and anti-κ and anti-λ human Ig. In 1996 CD 23 (FcεRII) was added to the panel. Peripheral blood mononuclear cells incubated with control MoAbs of matched isotype were included in each analysis. A typical B-CLL immunophenotype was defined as lymphocytes double positive for CD5/CD19 and CD19/sIg^{weak}.

Fluorescence in situ hybridization (FISH)

The probe against chromosome 12 is a 175 bp large α-satellite probe specifying the centromeric region (Oncor, Gaithersburg; MD, USA). Histopaque -1077 (Sigma-Aldrich, St. Louis; MO, USA) gradient separated peripheral blood mononuclear cells were obtained from all patients. The MNC fraction was resuspended in phosphate buffered saline to a concentration of approximately 5×10^9 MNC/L. One or two drops of this cell suspension were dropped on slides and allowed to air dry (approximately 2×10^5 MNC/slide). The films were frozen wrapped in foil paper at –20°C until their use. Bone marrow smears (n=42) and lymph node imprints (n=5) were also analysed. The films were frozen at –20°C. After thawing, the slides were hybridized according to the recommendations from the manufacturer and analyzed in a fluorescence microscope (Zeiss EPI-Fluorescence Axioskop 20). In each case 500 well spread cells were scored for trisomy 12. Cells with three distinct fluorescent signals were considered positive for the aberration. A sample was considered to be positive for trisomy 12 if >1.3% (mean±3SD) of the cells showed three fluorescent signals. This cut-off limit was chosen after analyzing 10 peripheral blood samples from healthy individuals. The proportion of MNC with 3 signals in normals was 0.2±0.3% (mean±SD), range 0-1.0%. Zero signals were found in 0%, one signal in 7.0±2.3%, range 1.2-8.8; and 2 signals in 92.7±2.2%, range 91.2-98.3 in the normal controls. A detailed description of the FISH method used has previously been published.[28]

In 38 cases, analysis for trisomy 12 was performed on cytological material from two different sites at the same time, either peripheral blood and bone marrow (43 paired samples) or peripheral blood and lymph node (5 paired samples). Since the FISH analysis of the bone marrow was not performed on separated MNC, a corrected bone marrow score was calculated by dividing the percentage of trisomy 12 positive cells with the percentage of lymphoid infiltration in the bone marrow. This correction was made to include only lymphoid cells of the bone marrow in the results.

Metaphase cytogenetics

Giemsa banding metaphase cytogenetics was performed in 7 patients with trisomy 12 detected by FISH. Peripheral blood was stimulated for 3-5 days with lipopolysaccharide W from *E. coli* (LPS) and phorbol-12-myristate-12 acetate (TPA) in 10% fetal calf serum.[7] Harvesting, fixation and Giemsa-Trypsin banding were carried out according to standard methods.[29] At least 15 metaphases were analyzed in each case, if possible.

Statistics

In order to evaluate the hypotheses of variables in contingency tables, the chi-square test was used or, in the case of small expected frequencies, Fischer's exact test. Statistical comparisons in order to test differences between groups were made by the use of the Mann-Whitney test. The Students t-test for paired observations was used to calculate the differences between paired observations. The actuarial survival was calculated according to Kaplan-Meier survival plots.[30] In addition, descriptive statistics and graphical methods were used to characterize the data. All analyses were made on the SAS system and the 5%, 1% and 0.1% levels of significance were considered. In case of statistically significant results the probability value (p-value) has been given.

Results

Trisomy 12 in peripheral blood, bone marrow and lymph nodes

Trisomy 12 was detected by FISH in peripheral blood lymphocytes in 18 of 118 (15%) and in the bone marrow in 8 of 38 (21%) patients with chronic leukemic B-cell disorders (Table 2). In each case an extra copy of chromosome 12 was found only in a subpopulation of the leukemic cells (2-54%). When analysing peripheral blood and bone marrow at the same time, the percentage of lymphocytes exhibiting trisomy 12 was significantly ($p<0.05$) higher in the bone marrow (40±29%; mean±SD; corrected score) than in peripheral blood (15±8%; mean±SD). Only patients with trisomy 12 in the peripheral blood showed the aberration in the bone marrow. Trisomy 12 was found in lymph node cells in one patient. This case also exhibited trisomy 12 in peripheral blood lymphocytes, but in a lower relative number than in the lymph node (21 v. 47%). Lymph node and peripheral blood cells were studied in four more patients without finding trisomy 12 in either cell type.

