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Moving from clinical to biological staging for the management of chronic lymphocytic leukemia

Introduction

Chronic lymphocytic leukemia (CLL) is a complex disease with a very heterogeneous outcome. It is generally assumed that not all patients with CLL die of their disease. However, a more recent analysis of data showed that patients who have progression of their CLL die predominantly of complications of the disease, especially from infections.[1]

Over the last decade, major advancements in our understanding of this hematologic malignancy have occurred. From the clinician's perspective the importance of the new knowledge is how it affects treatment. The assessment of prognosis in patients with CLL has been revolutionized. Thus, besides classical clinical parameters a number of biological features have shown to correlate with prognosis and to add prognostic value to Rai's and Binet's clinical stages.[2,3] These staging systems have been used satisfactorily to CLL for more than 25 years. The most important outcome of staging CLL was the realization that early-stage CLL need not be treated. A meta-analysis of clinical trials demonstrated that treatment of early-stage disease produced no survival advantage over a policy of delaying treatment until progression occurred.[4] For this *watch and wait* strategy the National Cancer Institute (NCI) has produced guidelines to specify what should put an end to the waiting.[5]

They formalize the recommendation that <20% of patients with CLL should be treated directly, but offer no advice to the >80% who have early-stage disease and who watch and wait and worry. In recognizing this, the NCI guidelines have including a dynamic measurement among the indications for treatment: namely, the lymphocyte doubling time (LDT).

Prognostic factors in CLL

Montserrat and colleagues analysed the LDT, defined as the time needed to double the peripheral lymphocyte count, in 100 untreated patients with CLL. LTD was shown to have independent prognostic significance.[6] This is a simple method of measuring proliferation rate that has been confirmed as a useful prognostic marker.[7] It has been incorporated into the NCI guidelines 5 in a more stringent form, requiring an LDT of <6 months before treatment is started. Its drawbacks are that it is not available at diagnosis, that the lymphocyte count may have a misleading transient rise during an infection or therapy with corticosteroids, and that the lymphocyte count may reach a

plateau after an initial rise.[8]

Cytogenetics is one of the most powerful prognostic tools for patient with acute leukemia. The introduction of interphase cytogenetics using fluorescent *in situ* hybridization (FISH) has greatly increased the sensitivity of cytogenetics. FISH has two advantages over conventional cytogenetics: 1) it allows for the detection of specific chromosome lesions in non-dividing cells which would be missed by metaphase analysis and 2) it is able to detect loss of chromosome material in the order of magnitude of one hundred-kb; deletions of this size are far beyond the resolution power of banding analysis. There are five common chromosomal abnormalities seen in CLL, four of which have been arranged in a prognostic hierarchy. The frequency of each cytogenetic lesion in larger study was as follows: 13q- 36%, trisomy 12q 14%; 11q- 17%; 17p- 7% other aberrations 8%, no demonstrable lesion 18%.[9]

The commonest, occurring in over 50% of cases, is deletion of part of the long arm of chromosome 13 (del 13q14).[10] Patients with this isolated del 13q14 have a good prognosis, with survival curves that are even better than those with a normal karyotype. Trisomy 12 is the next commonest abnormality, occurring in up to 25% of cases. It is associated with atypical morphology and unmutated IgVH genes, when it carries a worse-than-average prognosis.[11] However, those cases associated with mutated IgVH genes are usually benign.

Deletions at 11q23 occur in between 10% and 20% of cases[12] and are associated with a poor prognosis. Patients are typically male and have enlarged abdominal lymph nodes. With newer therapies remission rates are high, but early relapse is typical.

Patients with deletions of 17p[13] are only part of a larger group with aberrations of the p53 gene.[13–15] They occur in about 5% of untreated cases, and typically represent those that are unresponsive to modern therapy. Karyotypic evolution occurs,[16] and previously treated patients may have up to 30% 17p13 deletions. Survival of less than 2 years is commonplace.

Diverse deletions of part of the long arm of chromosome 6 occur in 6% of cases, almost always as a secondary event.

