The BCR-ABL positive leukemias: biology and clinical implications of interphase fish

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The precise knowledge of Philadelphia (Ph) chromosome status in leukemia is important for determining the most optimal therapy for evaluation of treatment response in patients with Ph-positive leukemia. The balanced translocation between the long arms of chromosomes 9 and 22, t(9;22) (q34.1; q11.1), is detected cytogenetically in over 90% of patients with chronic myelogenous leukemia (CML), 20-25% of pediatric and adult patients with acute lymphoblastic leukemia (ALL) and in less than 3% of patients with acute myelogenous leukemia (AML) (1-5). There are instances where quantitation of the Ph fail to establish accurate cytogenetic diagnosis. Patients treated with interferon have inaspirable marrow or a "dry tap" or insufficient number of metaphases to evaluate treatment due to the fact that interferon alpha has an inhibitory effect on cell division. Similarly, mitotic yield in many patients with ALL is insufficient for establishing the cytogenetic diagnosis. At the molecular level, the chimeric BCR-ABL gene is the pathogenic hallmark of the Ph chromosome (6-9). Methods for molecular detection, such as Southern blot analysis or PCR studies are not routinely performed in Cancer Cytogenetic Laboratories. In a routine diagnostic cytogenetic laboratory it is important to use a highly reproducible and reliable protocol which can be completed within a short time.

Fluorescence in Situ Hybridization (FISH) studies offer the advantage of identification of BCR and ABL fusion in non dividing (interphase) cells. This can improve diagnostic potential in difficult cases (10). The purpose of this study was to establish whether the BCR-ABL interphase FISH test, utilizing the commercially available dual color probe, can be clinically useful in the management of some leukemias.

Between July 1995 and December 1998 we prospectively investigated bone marrow and/or peripheral blood from 398 patients (453 specimens) with hematological disorders along with 139 control specimens. Patients were seen at the Mount Sinai Medical Center and its affiliated institutions in New York.

The sensitivity of interphase FISH was determined to be 96%, among 77 normal controls, utilizing first generation BCR-ABL probe from Vysis (Downers Grove, Ill). With the BCR-ABL ES second generation probe the sensitivity of interphase FISH test was determined, for 32 normal controls, to be 98%.

In all 452 specimens the BCR-ABL interphase FISH test provided useful results on the first attempt and in half the time required for standard cytogenetics. The scoring of hybridization signals in 200 nuclei was completed within 30 minutes including imaging for diagnostic documentation. The entire procedure together with scoring was usually completed within 3 hours including the results of a negative control. These findings provided information about cells independent of their cycling status. Determination that leukemia is Ph-positive through analysis of 30 metaphase cells using standard cytogenetics takes 6-7 hours and provides information only on proliferating cells. More importantly, in 147 specimens (128 were peripheral blood samples) standard cytogenetics failed to identify dividing metaphase cells while interphase FISH test documented clinical results. This represents 32.5% of all specimens studied (147/452).

100% PH – POSITIVE PATIENTS

Fifty-two patients (61 specimens) had CML and one patient (2 specimens) had ALL. The Ph chromosome was documented in 30 metaphases from each patient.

The mean percentage of cells with one BCR-ABL fusion signal was 76.3% ± 12.8 % (SD) and another 6.0% ± 17.5% (SD) cells had two copies of BCR-ABL fusion signal, a total of 82.3%. Molecularly "normal cells" constituted 18.4% ± 9.9% (SD) cells overall (range 3%-52%).

In comparing interphase FISH with conventional cytogenetics a discrepancy was observed in two patients. One patient had isoderivative 22: der(22)t (9;22) which was cytogenetically not detected but interphase FISH documented two BCR-ABL fusion hybridization signals. Interphase FISH in the second patient showed one ABL signal fused with BCR and the other ABL signal was missing from a cytogenetically normal chromosome 9.

PH-NEGATIVE, BCR-ABL FUSION POSITIVE PATIENTS

Five patients, three with CML and two with ALL, had Ph-negative karyotypes while interphase FISH studies showed a mean of 78.1% ± 9.3% cells with one BCR-ABL fusion signal and 3.3% ± 5.2% cells with two BCR-ABL fusion signals, a total of 81.4%.

This result highlights the importance of using interphase BCR-ABL FISH test.

