Strategies to improve the estimation of donor chimerism

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Abstract. *Background*: Reduced intensity conditioning regimens in peripheral blood stem cell transplantation (PBSCT) result in a period of mixed chimerism. Its regular monitoring is important to the patient's clinical assessment and measurement precision is essential to that process. 'Stutter' artefact peaks hinder chimerism estimation by mimicking minor proportions of DNA. We wished to assess the value of adding more loci to our current protocol and to look at the value of selecting only those markers unaffected by stutter artefact. *Methods*: Donor chimerism was monitored in 32 patients post PBSCT on five occasions using the Profiler Plus (Applied Biosystems) and Powerplex 16 (Promega) short tandem repeat (STR) kits. *Results*: Mean donor chimerism was not significantly altered by increasing the numbers of loci, but precision increased significantly ($p = 0.0002$). Removing loci affected by stutter from the Powerplex 16-associated measure increased error significantly ($p = 0.0024$), due to decreased sample size. *Conclusions*: Increasing available autosomal loci from 9 to 15 has increased precision of chimerism measurement and the time-consuming selection of loci compromised by stutter position alleles has proved unnecessary. © 2004 Elsevier B.V. All rights reserved.

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1. Introduction

Peripheral blood stem cell transplantation (PBSCT) is an effective method of treatment for haematological malignancies, but conditioning regimens result in a treatment-related mortality of 20–30%, deaths generally being related to infection or graft-versus-host disease (GVHD). Reduced intensity conditioning results in lower mortality and this produces a slower transformation from recipient to donor types, resulting in mixed chimerism continuing over several months. Serial chimerism studies are essential to monitor this process, to exclude non-alloimmune mechanisms of marrow suppression,

such as drug toxicity or viral infections, to predict graft failure [1] and schedule appropriate therapeutic interventions. We have been using the nine STR loci currently provided by Profiler Plus (Applied Biosystems) to monitor chimerism for more than 7 years [2].

For an accurate and precise assessment, sufficient numbers of polymorphic markers must be used to enable selection of enough informative splits between individuals, who are often closely related. 'Stutter' artefact peaks, four base pairs smaller than the true peak, can hinder chimerism estimation by mimicking minor proportions of DNA, making it difficult to get a true reflection of donor proportion where donor or recipient types are coincident with a stutter. We wished to assess the value of adding more loci and, in addition, the value of selecting only those markers unaffected by stutter artefact.

2. Materials and methods

2.1. Patient selection

Patients with a range of haematological malignancies treated by PBSCT were selected for study, including those with acute leukaemia, myeloma and lymphoma, under the care of St. Bartholomew's Hospital, London. Thirty-two patients who exhibited mixed chimerism from 1 month post PBSCT, and for whom five sequential assessments were available, were selected for the study.

2.2. Methods

DNA was extracted using Chelex 100 resin. Donors and recipients were profiled pre-transplant and recipients post-transplant, using both the Profiler Plus (PR) kit (Applied Biosystems) and the Geneprint Powerplex 16 (PO) kit (Promega) retrospectively. Analysis was undertaken using an ABI Prism 3100 Genetic Analyser (Applied Biosystems) and typed by reference to an allelic ladder run concurrently.

Percentage donor chimerism was calculated from the observed peak areas, making the assumption that the area contributed by one heterozygous allele, visible distinct in the mixed profile, was identical to that area contributing to a mixture of donor and recipient. In addition to calculation of donor chimerism using each of the STR kits, PR and PO respectively, values were also calculated for informative loci in the Powerplex 16 kit, but omitting loci where a donor allele coincided with the stutter peak region of the recipient allele, or vice versa (no stutter—PONST). In certain circumstances such removal seemed unnecessary: for example, leaving out a locus where the average donor chimerism was greater than 80% and where a particular donor allele was coincident with the stutter peak of the recipient. A further assessment was therefore made leaving out only those 'important' stutters (important stutter–POIST).

A repeated measures analysis of variance (ANOVA) was used to compare the 'between occasion' and 'between measure' means and standard errors (used as an assessment of precision) of the donor chimerism values at each of the five sequential time points.

3. Results

There was no significant difference in mean donor chimerism values obtained from each kit, whether or not selective loci were deleted ($p = 0.78$). Mean values did vary at the
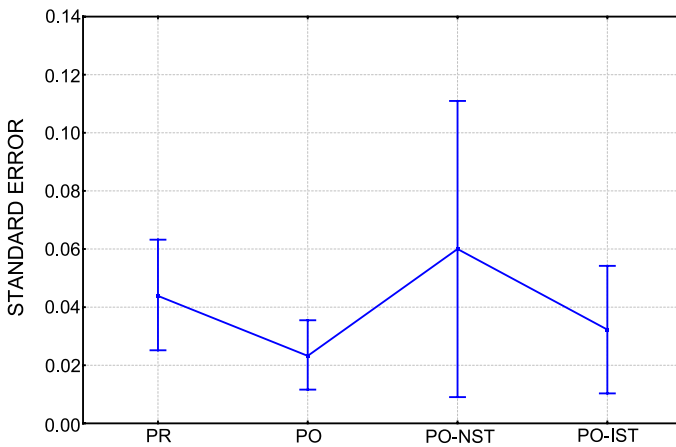
Fig. 1. Mean standard error of the mean donor chimerism, specific to method at all time points. Vertical bars denote 95% confidence interval.

different time points, which was to be expected as these reflected changes in clinical progress and the standard error was similar at different time points. There was, however, a significant difference in error between the different assessment measures ($p = 0.0002$), PO providing the smallest standard error (Fig. 1).

4. Conclusions

Increasing the number of available loci for our chimerism studies has allowed us to improve the precision of the measures that we provide for our clinicians, without any shift in mean values, enabling a trouble-free transition to the new methodology. Having a larger number of loci appears far more useful than methods to reduce the error produced by stutters, the selection of which is necessarily patient specific and therefore time-consuming.

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