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Comparison between automated and manual reading for evaluation of BCR/ABL FISH

◎Jee-yoon Shin[1)]、So-young Choi[1)]、Je-hyun Park[1)]、Je-sung Lee[1)]、Jeong-sung Du[1)]
Hwasun Chonnam University Hospital[1)]

[Introduction]
FISH (Fluorescent in *situ* hybridization) has become an essential tool for diagnosing and monitoring hematological disease. Testing for minimal residual disease requires precise and accurate normal cut-offs. There is no consensus in the field on the correct method of establishing a normal reference range. We discuss and compare automated analysis with manual analysis and settle reference range of cut-offs.
For chronic myeloid leukemia, the FISH detection of t(9;22)(q34;q11) in interphase nuclei is an alternative method to bone marrow karyotyping for monitoring treatment. Automation system suggests the circumvention of several drawbacks of manual analysis. In this study, the capabilities of a commercially available automated image acquisition and analysis system were evaluated by comparison study with manual reading method.

[Method]
Cell suspension was prepared from peripheral blood of 10 healthy controls. The commercially available BCR/ABL dual color, dual fusion (DF) probe kit (Vysis, Downers Grove, IL) was used for labeling the respective BCR and ABL gene regions. The cells and probes were denatured on a heating plate together at 80°C for 5 min. Hybridization was performed overnight at 37°C. Denaturation and hybridization was performed using Xmatrx system (Abbott, BioGenex, USA).
FISH analysis was performed by using both automated Bioview Duet™ (BioView, Ltd., Billerica, MA) system and manual reading. We also calculated cut-off value using BETAINV function of excel (Microsoft, Redmond, Washington, USA). Analysis of at least 200 nuclei on each preparation was performed.

[Result & Conclusion]
The cut-off value calculated from Bioview analysis for 3O3G2F pattern was 1.1%, and manual reading result is 1.2%. This result was similar to established cut-off value by the manufacturer. Otherwise, cut-off value calculated from Bioview analysis for 2O2G1F pattern was 18.6%, and manual reading result is 15.7%. The concordance of two analytic method was 96.5%~99.8% in major signal patterns. We suggest that 2O2G1F pattern of FISH for BCR/ABL1 gene rearrangement was observed when 3O3G2F pattern was lower than 1% in normal healthy person.
Our study indicates that Bioview system is a reliable method to analyse FISH for BCR/ABL1 gene rearrangement. And Bioview system represents a clinically useful tool for standardised and objective BCR/ABL1 gene rearrangement of hematologic malignancy patients.

Tel ; 82-61-379-7981