Flow cytometric detection of BCR-ABL fusion proteins in leukemia patients via an immunobead assay


INTRODUCTION

The BCR-ABL fusion gene results from the translocation t(9;22). It is the hallmark of chronic myeloid leukemia (CML), and is present in a poor-risk subgroup of precursor B cell acute lymphoblastic leukemia (ALL), which represents 25-30% of adult ALL and 3-5% of childhood ALL. Consequently, the detection of the BCR-ABL aberration is of utmost importance for diagnosis and classification of leukemia patients and can also be used as marker for monitoring of BCR-ABL leukaemias to evaluate treatment effectiveness.

So far, the BCR-ABL aberration has been detected by cytogenetics, FISH or PCR, all techniques that are time consuming and require special facilities.

We developed a simple flow cytometric bead assay for detection of the BCR-ABL fusion protein in cell lysates, using a bead-bound catching antibody against one side of the fusion protein and a fluorochrome-conjugated detection antibody against the other side of the fusion protein (see Figure 1).

RESULTS

1. Detection of all variants of the BCR-ABL fusion protein. For each patient, samples were tested in two separate immunobead assays. The results of two immunobead assays are shown in Table 1, together with the corresponding results of the CBA assay (BCR-ABL Protein Kit; BD Biosciences). The immunobead assay showed overall similar results in all patients, while the CBA assay showed a slightly higher positivity.

2. Protease inhibition needed in cell samples with many mature myeloid cells. Protein stability problems were encountered when cell samples contained high frequencies of mature myeloid cells.

3. Development and testing of standardized Research Use Only (RUO) kit. The immunobead assay was further developed and standardized by BD Biosciences into the Cytometric Bead Array (CBA) assay (BCR-ABL RUO kit; BD Biosciences). The CBA assay for Research Use Only (RUO) kit showed overall similar results in all patients.

4. High specificity of BCR-ABL fusion antibody

- No cross-reactivity was seen with cell samples from newly diagnosed (n=134) or relapsed (n=11) leukemia patients: 19 CML patients, 78 precursor-B-ALL, and 50 AML patients were negative in both assays.

- 19/19 CML were BCR-ABL positive in both assays (with very weak immunobead signals in one CML case).

- 17/78 precursor-B-ALL were BCR-ABL positive in both assays (mainly adults).

- 0/50 AML were BCR-ABL positive in both assays.

- 1 negative BCR-ABL RUO immunobead assay result of RQ-PCR.

- 2 Positive BCR-ABL RUO immunobead assay results of RQ-PCR.

- 100% of CML samples (n=19) were BCR-ABL positive in both assays.

- 84% of adult precursor-B-ALL samples (n=78) were BCR-ABL positive in both assays.

- 0% of AML samples (n=50) were BCR-ABL positive in both assays.

5. The anti-BCR antibody was developed against a non-homologous region of ~80 amino acids, encoded by exon 1, which allowed us to detect all known BCR-ABL variants, including p190, p210, and p230, as demonstrated by analysis of a series of well-defined cell lines. The assay appeared to be specific and sufficiently sensitive to detect BCR-ABL proteins in leukocyte samples of leukemia patients at diagnosis.

6. The anti-ABL antibody was developed against a homologous region of ~80 amino acids, encoded by exon 3, which allowed us to detect all known BCR-ABL variants, including p190, p210, and p230, as demonstrated by analysis of a series of well-defined cell lines. The assay appeared to be specific and sufficiently sensitive to detect BCR-ABL proteins in leukocyte samples of leukemia patients at diagnosis.

- For specific detection of BCR-ABL proteins in leukemic cells.

- Does not need special laboratory facilities other than a routine flow cytometer.

- Can be run in parallel to routine immunophenotyping (no extra technician time needed).

- Can be developed for development of developing single tube assays for combined evaluation of multiple different fusion proteins, e.g. per disease category.

- Can be used for specific detection of BCR-ABL proteins in leukemic cells.

CONCLUSIONS

We conclude that the flow cytometric immunobead assay is a fast and easy technique for specific detection of BCR-ABL proteins in leukemic cells.

1. The main advantages of the immunobead assay are:

- Not dependent on the breakpoint position in the BCR gene;

- Does not need special laboratory facilities other than a routine flow cytometer;

- Provides results within several hours;

- Can be run in parallel to routine immunophenotyping (no extra technician time needed).

2. Since differentially labeled beads allow multiplexing, it will be possible to develop single tube assays for combined evaluation of multiple different fusion proteins, e.g. per disease category.

3. Consequently, the flow cytometric immunobead assay can contribute to fast and easy diagnosis and classification of leukemias with fusion genes.

4. If sufficient sensitivity can be reached, also monitoring becomes possible.

Figure 1. Principle of the flow cytometric immunobead assay for fusion protein detection. The bead-bound catching antibody recognizes one side of the fusion protein and the fluorochrome-conjugated detection antibody recognizes the other side of the fusion protein.

Figure 2. Results of the flow cytometric BCR-ABL RUO immunobead assay.