We performed FISH using specific centromeric probes for chromosomes 3, 7, 11 and 18 on cultured peripheral blood cells, because these are frequently involved in plasma cell dyscrasias.[3,4,6] We evaluated 500 nuclei per probe. Trisomy 3 was detected in 34.8% of the cells and trisomy 18 in 27.2%. Disomy for chromosomes 7 and 11 (94.2% and 91.2% of the cells respectively) was observed.

The combination of MGG staining and FISH with a centromeric probe for chromosome 18 was performed as described by Anastasi *et al*.[5] Previously photographed cells were relocated for evaluation of FISH signals on peripheral blood lymphocytes and plasma cells. One hundred and fifty-two cells were studied: 82/116 plasma cells and 10/36 lymphocytes could be tested for FISH signals. Trisomy 18 was found in 65% of the plasma cells (52/82) and in 1 out of 10 lymphocytes (Figures 1 and 2). We observed that not all but a large proportion of plasma cells had trisomy 18, suggesting that the numerical cytogenetic abnormality could be a secondary change. In addition, we detected trisomy 18 in only one out of ten circulating lymphocytes. Although clonotypic rearrangements as defined by the bone marrow plasma cells in myeloma have been reported among blood lymphocytes, the precise nature of the peripheral blood B cells in MM remains unclear.[7-10] Our results suggest that peripheral blood lymphocytes probably belong to the malignant clone but we can not exclude a false FISH signal.

The present report shows the usefulness of the FISH technique in detecting numerical abnormalities not observed by conventional cytogenetic studies and that the combined MGG-FISH technique is a sensitive test for identifying the cell-lineage of cytogenetic abnormalities.

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***Keywords***

*MGG-FISH, cytogenetics, plasma cell leukemia, PCL, FISH*

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## References

1. Bernasconi C, Castelli G, Pagnucco G, Brusamolino E. Plasma cell leukemia: a report on 15 patients. Eur J Haematol 1989; 43(Suppl 51):76-83.
2. Dimopoulos DA, Palumbo A, Delasalle KB, Alexanian R. Primary plasma cell leukaemia. Br J Haematol 1994; 88:754-9.
3. Drach J, Schuster J, Nowotny H, et al. Multiple myeloma: high incidence of chromosomal aneuploidy as detected by interphase fluorescence in situ hybridization. Cancer Res 1995; 55(17):3854-9.
4. Zandecki M, Laï JL, Facon T. Multiple myeloma: almost all patients are cytogenetically abnormal. Br J Haematol 1996; 94:217-27.
5. Anastasi J, Vardiman JW, Rudinski R, et al. Direct correlation of cytogenetics findings with cell morphology using in situ hybridization: an analysis of suspicious cells in bone marrow specimens of two patients completing therapy for acute lymphoblastic leukemia. Blood 1991; 77:2456-62.
6. Drach J, Angerler J, Schuster J, et al. Interphase fluorescence in situ hybridization identifies chromosomal abnormalities in plasma cells from patients with monoclonal gammopathy of undetermined significance. Blood 1995; 86:3915-21.
7. Kay N, Leong T, Van Ness B, et al. Circulating CD19+ blood cells in multiple myeloma: phenotypic and molecular analysis of ECOG trial E9486/9487. Blood 1995; 86(suppl.1):275a.
8. Pilarski L, Masellis-Smith A, Szczepek A, Mant MJ, Belch AR. Circulating clonotypic B cells in the biology of multiple myeloma: speculations on the origin of myeloma. Leuk Lymphoma 1996; 22:375-83.
9. Billadeau D, Van Ness B, Kimlinger T, et al. Clonal circulating cells are common in plasma cell proliferative disorders: a comparison of monoclonal gammopathy of undetermined significance, smoldering multiple myeloma and active myeloma. Blood 1996; 88:289-96.
10. Zandecki M, Bernardi F, Genevieve F, et al. Involvement of peripheral blood cells in multiple myeloma: chromosome changes are the rule within circulating plasma cells but not within B lymphocytes. Leukemia 1997; 11:1034-9.

