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editorial

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# Towards a clinically relevant cytogenetic classification of chronic lymphocytic leukemia and related disorders

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B-cell chronic lymphocytic leukemia (CLL) accounts for approximately 30% of reported leukemia cases in western countries. It has an incidence of 2.3-3.3 per 100,000 people. Even though the disease more frequently affects elderly people (median age 60 years), a significant fraction of cases (10-15%) is diagnosed in subjects younger than 50 years old.[1] A growing body of evidence has been accumulated over the last decade demonstrating the variability of the clinical course.[2] This variability may reflect underlying differences in phenotypic and molecular cytogenetic features.

The awareness of the cytologic heterogeneity of CLL, which includes cases with dimorphic pictures and atypical immunophenotypic profiles,[3,4] prompted the French-American-British (FAB) group to formalize a proposal for the recognition of typical CLL and two related forms.[5] *CLL mixed cell type* denotes those cases with a spectrum of small to large lymphocytes, including lymphoplasmocytoid cells and cleaved lymphocytes and occasional (<10%) prolymphocytes.[6] CLL/PLL is the name for those cases having 10-55% prolymphocytes in a peripheral blood (PB) smear. Later on the term *atypical CLL* was adopted by several authors as a catch-all phrase to refer to any CLL with atypical lymphocyte morphology and/or immunophenotype.[7-10]

In the meantime, the introduction of efficient B-cell mitogens, along with the development of sensitive molecular cytogenetic techniques,[11,12] helped us to extend our knowledge on the cytogenetic profile of CLL significantly. Some excellent reviews have recently been published[13,14] summarizing the clinicobiologic significance of chromosome aberrations in CLL (see Table 1).

This issue of Haematologica publishes a paper by Hjalmar *et al*.,[15] who sequentially analyzed a spectrum of chronic leukemic B-cell disorders by FISH, confirming, in an unselected patient population, that: a) +12 is found in CLL with an excess of lymphoplasmocytoid cells, corresponding to CLL mixed cell type, and in indolent nonHodgkin's lymphoma with PB involvement; b) +12 is rare in typical CLL; c) +12 is usually detected at diagnosis and is not acquired during disease evolution; d) the prognosis in patients carrying +12 is worse than in patients without +12.

In addition, the same study draws the readers' attention to the fact that there were an unequal distribution of +12-lymphocytes at different sites [bone marrow (BM), PB and lymph nodes]. Only a fraction of the clonal B-cell population carried +12 and clonal expansion of +12 cells paralleled disease progression.

Unequal distribution of cytogenetically abnormal cells at different sites involved by disease has recently been observed in CLL[16] as well as in myeloid neoplasias,[17,18] possibly reflecting selective retention and/or destruction of leukemia cells due to as yet unclear mechanisms. In some cases different properties of adhesion to BM or lymph node stromal cells has been postulated to play a role in this process.[19] The acquisition of +12 is an early cytogenetic event in the history of CLL, although it probably does not represent the primary anomaly.[20-23] Indeed, the presence of +12 and of 13q deletion in two distinct populations of neoplastic lymphocytes[24] belonging to the same patient would suggest that these cytogenetic aberrations may be superimposed on an, as yet, unidentified submicroscopic primary change.

Clearly, the clinicobiologic significance of +12 and 13q– differ. The 13q14 deletion, involving a small region of DNA in which a putative oncosuppressor gene is located,[25] has been shown to affect the majority of lymphocytes[21] and to be associated with indolent disease. In contrast, +12 confers a worse prognosis and there is uncertainty as to whether it involves all neoplastic cells, as suggested by metaphase analysis, or only a fraction of the CD5/C19$^+$ cell population, as revealed by interphase FISH. The findings of Hjalmar *et al*.[15] that +12 cells preferentially home to the lymph node and BM, that the cells are not reduced or eliminated by chemotherapy[26]

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**Table 1. Chromosome abnormalities in CLL.**

| *Aberration* | *Frequency** | *Hematologic features* |
|---|---|---|
| +12 | 15-25% | CLL mixed-cell type therapy-demanding disease; relatively short survival |
| deletion at 13q14 | 10-40% | favorable prognosis and typical morphology if present as the sole change |
| deletion at 11q21 | 5-15% | typical CLL, massive lymphadenopathy, young age, short survival |
| deletions at 17p | 4-17% | more frequent in atypical CLL and in advanced disease, refractory to purine analogues |
| deletions at 6q | 3-6% | poorly defined; atypical morphology (few cases) |
| t(11;14)(q13;q32) | 2-5% | CLL/PL, frequent transformation into PLL; overlapping features with leukemic MCL |
| t(14;19)(q32;q13) | very rare | atypical CLL; aggressive disease |

**Wide varation in percentage figures due to the sensitivity of the method, i.e. conventional chromosome analysis or molecular cytogenetic techniques, and the heterogeneity of patient population. MCL: mantle cell lymphoma; PLL: prolymphocytic leukemia.*

and that their population expands as disease progresses, clearly support the hypothesis that this anomaly plays an important role in the natural history of the disease. It is reasonable to assume that +12 lymphocytes may divide in vitro with higher efficiency than neoplastic lymphocytes without +12 consequently, when mitogen-driven stimulation of the neoplastic clone is successful, more metaphases with +12 are found than +12-interphase cells.[20] Some technical considerations may be pertinent. There are no data in this, or in previous studies, concerning the efficiency of FISH in detecting +12 in a 100%-positive control sample and, as a matter of fact, up to 10-20% interphase cells may be interpreted as false null or monosomic cells in routinely processed slides. It should, therefore, be considered that underestimation of the size of the trisomic clone may occur as a consequence of sub-optimal hybridization efficiency.

Many of the uncertainties over the interpretation of the significance of trisomy 12 derive from the absence of information on the molecular genetic defects underlying this numerical change. Since +12 results from the duplication of one homologue, with retention of the other homologue, a gene-dosage effect might be involved in the general mechanism but no gene has been so far implicated. It is possible, of course, that not all cases with +12 have the same molecular defect, as is the case with trisomy 11 in myeloid neoplasia, which is associated in some, but not all cases, with *MLL* gene self-fusion.[27,28]

Finally, the data by Hjalmar *et al.* must be interpreted with the understanding that, due to the difficulty in studying metaphase cells in B-cell neoplasias with a low mitotic index, information on chromosome changes other than +12 is not available in the majority of their cases. As summarized in Table 1, rather a long list of chromosome changes has been shown to be specifically associated with distinct subsets of chronic B-cell lymphoproliferative disorders.[7]

Though not applicable in every patient in routine clinical practice,[29] molecular cytogenetic investigations in CLL and related disorders are drawing hematologists' attention to how disease subsets with unique clinical features may be identified.[30,33] Clarification of the clinicobiologic significance of recurrent chromosome changes in chronic (mature) lymphoproliferative disorders is crucial to the understanding of their clinical importance and may facilitate the compilation of a clinically useful cytogenetic classification of this spectrum of B-cells neoplasias.

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