**Introduction**

After stem cell transplant an assessment of engraftment can be made through monitoring the proportions of donor and recipient cells in a whole blood or bone marrow sample. Determining the mix of cells, or ‘chimerism’, aids scheduling of specific therapeutic interventions, such as withdrawal of immunosuppressive drugs or administration of donor lymphocyte infusions.

One method of measuring chimerism is by tracking a simplified genetic fingerprint that uniquely defines the donor and recipient. This technique is of great value, as the small amounts of DNA needed for the test can be obtained when the bone marrow is still hypocellular, soon after transplant. The whole blood analysis has a moderate sensitivity of 1-5%, but if blood components are sorted in to their specific lineages (B-cells, T-cells, myeloid cells etc) the sensitivity of this method can be increased over 10-fold. (Theide et al 2004). The aim of this work was to extend our analysis of whole blood chimerism to a lineage-specific test.

The additional information provided could help with understanding the dynamics of the engraftment process and in the future facilitate more effective treatment in the post-transplant period.

**Methods**

Our current method of chimerism analysis is based on analysis of polymorphic short tandem repeats (STR’s) to uniquely define the source of DNA extracted from leukocytes:

- DNA is extracted from the buffy coat layer of an EDTA blood sample using an automated magnetic purification method (Qiagen EZ1).
- PCR reactions using three STR markers are set up using a commercially available forensics kit (Promega PowerPlex16 Monoplex System).
- The differently sized fluorescent PCR products are detected and analysed on a capillary system genetic analyser (Applied Biosystems 3130xl).

- An assessment of the proportions of donor and recipient chimerism can be calculated from the analyser output using a simple algorithm measuring the peak height and area.
- The separate leukocyte lineages are isolated by cell separation using AutoMACS® immuno-magnetic separation technology.
- DNA is isolated from these purified fractions and analysed as whole blood.

**Results and Discussion**

- Extended validation tests using control samples indicated chimerism could be accurately assessed in the difference leukocyte lineages.
- Of eight patients studied, two patients displayed full donor chimerism in both the whole blood and lineage-specific analysis.
- Three patients with whole blood donor chimerism of 90-95%, displayed a similar level of donor chimerism in the myeloid fraction, but a more significant decrease in T-cell (CD3) fraction.

This patient received a stem cell transplant after refractory chronic myeloid leukaemia (CML) and so this result could give useful information as to whether the chimeric state is due to a recurrence of the original myeloid (CD33) clone. Reduced T-cell chimerism is strongly associated with graft failure (Mohy et al 2007) and the low donor CD3 value could also provide hard evidence of the success of the engraftment.

**Conclusions**

This study has confirmed that lineage-specific chimerism analysis represents a valuable adjunct to our whole blood studies. Due to its increased sensitivity it can be used to reveal mixed chimerism in specific leukocyte populations that are masked in the whole blood analysis. Information about the relative proportions of donor and recipient T-cells is important in understanding the dynamics of engraftment and predicting graft vs leukaemia and graft vs host effects. Consequently, T-cell chimerism represents a particularly useful part of the cell-specific analysis.

**References and Acknowledgments**


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