Two patients appeared to have monosomy 12 since only one fluorescent signal was found in 60% and 92%, respectively, of their peripheral blood lymphocytes. The corresponding figures for the bone marrow were 78% and 71%, respectively. The remaining 98 patients exhibited two fluorescent signals in 84-99% (median 92%), zero signals in 0-0.8% (median 0%), one signal in 0.6-14% (median 8%), and three

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Table 2. Results of FISH in 18 patients with trisomy 12. Percentage of hybridization signals in 500 peripheral blood lymphocytes.

	No. of fluorescent spots per cell			
Patient no.	*0*	*1*	*2*	*3*
8	0	1.2	92	6.8
20	0	7	90.4	2.6
25	0	1.8	62	36.2
33	0	7.8	82.6	9.6
34	0	6.2	91.6	2.6
44	0	6	81.2	12.8
49	0	3.8	83.6	12.6
72	0	5	65.6	29.4
88	0.2	7.6	88.6	4
97	0	3.6	83.6	12.8
126	0	9	75.8	15.2
130	0	6.6	84.8	8.6
154	0	8.5	79.5	12
157	0	1.8	54.6	43.6
207	0	3.4	71.6	25
236	0	2.6	70	27.4
237	0	3.8	76	20.2
241	0	4.6	81.6	13.8

Table 3. Number of patients with trisomy 12 in the different subtypes of chronic leukemic B-cell disorders and the finding of monoclonal band in serum and/or urine (Mband).

Diagnosis	*With trisomy 12*	*(Mband)*	*Without trisomy 12*	*(Mband)*
CLL	1	(0)	59	(7)
aCLL	9	(6)	28	(8)
CLL/PL	2	(1)	1	(1)
PLL	0	(0)	1	(0)
LL	2	(2)	4	(2)
LPCL	4	(1)	6	(2)
SLVL	0	(0)	1	(0)
	18	(10)	100	(20)

signals in 0-0.8% (median 0%) of the peripheral blood lymphocytes.

Association between trisomy 12, morphology, immunophenotype and cytogenetics

By morphology, 60 patients were classified as typical CLL, 37 as aCLL and three as CLL/PL. Among the aCLL cases, all had atypical lymphocytes with lymphoplasmacytoid features or cleaved nuclei and none had prolymphocytes. Trisomy 12 was significantly more common in aCLL (9/37, 24%) and CLL/PL (2/3, 67%) than in typical CLL (1/60, 2%) ($p<0.001$; Table 3). The corresponding figures for trisomy 12 among 92 CLL patients with typical and atypical immunophenotype were 7/73 (10%) cases, and 3/19 (16%), respectively (n.s., Table 4). Trisomy 12 was also found in a high frequency in LPCL, where four of 10 patients (40%) had the aberration. The immunophenotype of the PLL patient was CD5/19 and weak light chain positive but the morphology was typical for PLL. In the case with SLVL the flow cytometric analysis was inconclusive. Metaphase cytogenetics of peripheral blood was performed in seven of the cases with trisomy 12 by FISH. All of them had complex karyotypes, but trisomy 12 was only found in five of the cases (Table 5).

Table 4. Immunophenotype of the B-cell clone in patients with and without trisomy 12. Number of patients with typical and atypical immunophenotype in relation to morphology.

	With trisomy 12			*Without trisomy 12*		
Morphology	*CLL*	*aCLL*	*LPCL*	*CLL*	*aCLL*	*LPCL*
Immunophenotype						
*Typical**	0	7	2	48	18	1
Atypical°	1	2	2	6	10	1

**Typical immunophenotype: lymphocytes positive for CD5, CD19 and sIg^{weak}.*
°Atypical immunophenotype: not fulfilling the criterias for a typical immunophenotype.

Table 5. The karyotype in seven patients with trisomy 12 detected by FISH and a comparison between metaphase cytogenetic results and FISH results.