A number of serologic staging parameters such as beta-2-microglobulin (β2-M), thymidine kinase (TK) and soluble CD23 emerged as being independently discriminatory after accounting for the stage of the disease.[17–19] β2-M is an extracellular protein that is noncovalently associated with alpha-chain of the class I major histocompatibility complex (MHC), which is detectable in the serum. β2-M associated with adverse prognostic features at presentation and demonstrated higher values in CLL patients with worse survival.[17] Evaluated in the context of some other prognostic parameters, β2-M appears to maintain an independent prognostic value.[20]

Thymidine kinase (TK) is a cellular enzyme involved in a salvage pathway for DNA synthesis. Serum TK is probably related to the number of dividing neoplastic cells and is therefore a measure of proliferation. The ability of s-TK levels to detect a subgroup of patients with early, non-smoldering CLL at risk for a rapid disease progression seems particularly useful[19,21] and only the fact that it is measured by a radioimmunoassay, unpopular in routine laboratories, prevents it from being widely adopted.

CD23 is a surface functionally relevant molecule in B-CLL cells. Higher serum levels of its cleaved form (sCD23) detected indicate a worse prognosis.[19] However, its independent prognostic significance has not been proven. The value of some of these serum markers is currently limited by the lack of a standard assay method, variable cut-off points between series or the lack of validation in a prospective study. An attractive option is that of including

in prognostic models different serological markers that contribute individually to prognosis of CLL under the speculative assumption that their combined use, integrating different biological aspects of CLL, provides greater prognostic information, than of a single marker.[22]

The realization that there were effectively two types of CLL, one inclined to progress and one inclined not to, and that these tendencies were inbuilt and not acquired, came from a study of immunoglobulin heavy-chain variable region (IgV_H) genes.

For many years CLL was thought of as a tumour of pre-follicular B cells, possibly arising in the follicular mantle.[23] It would therefore be expected to lack somatic mutations in the IgV_H genes. Early gene sequences of tumour cells from patients tended to confirm this,[24,25] but reports of cases with evidence of somatic mutation began to appear. The demonstration that somatic mutations correlated with more benign disease was first presented in 1999 by two groups of investigators which demonstrated that patients with a memory cell immunophenotype with mutated IgV_H had a very favourable outcome and a low probability of developing progressive disease, whereas those with unmutated IgV_H genes were much more likely to develop progressive disease and to be associated with a shorter survival.[26,27] Median survival in the Hamblin *et al.* series was 8 years for patients without variable genes mutations, whereas median survival for patients with IgV_H genes mutations was of 25 years. This observation has since been confirmed by many other groups.[28-30] An exception to the rule is the tumour that uses the V3-21 gene segment.[31] Whether or not they carry somatic mutations, such cases are aggressive with short survival times.

Although there is a tendency for adverse karyotypic abnormalities to occur mainly in the subset with unmutated IgV_H genes, the distinction is not absolute, and Ig gene mutational status and karyotype are independent prognostic factors. The median survival for patients with unmutated Ig genes and 17p deletions is only about 3 years.[15,16]

However, IgV_H sequencing is difficult to perform in a routine diagnostic laboratory, and thus a search was made for a surrogate measurement that would have the same prognostic value.

CD38 is a membrane protein that marks cellular activation and maturation and has signalling activity. CD38 expression is associated with neoplastic cells showing atypical morphology diffuse bone marrow infiltration, high peripheral blood lymphocytosis and a less favorable overall prognosis.[32] Expression of CD38 on the surface of CLL cells is easily measured by flow cytometry.

Further studies revealed that CD38 and IgV_H gene mutation status often overlap.[33] Unfortunately, it soon became clear that, while CD38 is certainly a useful prognostic indicator, individual patients frequently show discordances between CD38 expression and IgV_H mutations.[33,34] Hamblin and colleagues estimated that approximately 30% of cases fell into that category, and these had a prognosis intermediate between those who had somatic mutations and were CD38-negative, and those who were unmutated and CD38-positive.[33] The same paper also demonstrated that CD38 expression could change during the course of the disease.[33] The original suggestion 26 was to use a 30% threshold, and this was confirmed by Hamblin and colleagues.[33] Subsequently levels of 7%[15] or 5%[35] have been suggested. The different threshold levels used might be explained by the observation of Ghia and colleagues[36] that 27.8% of 148 patients with CLL studied had a bimodal expression of CD38 and tended to progress no matter how high the population of CD38-positive cells. This tendency was not absolute, but 63.3% of the patients

with bimodal CD38 expression had progressed during the observation period. The majority of those with bimodal expression had >30% CD38-positive cells, but about a third had CD38 levels between 7% and 30%. The value of CD38 has since been confirmed by several series.[37]