## Cytogenetic and in situ hybridization findings in 27 patients with atypical B-cell chronic lymphocytic leukaemia

Sir,

Atypical B-CLL (aCLL) is a cytologically differentiated form of B-cell chronic lymphocytic leukemia (B-CLL) first described by the FAB group.[1] aCLL is the variant that has 10-55% of large lymphocytes, prolymphocytes and/or centrocytes.[1] The most common cytogenetic abnormality associated with aCLL is trisomy 12.[2-7] Other chromosomal abnormalities involve 4q, 6q21-q23, 11q, t(11;14)(q13;q32), 13q14, 17p and 17q.[4-6,8] The aim of the present study is to describe the cytogenetic findings in a series of 27 aCLL, focusing our interest on the detection of trisomy 12, del(13)(q14) and del(17)(p13) combining conventional cytogenetics (CC) with *in situ* hybridiza-

**Table 1. Cytogenetic and ISH features in 27 patients with aCLL.**

| Case | Karyotype (Conventional cytogenetics) | Mitogens used | % Abnormal metaphases | % Trisomy 12 by ISH | % Monosomy 13q14 by ISH | % Monosomy 17p13 By Ish |
|---|---|---|---|---|---|---|
| 1 | 46, XX [51] | PHA, PWM, LPS | 0% (0/51) | 24% | 1% | 0% |
| 2 | 46, XY [38] | PHA, PWM, LPS | 0% (0/38) | 1% | ND | ND |
| 3 | 46, XY [18] | PHA | 0% (0/18) | 2% | ND | ND |
| 4 | 47, XY, +12 [35] | PHA, PWM, DXS+PHA | | | | |
| | 46, XY [15] | PHA, PWM, DXS+PHA | 70% (35/50) | 81% | 0% | 3% |
| 5 | 46, XY [20] | NM | 0% (0/20) | 3% | 13.5% | 0.5% |
| 6 | 46, XX [18] | TPA, PWM | | | | |
| | 46, XX, +12, -17 [2] | TPA, PWM | 10% (2/20) | 37% | ND | ND |
| 7 | 46, XY [29] | PHA, PWM, DXS+PHA | 0% (0/29) | 2% | 1% | 1% |
| 8 | 46, XX [39] | PHA | 0% (0/39) | 42% | 2% | 0% |
| 9 | 46, XY[34] | PHA, LPS | 0% (0/34) | 1% | 2% | 2.5% |
| 10 | 46, XX [18] | PHA | | | | |
| | 46, XX, der(17)t(11;17)(p11;p11) [10] | PHA | 56% (10/28) | 3% | ND | ND |
| 11 | 46, XY [32] | PHA | 0% (0/32) | 27% | 1% | 0.5% |
| 12 | 46, XY [35] | PHA | | | | |
| | 47, XY, +12 [4] | LPS | 10% (4/39) | 66% | 2% | 0.5% |
| 13 | 46, XY [25] | PHA | | | | |
| | 45, XY, -13 [2] | PHA | 7% (2/27) | 30% | 1% | 1% |
| 14 | 46, XX [48] | PHA | 0% (0/48) | 60% | 1% | 3% |
| 15 | 46, XY [19] | PHA | | | | |
| | 47, XY, +12 [12] | PHA, LPS | 39% (12/31) | 60% | 2% | 0% |
| 16 | 46, XX [4] | PHA | | | | |
| | 47, XX, +12 [3] | PHA | 43% (3/7) | 70% | 2% | 0% |
| 17 | 46, XY [4]* | NM | | | | |
| | 47, XY, +12 [2]* | NM | 33% (2/6)* | 19%*30% | 1% | 4% |
| 18 | 46, XX [22] | PHA | 8% (2/24) | 70% | | |
| | 47, XX, +12 [2] | PHA | | | | |
| | 46, XX [20]* | NM]* | 0% (0/20)* | 20%* | ND | ND |
| | 46, XX [11]° | PHA° | 18% (3/14) ° | 39%° | | |
| | 47, XX, +ac [3]° | PHA° | | | | |
| 19 | 46, XX [24]* | PHA | 0% (0/24)* | 12%* | ND | ND |
| 20 | 46, XX [46] | PHA | 2% (1/47) | 2% | 6% | 2% |
| | 47, XX, t(3;15)(q27;q26) [1] | PHA | | | | |
| 21 | 46, XY [40] | PHA | 0% (0/40) | 9% | ND | ND |
| 22 | 46, XX [57] | PHA | 0% (0/57) | 2% | 0% | 0% |
| 23 | 46, XY [15] | PHA | 25% (5/20) | 1% | ND | ND |
| | 46, XY, del(7)(q32) [5] | PHA | | | | |
| 24 | 46, XY [30] | PHA | 0% (0/30) | 3% | ND | ND |
| 25 | 46, XY [23] | PHA, TPA | 0% (0/23) | 2% | 0% | 0% |
| 26 | 46, XX [5] | PHA, TPA | 75% (15/20) | 3% | ND | ND |
| | 46, XX, del(14)(q24), der(18)t(18;22)(q21-22;q11-13) del(20)(q11), -22, +mar [15] | | | | | |
| 27 | 46, XX [48] | PHA, TPA | 0% (0/48) | 22% | 2% | 0% |