UPN	*Metaphase karyotype: stemline/sidelines*
8	46, XX [8]/46, XX, del(13)(q14?)[5]/ 47, XX, +12, int del (13)[4]/45, X, -X[1]/45, XX, t(2;+), -17[1]/45, XX, csb3p21, -19[1]
25	47, XX, +12[7]/44-46, XX, +12[6]/46, XX[1]/45, XX, -3[1]/43, XX, -14, -17, -22[1]
88	48, XX, +12, +19[9]/47, XX, +12[2]/46, XX[1]/ 44, XX, -12, -21[1]/44, X,-X,-7[1]/47, XX, -4, -5, -7, +12?, +19, +2mar[1]/47, X, -X, +12, +19[1]/46, XX, -8, -17, +12, +19[1]/46, X, -X, -10, +12[1]/ 45, X,-X, -10, +12[1]/ 44, X, -X, -5, -7, -9, -14, -15, +19, add 9, +2 mar[1]
126	41-47, XY, +12[16]/46, XY[1]
154	43-47, XY, +12[10]/42-46, XY[4]
207	41-46, XY[7]
237	46, XX[3]/45, XX, -8[1]/ 45, XX, -12[1]/ 43, XX, -14, -17, -17[1]/ 42, XX,-4, -8, -11, -17[1]

UPN: unique patient number.

Association between trisomy 12 and clinical features

Clinical and laboratory features were compared between the 18 cases with and the 100 without trisomy 12 (Table 6). There were no significant differences between the groups regarding sex, age, hemo-

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Table 6. Clinical and laboratory features in 118 cases of chronic leukemic B-cell disorders patients with and without trisomy 12.

	Patients with trisomy 12	*Patients without trisomy 12*	*p-value**
Number of patients	18	100	
M/F	1.25	1.5	
Age (years; median)	66	70	
Hemoglobin (g/L; median)	124	132	
WBC ($x10^9$/L; median)	27	20	
Platelets ($x10^9$/L; median)	240	202	
LDH (g/L; median)	7.5	6.3	p=0.004
Tested at diagnosis	6/18	41/100	
Asymptomatic at diagnosis	7/18	61/100	
Required therapy	13/18	44/100	p=0.04
Time to treatment (month; median)	4	6	
Deceased	4/18	9/100	
Survival (month; median)	94	132+	p<0.05
P-/U-Monoclonal band	10/18	20/100	p<0.001
Hypogammaglobulinemia	6/18	29/100	

**Student's t-test and Fischer's exact tests were used.*

globin levels, WBC counts, platelet counts or B-symptoms (e.g. weight loss, night sweat and fever). Patients with trisomy 12 had a significantly higher level of S-LDH (p=0.004) and a significantly higher number of the patients required therapy during follow-up (p=0.04). Ten of 18 patients (56%) with trisomy 12 had a monoclonal band compared to 20 of the 100 patients (20%) without trisomy 12 (p<0.001; Table 6). The actuarial median survival time for patients with trisomy 12 was 94 months and was not reached in the other group (132+ months) (p<0.05; Figure 1). There was no difference between the groups in the degree of lymphadenopathy or the percentage of lymphocyte infiltration in the bone marrow (data not shown).

Sequential FISH analysis and clinical course in patients with trisomy 12

Nine patients with trisomy 12 were followed with repeat FISH analysis (2-6 times) during a period of 2 to 21 months (median: 12 months). The aberration was found in every test that was performed throughout the observation period, irrespective of whether therapy had been given or not. Seven of the patients had an increasing proportion of cells with trisomy 12. In 4 of the patients (57%) the increase was associated with a progressive disease. Two patients had a stable proportion of cells with trisomy 12 and a stable clinical course, one with and one without therapy. In two cases there was a decline in the proportion of trisomic cells after therapy with fludarabine (Figure 2).

