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Letter to Editor Open Access

Application of Sensitive AlleleSEQR Chimerism PCR from Peripheral Blood in Allografted AML/MDS Patients Treated with Pre-emptive Post-transplant Immunomodulation

Urpu Salmenniemi[1]*, Veli Kairisto[2], Jussi Leino[2], Mervi Putkonen[1], Marjut Kauppila[1], Tommi Salmi[1], Markku Oksman[3], Kari Remes[1] and Maija Itälä-Remes[1]

[1]*Department of Clinical Haematology, Turku University Hospital, Turku, Finland*
[2]*Laboratory of Molecular Genetics, Turku University Hospital Laboratories, TYKSLAB,Turku, Finland*
[3]*Department of Internal Medicine, Turku City Hospital, Turku, Finland*

PCR technique based on short tandem repeats (STR, microsatellites) is currently most widely used to monitor chimerism after allogeneic hematopoietic stem cell transplantation. Analysis of the proportion of donor and recipient derived cells enables an early detection of graft rejection or secondary graft failure. In malignant diseases increasing recipient chimerism has also been used to predict relapse, particularly in patients who lack disease-specific PCR marker to detect minimal residual disease (MRD). In these patients serial chimerism analyses may guide the use of post-transplant pre-emptive immunomodulation with the goal of avoiding clinical relapse. However, the sensitivity of the recently standardized STR-PCR, ranging between 0.8 and 1.6%, may not be sufficient to allow detection of impending relapse early enough in rapidly proliferating diseases like acute myeloid leukemia (AML) [1]. Therefore, we have tested the Abbott AlleleSEQR chimerism analysis system in AML patients who received pre-emptive post-transplant immunomodulation.

The patients were routinely investigated for chimerism with STR-PCR monthly from whole blood and from blood T-cell fraction during post-transplant period and from bone marrow CD34 positive precursor cell fraction at 2-3 month intervals. In high risk patients with AML or myelodysplastic syndrome (MDS), we have applied post-transplant immunomodulation with azacytidine, lenalidomide and/or donor lymphocyte infusions (DLI) based on findings in MRD and chimerism analyses the target being MRD negativity and full donor chimerism. In this retrospective study the routinely collected samples were additionally analyzed by Abbott AlleleSEQR chimerism assay.

The study included 143 samples from 15 patients (8 men, 7 women) transplanted for AML (n=13) or high risk MDS with blast excess (n=2) in years 2008- 2012. The median age was 59 (range 19-66) years and disease status at transplantation was refractory AML (n=6), AML in CR1 (n=4), AML in CR2 (n=3) and untreated MDS with blast excess (n=2). Sequential conditioning with FLAMSA induction (fludarabine, cytarabine and amsacrine) followed by high dose cyclophosphamide and total body irradiation was used in 8 patients, myeloablative in 4 and reduced intensity conditioning (RIC) in 3 patients. Matched unrelated donor was used for 11 and HLA-identical sibling donor for 4 patients.

The CD3-positive cells (T-cell fraction) were enriched from fresh blood samples and the CD34-positive cells from fresh bone marrow samples using magnetic beads according to manufacturer instructions (Dynabeads® CD3+ and Dynal®CD34+ Invitrogen™). DNA was isolated from total cell fraction using the NucleonBACC3 kit (GE Healthcare); from CD3-positive cell fraction by the EZNA-kit (Omega Biotek); and from CD34-positive cell fraction by cell lysis with Proteinase-K.

Pre-transplant samples from patients and their respective donors were screened for the most informative markers for chimerism analysis. The initial screening panel for STR-PCR analyses included seven polymorphic short tandem repeats markers among which 2-3 most informative were selected for followup. All STR-PCR analyses were performed with the ABI 7500 PCR system and the ABI 3700 capillary electrophoresis system as previously described [2]. 100 ng of DNA was used in the PCR-reactions. For the CD3 and CD34 –selected samples the concentration of DNA could not always be measured spectrophotometrically because of the low amount of sample available. Such samples were pre-analysed by quantitative PCR targeting the albumin gene. Up to eight PCR-cycles were then added to the actual analytical reaction as guided by the delta cycle threshold value reached in the reference gene albumin PCR.

