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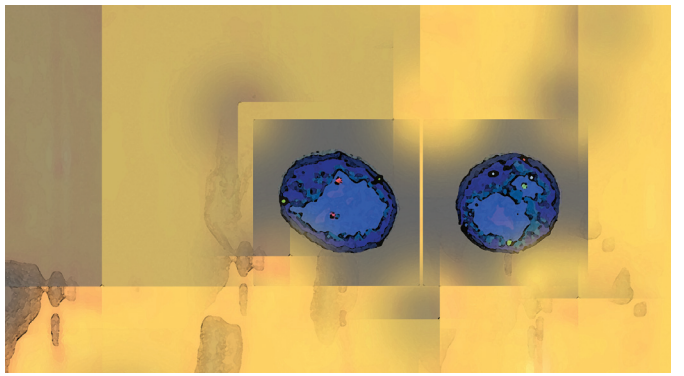

Fluorescence in-situ hybridization (FISH) analysis for B-CLL patients

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Introduction

Chronic lymphocytic leukemia (B-CLL) is the most common leukemia in adults and has a highly variable clinical course.
The genetic characterization of chronic lymphocytic leukemia has made significant progress over the last few years. Clonal chromosomal abnormalities are detected in 40 - 50% of cases by conventional cytogenetic analysis. However, chromosome banding studies are still hampered by the problem of the low in vitro mitotic activity of B-CLL cells.
In the past years new molecular cytogenetic methods, such as fluorescence *in-situ* hybridization (FISH) with specific DNA probes, have improved the ability to detect chromosomal abnormalities in about 80% of B-CLL cases. It is reported, that several of the most common chromosomal abnormalities have clinical correlation (1, 2).

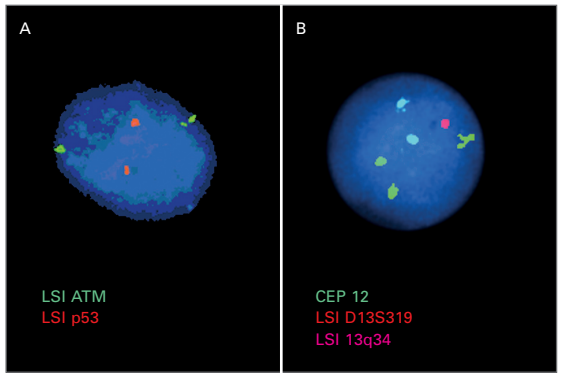

Fig. 1:
Fluorescence in-situ hybridization with "CLL Probe Panel": the left normal cell shows two signals for "LSI ATM" (green) and "LSI p53" (red); the right cell shows three signals for "CEP 12" (green; trisomy 12), one signal for "LSI D13S319" (red; monolallelic deletion 13q14.3) and two signals for "LSI 13q34" (blue; normal signal distribution).

Another predictor of the clinical course in patients with B-CLL is the expression of mutated or unmutaded immunoglobulin heavy-chain variable region (IgVh) gene rearragements. Another marker is ZAP70 ("70-kD zeta-associated protein"), a protein tyrosine kinase, known to be of importance in T-cell signaling, but absent in normal peripheral B-Cells. In aggressive disease course, the B-CLL cells usually express an unmutated IgVh-gene together with ZAP70, whereas in indolent disease, the B-CLL cells usually express mutated IgVh-genes but lack expression of ZAP70 (3). The analysis of the mutation status of the IgVH-gene is highly extensive and thus not suited for routine diagnostics. Since the analysis of the ZAP70 expression by flow cytometry has become routine in clinical laboratories, ZAP70 studies in CLL-patients are more amenable as a predictor of the clinical course.

The method

Fluorescence *in-situ* hybridization (FISH):
FISH provides a direct way of detecting specific DNA sequences in interphase nuclei and metaphase chromosomes. With different DNA probes specific genomic segments can be visualized and examined microscopically.
If the indication for the analysis is the clinical or suspected diagnosis of B-CLL, FISH is performed using unstimulated, short-term incubated cultures or smears of heparinized peripheral blood and/or bone marrow samples. After harvesting, cells are placed onto slides and FISH is carried out with DNA probes described in this information.
Since the cells are examined at a stage of the cell cycle known as interphase, this approach is also referred to as "interphase cytogenetics".

Flow cytometry:
This is a method for quantitating cellulary components or structural features of single cells in a cell suspension by optical means. Since different cell types can be distinquished by quantitating structural features, flow cytometry can be used to count cells of different types in a mixture.

The analysis

Fluorescence *in-situ* hybridization (FISH):
Different FISH-probes are tested to detect the known prognostically significant genetic lesions that can be found in the majority of patients with B-CLL.
The most common abnormality with an incidence of 55% is deletion (13)(q14), followed by deletion (11)(q22-q23), trisomy 12, deletion/aberrations of (14)(q32), deletion (17)(p13), deletion (6)(q21) [incidence: 18%,16%, 8%, 7% and 6%, resp.] (1, 2, 4). These chromosomal aberrations are independent prognostic markers identified by multivariate analysis in subgroups of patients with rapid disease progression and short survival times. Patients with deletion (13)(q14) as the sole abnormality were reported to have a prolongated survival, while those with deletion (6)(q21), trisomy 12, t(14)(q32), (11)(q22-q23) or deletion (17)(p13), appear to have a shortened survival (1, 2, 4) in comparison to patients without chromosomal aberrations.
The following DNA probes are offered and tested in combination (Vysis/Abbott or Kreatech):

ON6q21/SE6	(6q21/6p11-q11)
LSI ATM	(11q22.3)(ataxia teleangiectasia gene)
CEP 12	(12p11.1-q11)
LSI D13S319	(13q14.3)
LSI 13q34	(13q34)
LSI IGH	14q32)
LSI p53	(17p13.1)

Flow cytometry:
Increased expression of ZAP70 by B-CLL cells is significant associated with the presence of unmutated IgVh-genes. Therefore, ZAP70 analysis applying flow cytometry is a reliable alternative method to the extensive DNA sequencing of the IgVh-gene.
Expression of ZAP70 in B-CLL cells is associated with a poor prognosis and an aggressive clinical course (3).

Requirement and results

For cytogenetic analyses at least 2 ml of heparinized peripheral blood or bone marrow are necessary. For flow cytoemtry 2,5 ml of an EDTA peripheral blood probe are required. For both analyses, the specimen should drawn on the day of shipping and should not be frozen. FISH on fixed cell suspensions (high cell density required) can also be accepted. A specified number of cells are investigated by FISH in accordance with national and international guidelines (5). The report states the number of cells investigated and chromosomal abnormalities found. These chromosomal abnormalities can be relevant for the therapeutic strategy (6). Results are reported within 6 to 8 days after receipt of the specimen in the laboratory.