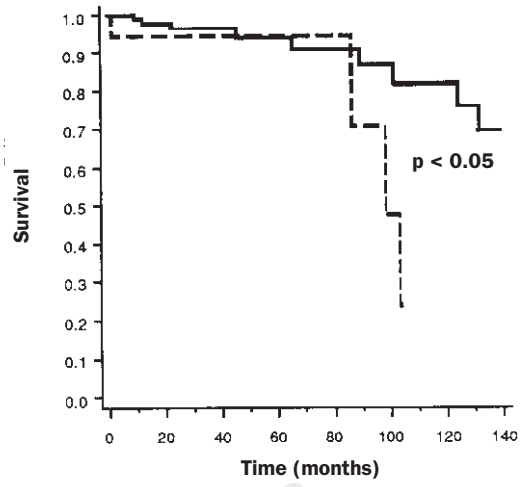

Figure 1. Kaplan-Meier survival plots. Patients with trisomy 12 (- - -) and patients without trisomy 12 (—).

Sequential FISH analysis and clinical course in patients without trisomy 12

Thirty-one patients without trisomy 12 in the first sample were followed with repeat FISH analysis (2-4 times) for a median observation time of 10 months (range 1-21 months). An extra copy of chromosome 12 did not appear in subsequent tests in any of the patients, despite the fact that 17 of the 31 patients (55%) had clinically progressive disease, which in 14 cases (82%) required therapy.

Discussion

We have used FISH to study the occurrence of trisomy 12 in an unselected group of patients with chronic leukemic B-cell disorders from a well defined geographic area in Sweden. Trisomy 12 was found in 15% of all patients, and in 12% of the patients with CLL. A number of earlier reports on trisomy 12 in chronic leukemic B-cell disorders,[6,31,32] particularly in CLL[9-12,14,15] have showed a frequency of trisomy 12 varying between 8 to 30% of the patients. The different results are probably because of selection mechanisms and the use of different cytogenetic techniques. Patient materials with many referral cases are likely to include fewer cases with an indolent clinical course and thus relatively more patients with a progressive disorder, where trisomy 12 is more common.[14-19,33] Moreover, by FISH it is possible to detect almost twice as many cases of trisomy 12 than by conventional metaphase cytogenetics.[9-12,22,34] Therefore, it is likely that our use of FISH in an unselected material of patients accurately reflects the true prevalence of trisomy 12 in chronic leukemic B-cell disorders.

CLL can be divided by cytomorphology into typical

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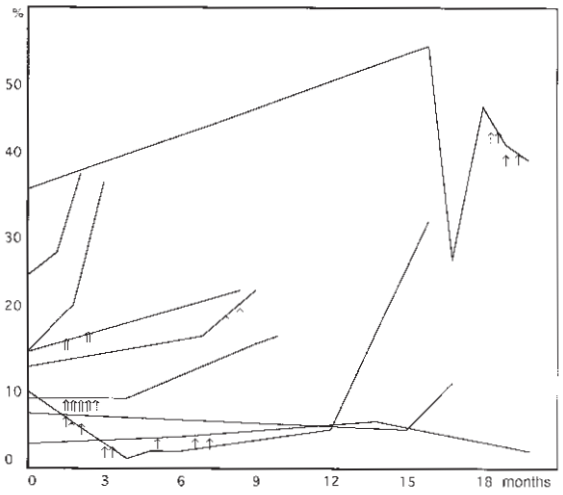

Figure 2. The evolution of trisomy 12 over time in nine individual patients. ⇑ = course with alkylating drug; ↑ = course with nucleotide analog).

CLL and atypical CLL (aCLL).[14,25] Que *et al.*[11] showed that trisomy 12 was more commonly found in aCLL than in typical CLL. Several studies have later confirmed this observation,[14,15,23,33-37] showing trisomy 12 in 20 to 65% of aCLL. No case of typical CLL with trisomy 12 was reported in one study,[36] while others showed up to 13%.[14,15,23,33,35,37] In the present study we found trisomy 12 in 24% of patients with aCLL but only in one case (2%) with typical CLL. However, this particular patient had an atypical immunophenotype without CD5 expression. Thus, when typical CLL was defined by both morphology and immunophenotyping we did not find any case with trisomy 12.