It is important to identify Ph-negative BCR-ABL positive CML patients as they could benefit from interferon treatment. Identification of BCR-ABL positive ALL patients is extremely important because their prognosis is very poor and treatment should be aggressive. Localization of BCR-ABL fusion gene was on 9q in two patients (one with CML and one with ALL) and on 22q11 in two patients with CML and one patient with ALL. Chromosomal localization of BCR-ABL fusion from patients with Ph-negative leukemia may show in future to be of prognostic value. In the three of four reported patients with Ph-negative, BCR-ABL positive CML, who had colocalization of BCR and ABL on 9q, the clinical course and the evolution of the disease was rapid (11-13). Two of our three patients had a rapid clinical course and colocalizaton of BCR-ABL on 9q. Two other patients underwent bone marrow transplantation. It has been reported that in nine patients with Ph-negative CML and a normal karyotype, RT-PCR showed the presence of BCR-ABL fusion and the absence of ABL-BCR transcript (14). In each case the ABL protooncogene was documented by metaphase FISH to be translocated to a normal chromosome 22, while the 3' BCR sequence that would have been translocated to 9q34.1 in Ph-positive CML, remained on 22q11. However, even in complex, variant and masked Ph translocations, including our patient with t(9; 21; 22), BCR and ABL were colocalized on 22q, strongly suggesting that the BCR-ABL fusion transcript is implicated in the majority, but not all, of these patients and that they should be considered as Ph-positive. The observation that to date 6 patients had colocalization of ABL and BCR on 9q suggests that reciprocal ABL-BCR, although rare does occur in Ph-negative CML and ALL. The observation that two fusion signals were detected on two chromosomes 9 in a patient with Ph-negative ALL and trisomy 9, provides evidence that +9 in this patient was the result of replication of the abnormal 9 and may be equivalent to the double Ph chromosome seen in blast crisis of CML.

PH-MOSAICISM

Seven patients showed Ph mosaicism in nine samples. When conventional cytogenetic and interphase BCR-ABL were compared the results were concordant in four patients. Two patients had an insufficient number of metaphases and thus an inaccurate percentage of the Ph chromosome was obtained. Discrepant results were obtained in one patient with ALL. The bone marrow cytogenetics showed 17% of Ph-positive metaphases while interphase FISH revealed 48% BCR-ABL positive cells.

PH – NEGATIVE MYELOPROLIFERATIVE DISORDERS

At the time of the study 117 patients (122 specimens) had Ph-negative status. The mean number of cells with BCR-ABL fusion signal was 2.2% ± 1.4% and the mean number of cells with a normal distribution of BCR and ABL hybridization signals was 97.3% ± 1.8%. From the diagnostic point of view 4% of cells with a BCR-ABL fusion signal may be considered as non-specific background "noise". Results on 32 normal controls using ES probe demonstrated higher sensitivity and the cut-off reference point at 0.91%.

One patient was found to have 3 BCR and 2 ABL signals without fusion. The karyotype of the patient was complex and included trisomy 19 and monosomy 22. Metaphase FISH study revealed that BCR probe was localized to a normal chromosome 22 and to both arms of a chromosome, originally identified by cytogenetics as +19. Therefore metaphase FISH

identified i(22)(q10) and the patient did not have trisomy 19 but isochromosome of the long arms of #22.

POST TRANSPLANT PATIENTS

To determine whether interphase BCR-ABL FISH test may be used to detect minimal residual disease we studied 103 specimens from 33 patients (32 with CML and 1 with ALL). All patients were 100% Ph-positive at diagnosis and in confirmed remission up to 8.7 years following allogeneic BMT. The mean number of cells with BCR-ABL fusion following bone marrow transplantation was 2.6% ± 1% and the mean number of cells with a normal distribution of BCR and ABL was 96.8% ± 2.6%. Fourteen specimen from four patients who were in cytogenetic relapse after BMT had a mean of 61.2% ± 28.7% (range 3% ± 98%) cells with fusion signal.

We utilized a combined dual color XY and dual color BCR-ABL probes to determine minimal residual disease. Percentage of host and donor cells was determined by scoring 1,000 nuclei for their XX vs. XY status. By evaluating whether BCR-ABL fusion signal was in XX or XY cell it was possible to determine the frequency of fusion signal in donor versus host cells. Between 0.5% and 4% cells with colocalization of BCR and ABL were found in donor cells of patients in continuous remission.

CYTOGENETICALLY INADEQUATE SPECIMENS

Fifty-nine patients had cytogenetically inadequate specimens. These specimens included bone marrow specimens without mitosis, peripheral blood samples with less than 3% blasts, "dry tap" specimens and fibrosis of the marrow. 40 of the 58 specimens were peripheral blood samples. Twenty-five specimens had a mean of 71% +17% cells with BCR-ABL fusion signal and the other 34 specimens had a mean of 2% +1.5% cells with BCR-ABL fusion signal. These findings demonstrate the higher sensitivity of interphase BCR-ABL FISH test over G-banded metaphase analysis.

PATIENTS ON INTERFERON THERAPY

Obtaining a sustained major genetic response in interferon-treated patients

is important because of the correlation with prognosis (15-16). Since interferon alpha has an inhibitory effect on cell division, bone marrow cytogenetic analysis

Is frequently uninformative due to the small number of dividing cells. We evaluated 24 patients (80 specimens) on interferon therapy. Metaphase spreads were not available in 33 of 80 specimens and the cytogenetic analysis was inadequate in additional 6 specimens. Thus, in 48.5% of specimens conventional cytogenetics could not provide diagnostic results while BCR-ABL interphase FISH provided results in all studied specimens. Based on these results, we suggest that effective monitoring of interferon therapy may be established using BCR-ABL interphase FISH test, and this may be performed, if necessary, in conjunction with standard cytogenetics.

