Review of: "High Frequency of Post-Transfusion Microchimerism Among Multi-Transfused Beta-Thalassemic Patients"

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This study describes the formation of transfusion-associated microchimerism (TAM) in multi-transfused beta-thalassemic patients. This study is interesting as it describes TAM in a population group not well-studied for this phenomenon. Studies to describe TAM are complicated to perform, particularly as they require adequate patient numbers, correct timing for sample collections and appropriately sensitive assays. The authors of this study managed to bring these factors together and describe a very high rate of TAM (over 81%) in transfusion dependent patients. This rate is significantly higher than when TAM has been analysed in other patient settings, such as trauma patients, where only 10% of patients were found to have TAM. The authors suggest that changes in NK and regulatory T-cell and high serum ferritin levels are linked to the potential incidence of TAM in in multi-transfused beta-thalassemic patients.

The study has many enviable positives. The authors were able to sample the same patients regularly for a period of time and were able to collect fresh samples during the transfusion regime to enable flow cytometry analysis of lymphocyte subpopulations. However, there are still some study design aspects that were missed opportunities for improving the understanding of TAM.

First was the choice of control group size and use of male only donors in that group. Of the 21 never-transfused male donors used, 6 were positive for microchimerism. The authors concluded this result was due to potential naturally acquired persistent maternal microchimerism.

Second, in studies of TAM, females are generally not recruited into study cohorts or used in ‘control’ cohorts because of the risk of being unable to distinguish between TAM and naturally acquired foetal-maternal microchimerism. The authors do use female patients in their study cohort so perhaps finding samples from younger nullipara female donors to act as controls would also have been appropriate for comparison.

It is also unclear why only 21 control participants were recruited and exactly how this sample size was defined. Also, how was it determined that these individuals had never had a blood transfusion? The ability to access fresh control patient samples to analyse the immunophenotype of non-transfused individuals and compare it to the transfused cohort was also a missed opportunity. Did the control patients with microchimeric allelic patterns have similar T-cell and NK-cell responses to those who had microchimerism following blood transfusion? Perhaps this data would have been valuable to determine if suspected persistent maternal microchimerism might occur due to similar lymphocyte subpopulation profiles as for TAM.

Of the 44 patients who exhibited long-term microchimerism, how many were female? The data presented is not clear but this would be valuable to know. If persistent maternal microchimerism is present, more females would be expected to have long-term microchimerism, although the authors state that only 8 patients from their cohort had been previously

pregnant. Of those 8, 100% of them reported microchimerism but it is unclear if this was in the long, short or mixed term timepoints. Regardless of whether the females were reported with previous pregnancies or not, the number of microchimeric alleles detected are similar among them. Therefore, perhaps females are more conducive to forming microchimerism. Again, perhaps an analysis of never transfused, never pregnant females would have been valuable.

As with other TAM studies, leucodepletion of RBC units prior to transfusion appeared not to have influenced the formation of TAM, however the high rate of detection in this study is still surprising. Patients with autoimmune disorders appeared to have more chimeric alleles, however the disorders were not listed in the data.

The control patients have fewer alleles than patients. This finding was not explained further by the authors. C_T values of the detection in controls might be useful to assess how strong the signal was in these individuals. Was the positivity borderline i.e. $C_T \geq 41\text{-}42.9$? Given the incredibly high rates of detected chimerism compared to previous studies, the C_T values used to classify each patient and control would have been valuable to provide. The authors do state that there may be some false positives in this cohort.

Figure 3 is also hard to interpret without knowing how strongly each allele was detected at each timepoint. Did taking samples 14-16 days following their previous blood transfusion account for high detection of short term microchimerism?

For the demographics data it is hard to interpret due to lack of knowledge about long term vs short term breakdowns.

When discussing these results under the heading ‘association of TA-MC with potential contributing factors’, the authors suddenly indicate that there are 11 patients without established TAM but in figure 2 there are 9 patients without TAM. I presume the extra 2 patients had very transient TAM present(?).

More investigation is needed of splenectomised individuals. Despite being borderline in this study, in another TAM study with trauma patients from Australia, spleen injury was associated with TAM detection. It is an exciting potential pathway for TAM establishment. The slight potential changes observed of regulatory T-cell and NK-cell patterns in splenectomised patients is intriguing and fascinating for the field.

Generally the flow data presented is very exciting as it does demonstrate that for people with microchimerism, immune system perturbation really appears to play a role in recognising and removing the donor leucocytes following transfusion. However intriguing, the patient numbers examined are very small as I am sure the authors are aware.

The fact that persistent repeated RBC transfusions could induce immune tolerance is a potential reason why such high levels of microchimerism were detected. Indeed a recipients ‘reaction’ to transfusion would be of interest to follow. It is hard to determine how many transfusions participants had prior to this study being conducted. It can be argued that the only way to determine if persistent repeated RBC transfusion induces tolerance, is to analyse transfusion naïve individuals and follow them with PCR analysis for microchimerism but also immunophenotype profiles over a course of transfusion therapy.

I am excited to see these findings and look forward to future studies to find other features of potential TAM establishment. Study design and cohort choice is vital to ensure that informed data interpretation can be made.