References


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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).

Another predictor of the clinical course in patients with B-CLL is the expression of mutated or unmutated immunoglobulin heavy-chain variable region (IgVH) gene rearrangements. 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 genetic 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 cellular components or structural features of single cells in a cell suspension by optical means. Since different cell types can be distinguished 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 (11)(q22-23)(1)(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):
- CEP 12
- LSI ATM
- LSI p53
- LSI D13S319
- LSI 13q34
- LSI IGH
- LSI p16
- LSI p13.1

Flow cytometry: Increased expression of ZAP70 by B-CLL cells is significantly associated with the presence of unmethylated 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 cytometry 2.5 ml of an EDTA peripheral blood probe is required. For both analyses, the specimen should be shipped on dry ice 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 aberrations 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.