*NM: No mitogen; Ac: acentric chromosome;*bone marrow sample; °pleural effusion sample; ND: not done (not enough sample to perform ISH).*



tion (ISH) techniques.

Twenty-seven patients with aCLL, diagnosed between 1989 and 1997, were included in this study. Diagnosis was made in all cases according to standard clinical, cytologic and immunologic criteria. CC analyses were carried out on lymphoid cells from peripheral blood stimulated with different mitogens. ISH was performed in all patients using a biotin- or a spectrum green-labeled chromosome 12-specific $\alpha$-satellite DNA probe (Oncor/CEP 12, VYSIS). In those patients from whom material was available (17/27) a spectrum orange-labeled 13q14 band DNA probe and a spectrum orange-labeled 17p13.1 band DNA probe (LSI 13 and LSI p53, VYSIS) were applied to cultured peripheral blood cells. A minimum of 200 nuclei per case were analyzed.

CC and ISH studies are described in Table 1. Clonal karyotypic aberrations were detected in 10/27 cases (37%), six (22%) had trisomy 12, four as a sole anomaly (15%). Other chromosomes involved were 7, 11, 13, 14, 17, 18, 20 and 22. Seventeen out of 27 patients (63%) showed trisomy 12 by ISH but only six cases (22%) by CC. The percentage of trisomic cells ranged between 9 and 81%, the median value being 40%. One patient showed 13q14 monosomy, no patient had monosomy 17p13.1.

Table 2 summarizes the main cytogenetic findings in aCLL reported by other authors. We have confirmed our earlier observation[4] that trisomy 12 represents a distinct morphologic subgroup of B-CLL. The most outstanding finding is the different percentage of trisomy 12 depending on the method used: 22% (6/27) using CC and 63% (17/27) when ISH. In the present series and in others,[2,4-6] the incidence of trisomy 12 was higher with ISH studies were performed. Tabernero *et al*.[9] observed a significant association between trisomy 12 and a high WBC count, but not with clinical stage. In our series, in agreement with others[3-4,9] we did not find any association between the karyotype (normal/abnormal/trisomy 12) and the clinical Binet/Rai stage nor between the karyotype and the WBC count. As far as concerns other chromosome anomalies detected by CC, we observed a del(7)(q32), a monosomy 17 associated with trisomy 12, a der(17) t(11;17) (p11;p11) and a complex karyotype involving del(14) (q24) and der(18)t(18;22)(q21-22;q11-13).

**Table 2. Comparison between % +12 detected by conventional cytogenetics and by FISH in aCLL series.**

| Series | Number of aCLL patients analyzed by CC/ISH | Clonal karyotypic aberrations % | +12 detected by conventional cytogenetics % | +12 detected by in situ hybridization % |
|---|---|---|---|---|
| Criel *et al.*, 1994[2] | 21 | 57 (12/21) | 48 (10/21) | 57 (12/21) |
| Matutes *et al.*, 1996[3] | 39 | 72 (28/39) | 56 (22/39) | ND |
| Woessner *et al.*, 1996[4] | 13 | 62 (8/13) | 31 (4/13) | 54 (7/13) |
| Criel *et al.*, 1997[3] | 67 | 55 (37/67) | 36 (24/67) | ND |
| Bigoni *et al.*, 1997[6] | 43 | 63 (27/43) | 21 (9/43) | 33 (14/43) |
| Hjalmar *et al.*, 1998[7] | 37 | ND | ND | 24 (9/37) |
| Present series | 27 | 70 (19/27) | 22 (6/27) | 63 (17/27) |