In allele SEQRanalysis 34 biallelic insertion/deletion loci across the entire human genome were screened to identify one recipient specific and two donor specific markers. To reach high sensitivity 500 ng of DNA was used in each of the triplicate reactions. For the scarce CD3 or CD34 –selected samples as much DNA as possible was used. The real-time PCR was performed with the ABI 7500 as recommended by the manufacturer and Abbott AlleleSEQR software was used to analyze the real-time PCR data [3,4].

In all, 143 samples were analyzed by STR-PCR and AlleleSEQR-PCR (whole blood n=83, T-cell fraction n=26 and bone marrow CD34 cell fraction n=34). There were 1-21 samples per patient collected 24 to 640 days after transplantation. The concordance of the parallel measurements is shown in Table 1. Around 60% of samples were in accordance i.e. showing either 100% chimerism or <100% chimerism with both methods.

Majority of discordant samples showed full donor chimerism by

	AlleleSEQR 100% donor	AlleleSEQR <100% donor	
STR-PCR 100% donor	43 (30.1 %)	52 (36.4 %)	95 (66.6 %)
STR-PCR <100 % donor	3 (2.1 %)	45 (30.8 %)	48 (33.6 %)
	46 (32.2 %)	97 (67.8 %)	143 (100%)

Table 1: Concordance of parallel measurements by AlleleSEQR and STR-PCR.

***Corresponding author:** Urpu Salmenniemi MD, Department of Clinical Haematology, Turku University Hospital, Hämeentie11,20521, Turku, Finland, Tel:+35823130169; Fax:+35823133043; E-mail: urpu.salmenniemi@tyks.fi

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STR-PCR but <100% chimerism by allele- PCR. The median donor chimerism by AlleleSEQR in the 52 samples that showed 100% donor chimerism by STR-PCR was 99.98% (range 98.200% - 99.999%). To test the sensitivity and analytic linearity of the AlleleSEQR-PCR, DNA of one of the recipients was mixed with the DNA of the respective donor using 1:2 dilution steps down to the mixture of 1:16 384, and the range of linear measurement was shown to span to the lowest mixture.

Eight patients were MRD positive after transplantation, and 58 whole blood chimerism analyses were performed in them: 52 of the 58 samples showed mixed chimerism by AlleleSEQR-PCR, but only 22/58 by STR-PCR. The behaviour of MRD and chimerism analyses of three patients receiving post-transplant immunomodulation is shown in Figure 1. Two of these patients have experienced a relapse and the last whole blood chimerism analysis was performed 17 and 31 days before the diagnosis of relapse (Figure 1, patients B and C). Both patients showed full donor chimerism by STR-PCR while AlleleSEQR showed a trend (>5 fold changes in serial analyses) towards higher recipient chimerism.

Twelve patients had more than one whole blood chimerism analyzed and the last samples were taken the median of 367 days (117-

