

Can minor bcr/abl translocation in acute leukemia be discriminated from major bcr/abl by extra-signal fluorescent *in situ* hybridization analysis?

We investigated whether bcr/abl extra signal(ES)-probe can discriminate minor-bcr (m-bcr) and major-bcr (M-bcr). The number of fusion signals was two in m-bcr and one in M-bcr with ES-fluorescent *in situ* hybridization (FISH), while with double-FISH, there were two fusion signals in both m-bcr and M-bcr. It was possible to discriminate them by ES-FISH, whilst it was impossible by double-FISH.

The detection of Philadelphia (Ph) chromosomes plays a vital role in the diagnosis of chronic myeloid leukemia, in the monitoring of therapeutic effect, and in the detection of minimal residual disease after bone marrow transplantation. It is meaningful not only to detect the presence of the Ph chromosome, but also to have a quantitative measure of the Ph-positive clones. Thus, a quantitative method of follow-up with high specificity and sensitivity has become of great importance. A major drawback of current polymerase chain reaction (PCR) studies is the lack of quantification of PCR data, though real time quantitative PCR has been developed. Fluorescent *in situ* hybridization (FISH) can detect malignant clones quantitatively with high accuracy and does not need dividing cells. However, the discrimination between minor and major bcr/abl translocations (m-bcr and M-bcr, respectively) was impossible by FISH. Recently extra signal-FISH (ES-FISH) has been introduced; this uses an abl probe spanning the breakpoint of the abl gene. When there is a bcr/abl translocation, the labeled abl gene splits into two signals, resulting in one fusion signal and an extra signal, and this extra signal enables the discrimination of false positive signals (Figure 1-A,B,C). The purpose of this study was to investigate whether M-bcr and m-bcr can be discriminated by the ES-FISH method. We performed ES-FISH on archival bone marrow cells from 13 cases with m-bcr (7 acute lymphoblastic leukemia, 6 acute biphenotypic leukemia) and 70 cases with M-bcr confirmed by RT-PCR and with the Ph chromosome by conventional cytogenetics, including 50 bone marrow specimens without hematologic malignancies to establish a cut-off value. Bone marrow and peripheral blood samples were processed by conventional cytogenetic procedures with GTG (G bands by trypsin using Giemsa) banding. ES-FISH was performed according to the manufacturer's instructions, using a bcr/abl ES dual color probe (LSI bcr/abl ES Dual Color Translocation Probe, Vysis Inc, Downers Grove, IL, USA). Double-FISH (D-FISH) was performed, using bcr and abl1 probes for D-FISH (Oncor Inc, Gaithersburg, MD,

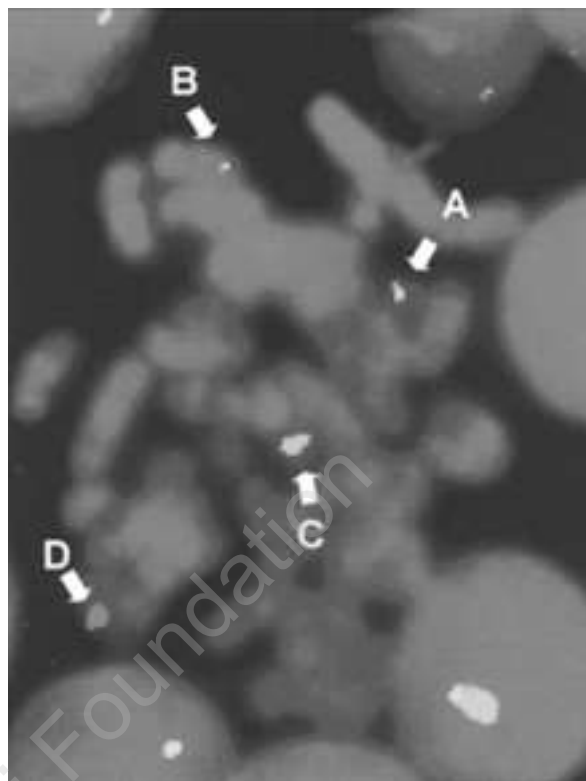


Figure 2. Comparison of 2 fusion signals of m-bcr FISH performed on metaphase. A: larger fusion signal on chromosome 22 B: smaller fusion signal on chromosome 9 C: green signal on normal chromosome 22 D: orange signal on normal chromosome 9.