*The table only includes those series in which a distinction between aCLL patients and tCLL patients was made. CC: conventional cytogenetics; ISH:* in situ *hybridization; aCLL: atypical chronic lymphocytic leukemiat; CLL: typical chronic lymphocytic leukemia.*

Despite the fact that del(13)(q14) is associated with CLL, only one case with this deletion was detected by ISH.[6] Döhner *et al.*[10] observed that abnormalities of chromosome 17 occur at low frequency (9-15%), most of them being structural abnormalities affecting the short arm, where the TP53 tumor suppressor gene is located. However, among our cases analyzed by ISH no monosomy of 17p13 was detected. Summing up, it is accepted that aCLL constitutes a subgroup distinct from typical CLL (tCLL), with different morphologic features, cytogenetic findings and clinical evolution. Despite the fact that large B-CLL series combine cytogenetic and ISH studies, few authors have distinguished between tCLL and aCLL. Further series would be warranted in order to consider both subtypes of B-CLL separately so as to obtain a better characterization of their cytogenetic and molecular behaviors.

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***Keywords***

*Atypical B-cell chronic lymphocytic leukaemia (aCLL), cytogenetics, trisomy 12, 13q14, 17p13, in situ hybridization*

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## References

1. Bennett JM, Catovsky D, Daniel MT, et al. The French-American-British (FAB) Cooperative Group: proposals for the classification of chronic (mature) B and T lymphoid leukaemias. J Clin Pathol 1989; 42:567-84.
2. Criel A, Wlodarska I, MeeusP, et al. Trisomy 12 is uncommon in typical chronic lymphocytic leukaemias. Br J Haematol 1994; 87:523-8.
3. Matutes E, Oscier D, García-Marco J, et al. Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical and laboratory features in 544 patients. Br J Haematol 1996; 92:382-8.
4. Woessner S, Solé F, Perez-Losada A, Florensa L, Vilà RM. Trisomy 12 is a rare cytogenetic finding in typical chronic lymphocytic leukemia. Leukemia Res 1996; 5:369-74.
5. Criel A, Verhoef G, Vlietinck R, et al. Further characterization of morphologic defined typical and atypical CLL: a clinical, immunophenotypic, cytogenetic and prognostic study on 390 cases. Br J Haematol 1997; 97:383-91.
6. Bigoni R, Cuneo A, Roberti MG, et al. Chromosome aberrations in atypical chronic lymphocytic leukemia: a cytogenetic and interphase cytogenetic study. Leukemia 1997; 11:1933-40.
7. Hjalmar V, Kimby E, Sundström Ch, et al. Trisomy 12 and plasmacytoid lymphocytes in chronic leukemic B-cell disorders. Haematologica 1998; 83:602-9.
8. Pérez Losada A, Wessman M, Tiainen P, et al. Trisomy 12 in chronic lymphocytic leukemia: an interphase cytogenetic study. Blood 1991; 78:775-9.
9. Tabernero MD, San Miguel JF, García JL, et al. Clinical, biological and immunophenotypical characteristics of B-cell chronic lymphocytic leukemia with trisomy 12 by fluorescence in situ hybridization. Cytometry 1995; 22:217-22.
10. Döhner H, Stilgenbauer S, Fischer K, Bentz M, Lichter P. Cytogenetic and molecular cytogenetic analysis of B cell chronic lymphocytic leukemia: specific chromosome aberrations identify prognostic subgroups of patients and point to loci candidate genes. Leukemia 1997; 11:S19-S24.

## Massive hemolysis in *Clostridium perfringens* infection

Sir,

*Clostridium perfringens* infection is a very rare cause of massive intravascular hemolysis, but it should always be kept in mind since early treatment can rescue patients from an otherwise rapidly fatal outcome. We report a case which illustrates the very fast course of this disease, and outline some features that can help to reach a prompt diagnosis.

A 77-year-old woman presented with jaundice and stupor of recent onset. Her temperature was 38ºC and