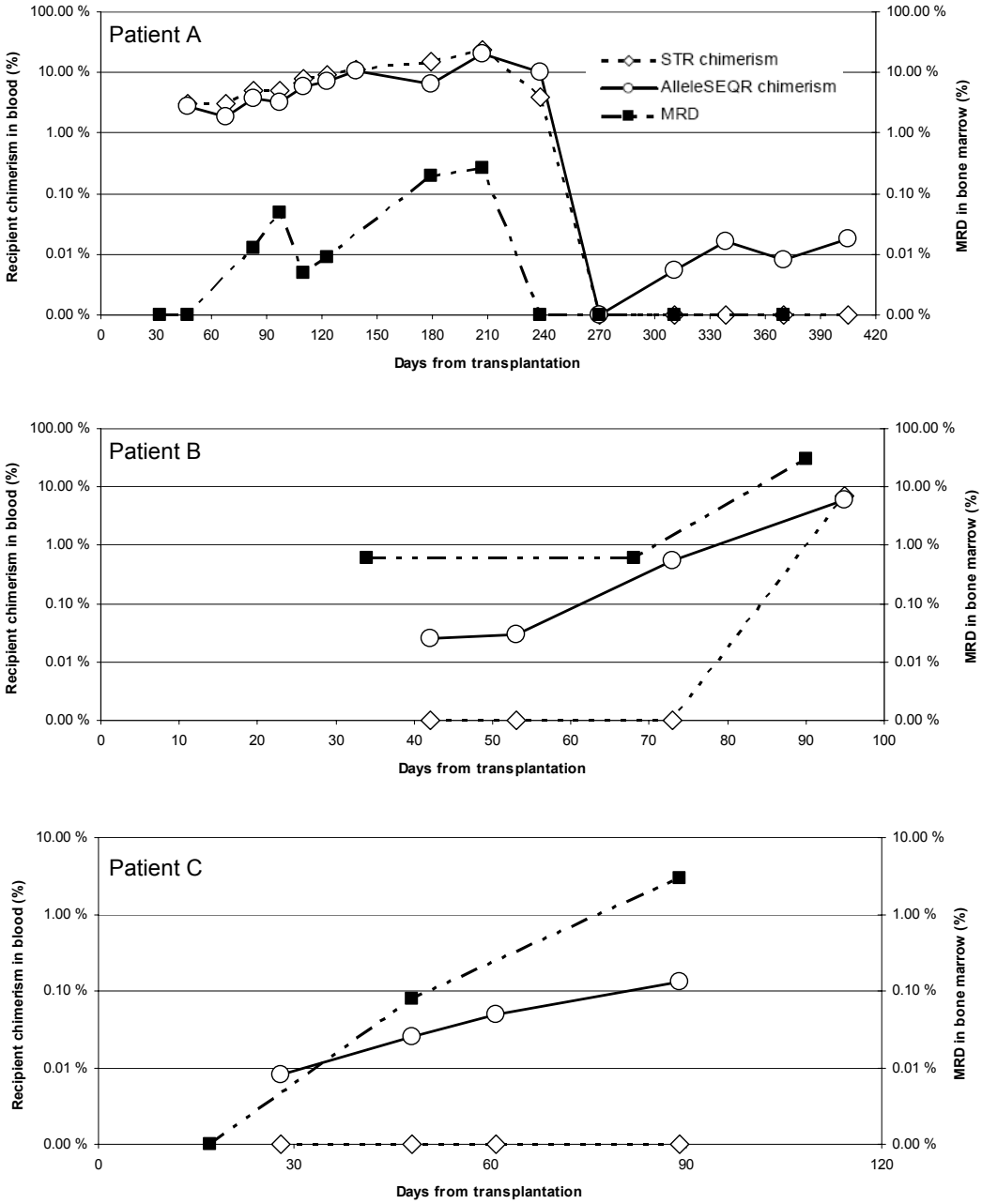

Figure 1: Comparison of MRD and chimerism analyses in three patients transplanted for refractory AML. Patient A received post-transplant immunomodulation (azacitidine, lenalidomide and DLI) due to mixed STR-chimerism and MRD positivity, and subsequently achieved MRD negativity and full donor chimerism(>99.99% by AlleleSEQR-PCR). In patients B and C the result of chimerism analysis by AlleleSEQR-PCRparallel the MRD while STR-PCR indicates full donor chimerism. Patient B experienced hematological relapse 90 days post-transplant.

640 days) after transplantation. Among these samples 9/12 showed 100% donor by STR-PCR whereas only one sample showed full donor chimerism also by AlleleSEQR-PCR.

Our results suggest that AlleleSEQR-PCR is well suited for sensitive detection of minor amounts of recipient DNA in near complete donor chimerism. Low level of recipient DNA was detected in most patients irrespective of conditioning treatment (myeloablative versus reduced intensity conditioning). Similar findings have been observed when chimerism has been studied with sensitive PCR technique applying the amplification of Y-chromosome-specific DYZ1 sequence in male patients receiving grafts from female donor [5]. In that study, 6/11 patients with mixed chimerism converted to full donor chimerism by 15 months post-transplant (sensitivity level of the analysis was 0.01%). Although most of our patients (11/12) showed mixed microchimerism by AlleleSEQR-PCR in their last analysis, the level of mixed microchimerism was below 0.01% in 5 patients.