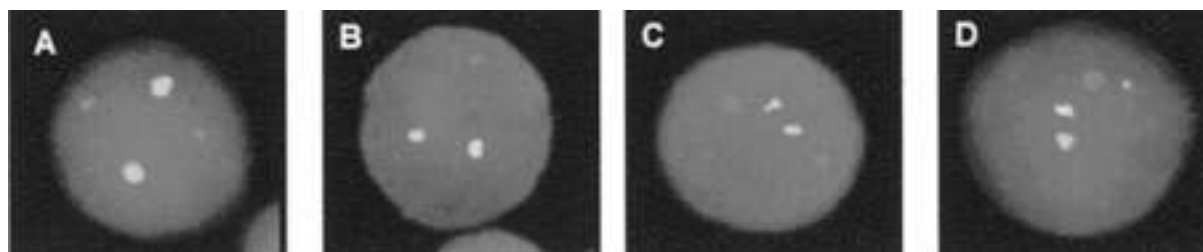


Figure 1. Signals of bcr/abl rearrangement by ES-FISH. A: 2G2O signal in normal cell without bcr/abl rearrangement; B: 1G101F signal of false positive bcr/abl rearrangement in ES-FISH; C: 1G201F signal of major bcr/abl rearrangement in ES-FISH; D: 1G102F signal of minor bcr/abl rearrangement in ES-FISH. Note that one fusion signal is larger than the other. The larger one is on chromosome 22 and the smaller one on chromosome 9.

USA) according to the manufacturer's instructions. RT-PCR for bcr/abl was performed using the method of Melo et al.¹

Among 13 cases positive for m-bcr RT-PCR, 11 cases showed bcr/abl translocation signals by FISH and all of them showed 2 fusion signals and 101G. The signals of m-bcr were discriminated by 2F101G, and those of M-bcr by 1F201G (Figure 1 C-D). In m-bcr, one of the 2 fusion signals was relatively larger than the other in interphase cells. When we analyzed the 2 fusion signals in the metaphase, the larger one was on chromosome 22, and the smaller on chromosome 9 (Figure 2). All of the 70 M-bcr positive cases showed 1F201G. The point of breakage onto the hybridized probe for the bcr region caused the difference. In M-bcr, the breakpoint was located at the end of the labeled area of the bcr gene. As a result of translocation, 1 fusion signal, 1 residual abl signal, 1 abl signal in intact chromosome 9, and 1 bcr signal in intact chromosome 22 were seen. In m-bcr, the breakpoint was located in the middle of the labeled area of the bcr gene. As a result of translocation, 2 fusion signals, 1 abl signal in intact chromosome 9, and 1 bcr signal in intact chromosome 22 were seen. We performed D-FISH on specimens from 8 cases with m-bcr and 10 with M-bcr. All of them showed the same signals (2F1G10), and the sizes of the 2 fusion signals were similar (Figure 1). D-FISH was shown to be a very sensitive method (false positivity 0.64%),² but the discrimination of m-bcr from M-bcr was impossible by D-FISH. In the present study, ES-FISH enabled us to discriminate m-bcr from M-bcr, and the false positive signal was also discriminated by F101G1 from genuine bcr/abl fusion by F102G1. In addition, the cut-off value in our study was 0.22%, which is lower than that reported by D-FISH (0.64%).² With S-FISH, up to 10% of false-positive fusion were reported (results of a College of American Pathologists survey published in 1999), which made it difficult to detect minimal

residual disease (MRD) quantitatively. On the other hand, in the present study, ES-FISH made it possible to detect MRD quantitatively by decreasing the false positive signal to 0.22%. We conclude that ES-FISH can discriminate M-bcr and m-bcr, and can be effectively utilized in the quantitative follow-up of patients positive for bcr/abl.

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