In a pioneering gene-expression profiling study in CLL, a panel of genes has been identified in which the expression of a small subgroup of genes, including those encoding ZAP70, IM1286077, and C-type lectin, correlated with the mutational status of IgV_H genes.[38] ZAP70 is an enzyme that is normally expressed in T lymphocytes and that is critical for the activation of T cells by antigen. ZAP70 is an unexpected finding in a B-cell tumour, since the protein has not been reported in normal circulating B cells. ZAP-70 expression was shown correctly to predict the IgV_H mutation status in 93% of cases of CLL. Four patients with mutated IgV_H genes had high ZAP-70 expression, and three with unmutated IgV_H genes had low ZAP-70 expression. Both ZAP-70 expression and IgV_H mutational status were equally able to predict time to requirement for treatment.[39] For routine assessment of ZAP-70, a flow cytometry assay is required.

This has proved difficult, but three different methods have been reported.[40-42]

It is not completely clear that ZAP-70 is stable over time. Levels are higher in cells from marrow rather than the blood,[43] and it is known that it can be up-regulated in response to T-cell stimulation.[44] Villamor and colleagues showed changes (usually from positive to negative) in nine out of 111 patients with at least two estimates separated by a median of 29 months.[45]

A risk-adapted therapeutic approach?

The development of newer prognostic factors, such as the mutational status of the immunoglobulin heavy-chain variable genes, cytogenetics, CD38 expression, and some serum markers, has allowed for further discrimination of patients into risk categories. Thus, progressive and smoldering forms of the disease can now be separated more accurately than was previously possible using Rai's and Binet's staging systems. Indeed, Binet's stage A corresponds to a good prognostic group, comprising almost 65% of CLL patient cases. During the course of the disease, 25% die from CLL-related causes, 40% progress to stages B and C, and 50% ultimately require treatment. Most of the modern prognostic factors were discovered by retrospective analysis of single-centre series, but they have now been evaluated by four prospective studies. In the CALGB 9712 randomized phase-II study of fludarabine and rituximab, given according to two different schedules, there was no difference in response rate among those with different prognostic markers, but significantly shorter median progression-free survival (PFS) and overall survival (OS) in those with unmutated IgV_H genes and those with high-risk interphase cytogenetics (del 11q or del 17p).[46] In a similar Italian study with fludarabine and rituximab, a much shorter PFS was found in patients who were ZAP-70-positive or CD38- positive.[47]

In the German CLL4 phase-III trial which compared fludarabine (F) with fludarabine plus cyclophosphamide (FC), non-response to FC, poor PFS and OS correlated with deletions of 17p.[48,49] This was confirmed by the British CLL4 trial[50] which randomized treatment between F, FC and chlorambucil. For all treatment groups a finding of 17p deletion in more than 20% of cells by FISH was associated with non-response and poor PFS and OS. In addition, in a multivariate analysis, unmutated IgV_H genes, but not expression of either CD38 or ZAP-70, correlated with response, PFS, and OS. There was also an association of del 11q with early relapse.

We have enough information to suggest how

these prognostic markers ought to be used. In particular, according to the molecular staging, high risk patients (i.e. 17p) should be offered, preferably in multinational clinical trials, treatment with innovative strategies (MoAb based strategies, high dose steroids, innovative transplantation approaches).

Thus, the following recommendations for a risk adapted treatment of a CLL patient seem justified by scientific evidence: 1) The prognostic assessment of a patient should no longer rely exclusively on the Binet or Rai stage. There is good evidence that IgV_H mutational status should be used to help stratify treatments in clinical trials. CD38 expression and/or serum TK might possibly be used in association. 2) The concept that patients in early stage should not be treated need to be re-challenged and investigated mainly if those patients present poor risk markers.

Finally, there is no evidence to support treating such patients outside a clinical trial.

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