The majority of our patients with aCLL had atypical lymphocytes with lymphoplasmacytoid features, rather than prolymphocytes. This is in contrast to some earlier reports where aCLL with prolymphocytic features dominated.[14,22,33] The reason for the relative overweight of patients with lymphoplasmacytoid aCLL in our material is not clear, but might reflect epidemiological differences (Matutes, unpublished observation). The finding of trisomy 12 in two of three CLL/PL patients concur with Criel *et al.*,[33] but the main finding in our study is that aCLL with lymphoplasmacytoid differentiation is strongly linked to trisomy 12. The aberration was also seen in 40% of cases with LPCL, confirming the earlier observations[20,21] of an association between trisomy 12 and Waldenström's macroglobulinemia. In addition, we could show a significant correlation between trisomy 12 and the presence of a monoclonal band. Our findings suggest that there is a closer relationship between trisomy 12 and B-cell disorders with lymphoplasmacytoid differentiation than earlier thought.

When simultaneous samples from peripheral blood and bone marrow were studied by FISH there was complete concordance between the findings in the two cell types. All patients with trisomy 12 in the bone marrow also showed the aberration in peripheral blood, while the negative cases where negative in peripheral blood as well. The same was observed also in the few cases where lymph node biopsies and peripheral blood were compared. An unexplained finding was the significantly higher proportion of trisomic cells in the bone marrow compared to the peripheral blood. Trisomy 12 has been linked to proliferating cells,[38,39] and it might be that the leukemic growth fraction is higher in the bone marrow than in the peripheral blood. The higher proportion of trisomy 12 in the bone marrow might also be an explanation of the varying frequency of trisomy 12 found in different studies. Most studies are performed on both bone marrow and peripheral blood and the results are given without specifying the origin of the sample.

The present results clearly show that it is sufficient to analyze the peripheral blood in order to detect cases with trisomy 12 in this group of patients. Similarly to earlier reports,[15,22,35,40] trisomy 12 was found only in a subpopulation, in our study ranging from 2 to 59% of the leukemic cells. This may indicate that trisomy 12 is a secondary event in the leukemogenesis of CLL.[22] Repeat FISH analysis in this study showed that no patient acquired trisomy 12 during the observation period even in cases with progressive disease and that trisomy 12 was a constant finding among the patients that exhibited the aberration initially. Therefore the appearance of the trisomy 12 clone must be an early event which takes place before the leukemia becomes clinically manifest. An exception is the acquisition of trisomy 12 reported in some CLL cases undergoing Richter transformation.[22,41]

In a number of studies, trisomy 12 in CLL has been associated with a clinically more advanced disease and a worse prognosis.[13-19,33,42] We found that patients with trisomy 12 had significantly higher levels of S-LDH, which is a marker of disease activity,[2] needed therapy more often and had a shorter survival compared to the disomic group. However, the concept of trisomy 12 as an independent prognostic marker in CLL was recently challenged in favor of an atypical lymphocyte morphology or an atypical immunophenotype.[37,43] It might be that trisomy 12 is not associated with a worse prognosis per se but is a marker of a distinct disease entity with atypical leukemic cells and an aggressive clinical course. In one study, chromosome 17 abnormalities seemed more important for prognosis than an extra chromosome 12, especially when trisomy 12 appeared as a single aberration.[37] In our study metaphase cytogenetics was done in seven cases only, all showing complex chromosomal rearrangements. One patient had both trisomy 12 and del 13q which is uncommon but previously described.[39,44] Another patient

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had a clone that showed both trisomy 12 and trisomy 19. The impact of trisomy 19 in chronic B-cell lymphoproliferative disorder is unknown and has been reported in only a few cases.[45,46] In two cases it was not possible to identify the extra chromosome 12 in metaphase preparations, thus again showing the advantage of FISH over conventional cytogenetics in detecting defined chromosomal aberrations. Interestingly, we found two cases with monosomy 12 but the significance of this is not known.

Contributions and Acknowledgments

Dr. Hjalmar, Dr. Kimby and Dr. Hast were the principal investigators, designed the study and wrote the paper with Dr. Matutes. Dr. Hjalmar, Dr. Jacobsson and Ingrid Arvidsson performed the FISH analysis. Dr. Matutes and Dr. Sundström reviewed all specimens included in the study.

The criteria for the order in which the authors appear is based on the amount of work engaged in the study except for Dr. Hast who is the last name since he is the head of the department were the study was performed.

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Disclosures

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