As shown in this study, the majority of patients may present with low level of recipient chimerism transiently during post-transplant period. In addition to normal recipient hematopoiesis or residual malignant cells, very low level of mixed chimerism might represent contamination by non-hematopoietic cells. This issue was addressed by Petit et al. [5]. They concluded, however, that contamination was unlikely,because residual cells were detected at comparable level in whole blood and sorted lymphocytes and they were not detected in most long-term samples.

Despite the unspecificity of chimerism analysis, several studies have shown that mixed chimerism may predict impending relapse [6,7]. Similarly, studies have shown that relapses occur relatively shortly after decrease in donor chimerism and that the level of mixed chimerism at the time of hematological relapse is very variable. Thus to accurately predict impending relapse, the test for chimerism has to be sensitive, taken frequently, and the trend in degree of mixed chimerism is significant. Peripheral blood analysis of chimerism by AlleleSEQR fulfils these criteria. Although the sensitivity and/or specificity of the chimerism analysis may be improved by analyzing the CD34-cell fraction in bone marrow, the sampling of marrow at short intervals is cumbersome.

We speculate that AlleleSEQR-PCR might be particularly useful in patients with high risk of relapse who lack sensitive disease-specific PCR-marker for MRD analysis. In these patients AlleleSEQR-PCR with sensitivity level of 0.001% could serve as a surrogate of MRD. This is exemplified by patients B and C (Figure 1) whose proportion of recipient specific DNA increased in peripheral blood while molecular relapse developed in bone marrow and the less sensitive STR-PCR still indicated full donor chimerism. Patient A was treated with DLI. The treatment seemed to eradicate measurable MRD and turned STR-PCR to indicate full donor chimerism. Initially after the DLI also the AlleleSEQR-PCR indicated 100% donor chimerism but subsequent analyses showed about 0,01% level of recipient specific DNA in blood samples. This is in concordance with the results obtained from bone marrow CD34 cell fraction showing about 99% donor chimerism.

In summary blood AlleleSEQR-PCR is well suited for the chimerism analyses and is significantly more sensitive than STR-PCR. The method is faster and the total costs are less compared to STR-PCR. Further studies are needed to confirm its significance in predicting impending relapse, and whether frequently repeated analyses from peripheral blood could obviate the need for bone marrow sampling.

References

1. Lion T, Watzinger F, Preuner S, Kreyenberg H, Tilanus M, et al. (2012)The EuroChimerism concept for a standardized approach to chimerism analysis after allogeneic stem cell transplantation. Leukemia 26: 1821-1828.
2. Thiede C, Lion T (2001) Quantitative analysis of chimerism after allogeneic stem cell transplantation using multiplex PCR amplification of short tandem repeat markers and fluorescence detection. Leukemia 15: 303-306.
3. AlleleSEQR® Chimerism Assay (2009) - Operator's Manual. Celera corporation, Alameda, USA.
4. Gendzekhadze K, Gaidulis L, Senitzer D (2013) Chimerism Testing by Quantitative PCR Using Indel Markers. Methods in Molecular Biology 1034: 221-237.
5. Petit T, Raynal B, Socié G, Landman-Parker J, Bourhis JH, et al. (1994) Highly sensitive polymerase chain reaction methods show the frequent survival of residual recipient multipotent progenitors after non-t-cell-depleted bone marrow transplantation. Blood 84: 3575-3583.
6. Bornhäuser M, Oelschlaegel U, Platzbecker U, Bug G, Lutterbeck K, et al. (2009) Monitoring of donor chimerism in sorted CD34+ peripheral blood cells allows the sensitive detection of imminent relapse after allogeneic stem cell transplantation. Haematologica 94: 1613-1617.
7. Tobiasson M, Olsson R, Hellström-Lindberg E, Mattsson J (2011) Early detection of relapse in patients with myelodysplastic syndrome after allo-SCT. Bone Marrow Transplantation 46: 719-726.

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