BONE MARROW VERSUS PERIPHERAL BLOOD

Very little is known about the distribution of Ph-positive cells in peripheral blood. In order to determine whether peripheral blood can be used instead of bone marrow cells to document BCR-ABL positive leukemia we compared the results of bone marrow and peripheral blood on the same date in 16 patients (15 with CML and 1 with ALL). High linear correlation was established with a regression coefficient of 0.996. High correlation in the majority of studied patients reflects that the percentage of BCR-ABL positive cells in bone marrow was comparable to that in peripheral blood.

In conclusion, the most important advantage of interphase BCR-ABL FISH methodology over cytogenetic investigation is the independence from dividing cells, which permits the use of peripheral blood cells for diagnostic purposes. Our results clearly showed that peripheral blood cells may be used to establish diagnosis, to monitor interferon therapy, to detect minimal residual disease after bone marrow translantatation and after donor lymphocyte infusion. The most important observation in this study was the high reliability and reproducibility of the test which can be completed within 3 hours and provides information in cytogenetically inadequate specimens as well as in specimens which are Ph-negative but had BCR-ABL fusion signal.

REFERENCE

1. Cortes JE, Talpaz M, Kantarajian H: Chronic myelogenous leukemia: A review. The American Journal of Medicin 100: 555, 1996.
2. Westbrook CA, Hooberman AL, Spino C, et al: Clinical significance of BCR-ABL fusion gene in adult acute lymphoblastic leukemia: A Cancer and Leukemia Group B Studies Blood 80: 2983, 1992.

3. Russo C, Carrol A, Kohler C, et al: Philadelphia chromosome and monosomy 7 in childhood acute lymphoblastic leukemia: A Pediatric Oncology Group Study. **Blood** 77: 1050, 1991.
4. Suryanarayan K, Hunger SP, Kohlert S, et al: Consistent involvement of the *bcr* gene by 9;22 breakpoints in pediatric acute leukemias. **Blood** 77: 324, 1991.
5. Najfeld V, Cuttner J, Figur A, et al: P185 *BCR-ABL* in two patients with late appearing Philadelphia-chromosome positive acute non lymphocytic leukemia. **Leukemia** 3: 841, 1989.
6. Heisterkamp N, Stam K, Groffen J, et al: Structural organization of the *bcr* gene and its role in the Ph translocation. **Nature** 315: 758, 1985.
7. Davis RL, Konopka JB, Witte ON: Activation of the *c-abl* oncogene by viral trasduction or chromosomal translocation generates altered *c-abl* proteins with similar in vitro kinase properties. **Mol Cell Bio** 5: 204, 1985.
8. Clark SS, McLaughlin J, Timmons M, et al: Expression of a distinctive *BCR-ABL* oncogene in Ph-positive acute lymphocytic leukemia (ALL). **Science** 239: 775, 1988.
9. Clark SS , McLaughlin J, Crist W, et al: Unique forms of the abl tyrosine kinase distinguish positive CML from Ph-positive ALL, **Science** 235: 85, 1987.
10. Tkachuck DC, Westbrook CA, Andreef M , et al: Detection of *bcr*-abl in chronic myelogenous leukemia by *in situ* hybridization. **Science** 250: 559, 1990.
11. Brunel V, Sainty D, Costello R, et al: Translocation of BCR to chromosome 9 in Philadelphia-negative chronic myelod leukemia. **Cancer Genetics Cytogenetics** 85: 82, 1995.
12. Hagemeijer A, Bujis A, Smith E, at al: Translocation of BCR to chromosome 9: a new cytogenetic variant detected by FISH in two Ph-negative BCR-ABL positive patients with chronic myeloid leukemia. Genes, Chromosomes and Cancer 8: 237, 1993.
13. Nacheva E, Holloway VT, Brown K, et al: Philadelphia-negative chronic myeloid leukemia: detection by FISH of BCR-ABL fusion gene localized either to chromosome 9 or to chromosome 22. **British Journal of Haematology** 87: 409, 1993.
14. Lazaridou A, Chase A, Melo J, et al: Lack of reciprocal translocation in BCR-ABL positive Ph-negative chronic myelogenous leukemia. **Leukemia** 8: 454, 1994.
15. Lino T: Monitoring of residual disease in chronic myelogenous leukemia: methodological approaches and clinical aspects. **Leukemia** 10: 896, 1996.
16. Faderl S, Talpaz M, Kantarajian HM, Estrov Z: Should polymerase chain reaction analysis to detect minimal residual disease in patients with chronic myelogenous leukemia be used in clinical decision making? **Blood** 93: 2755, 1999.