

A Sensitive Alternative to Fragment Analysis–Based Methods in Chimerism Monitoring: Microchimerism Analysis Using Next-Generation Sequencing Technology

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Abstract

Objective: Chimerism monitoring following allogeneic hematopoietic stem cell transplantation (allo-HSCT) is of critical importance for evaluating graft success and the early detection of potential transplantation-related complications. Conventional methods such as short tandem repeat (STR) analysis and quantitative PCR (qPCR) are limited in terms of sensitivity. Next-generation sequencing (NGS) has emerged as a groundbreaking approach, offering unparalleled sensitivity and accuracy for the detection of donor and recipient cell populations. The aim of this study was to compare NGS-based chimerism analysis with the STR method and to assess its analytical sensitivity.

Materials and Methods: This study included 20 patients who underwent allogeneic bone marrow transplantation at the Department of Hematology, Istanbul Faculty of Medicine, along with 10 international external quality control samples. A total of 41 samples were analyzed at the Tissue Typing Laboratory of Istanbul Faculty of Medicine. DNA samples were obtained from peripheral blood, bone marrow, intra-abdominal ascitic fluid, and liver biopsy specimens. DNA isolation was performed using the Promega Maxwell RSC kit, after which all samples were analyzed using both STR and NGS methods. STR analysis was performed using the Qiagen IDPlex Plus kit with fragment analysis, while NGS-based analyses were carried out using the One Lambda Devyser™ Chimerism NGS Assay and the GenDx NGSTRack kit.

Results: Of the 41 samples analyzed by STR, 23 were reported as complete chimerism. However, NGS-based analysis revealed that 21 of these samples actually exhibited microchimerism or mixed chimerism. Additionally, quantitative differences in donor–recipient cell proportions were observed among samples identified as mixed chimeric.

Conclusion: NGS-based chimerism analysis demonstrates significantly higher sensitivity compared to conventional STR methods and enables the early detection of minimal residual disease. The presence of microchimerism in many samples previously classified as “complete chimerism” by STR indicates that NGS is a more reliable method for predicting early graft failure and relapse risk. The integration of NGS into routine clinical practice has the potential to initiate a new era in post-transplant patient management following hematopoietic stem cell transplantation.

Keywords: Chimerism, NGS, microchimerism, monitoring, HSCT

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