Summary of positive PMCA ("blood test for vCJD") result for CJD Incident Panel meeting 20 January 2011

Introduction

The Scottish National Blood Transfusion Service (SNBTS) has been working in collaboration with colleagues from the National CJD Surveillance Unit and Etablissement Francais du Sang (EFS) on the adaption of the Protein Misfolding Cyclic Amplification (PMCA) assay to peripheral blood. As part of this process, 250 routine Scottish blood donor samples were used as negative controls. These were buffy coat samples generated as surplus / discard during routine blood donation processing. The blood was donated under standard generic consent given by all Scottish blood donors and samples were anonymised and unlinked from the donors. Somewhat surprisingly, one sample tested strongly positive on PMCA.

Further work carried out to attempt to validate the positive result

In order to try to establish whether this was a true or false positive result the amplicon from the PMCA assay was injected into humanised transgenic mice under the aegis of our collaborator Dr Hubert Laude in Paris. Dr Laude has recently reported to us that of four[1] mice in the test group which have died of inter-current illness, three[1] showed evidence of PrP^{TSE} accumulation in their spleens. Positive control mice which also died of inter-current illness showed similar positivity whilst negative control mice were negative as expected. The Western blots are consistent with a vCJD glycosylation pattern. The technical details are summarised as an Appendix.

The study has another 12-18 months to run before the full suite of mice are sacrificed, which will allow formal analysis of the prevalence of splenic and neurological disease, and full Western blotting and lesion profiling. There is a possibility that the positive PMCA result represents cross-contamination; we believe this to be unlikely because the sodium chloride precipitation of these blood samples was undertaken shortly after they were drawn in June 2009 in the SNBTS Cat 3 containment facility at Ellen's Glen Road. No known vCJD positive was handled by that facility until the Autumn of 2009. Clearly the possibility of cross-contamination cannot be formally excluded at this stage.

Issues raised

The finding of one strongly positive result in 250 random donor samples was unexpected. We feel, however, that the evidence of true positivity is now sufficiently strong that we ought to take precautionary action in relation to the donors.

Although the samples were anonymised and unlinked, the fact that they were processed in small batches allows a relatively high degree of imputability and the positive sample has been narrowed down to four individuals who donated on the same date in June 2008[1]. Of these,

- one is no longer on service
- one has not donated again since that time
- two are currently active donors. These donors have been placed on medical hold as an interim measure to prevent donations entering the blood supply. However this is not a long-term solution since although they will not be called to donate, they may choose to do so and would then need to be deferred and informed of the reason.

[1] Corrected 03.03.2011

Proposal

Our proposal is to now contact the four donors, explain the current situation and ask them to contribute a further sample for repeat testing.

- Assuming that all four agree to provide a further sample, this would allow us
  - to confirm that one of these four donors is positive (exclude cross-contamination)
  - reassure the other three donors and return them to service if they so wish.

- If one or more of the donors decline to donate a further sample, they could be returned to service so long as one of those who does agree to donate a sample is confirmed as the positive donor.

- If one or more donors decline to donate a further sample and we cannot identify a positive donor amongst those who do donate a sample, the untested donor(s) would have to be removed from the donor panel as indeterminate.

- If all 4 donors test negative on repeat sampling (x2) we would probably take the view that the positive PMCA result was a reflection of cross-contamination and return all 4 to service (where eligible).

SNBTS would handle the contact and notification of the four donors through our normal donor medical structure in the first instance. Professors Bob Will and Richard Knight from National CJD Surveillance Unit have kindly agreed to see and offer counselling/support to a confirmed positive donor if that eventuality arises.

Questions for the CJD IP

1. Is the Panel content with our proposed approach to managing this cohort of donors?

2. If we are able to identify a specific confirmed positive donor, is the Panel content with the measures we propose for deferral and counselling and should he or she be managed as if 'at risk for public health purposes' or "as if infected"?

3. Is the Panel content in that context that the other donors confirmed negative on repeat testing are returned to service?

4. If all 4 donors test negative on repeat testing on at least two occasions, is the Panel content that they are considered not infected, and managed accordingly with return to normal service?

5. In the eventuality that some donors choose not to donate a further sample, but one of the other donors tests positive, is the Panel content that the untested donor(s) should be returned to service?

6. In the eventuality that some donors choose not to donate a further sample, and the other donors are negative on repeat testing, does the Panel agree that the former will need to be permanently deferred as indeterminate? In this

eventuality should these individuals be managed as if 'at risk for public health purposes'?

7. How should previous recipients of blood components from a confirmed positive donor or from those who remain indeterminate be managed?

Your guidance on these matters would be most welcome.

Marc Turner.
Medical Director SNBTS

15th January 2010[1].

Appendix:
Summary of results obtained from screening 250 normal plasma samples by sPMCA/CDI.

As part of an ongoing project to evaluate the application of serial Protein Misfolding Cyclic Amplification (sPMCA) in combination with Conformation Dependent Immunoassay (CDI) for the amplification and subsequent detection of disease associated PrP^{Sc}, as a surrogate marker for vCJD infectivity, in human plasma, we have now tested 250 normal plasma samples. For screening, aliqoots of each plasma sample were treated with NaCl to precipitate any PrP^{Sc} and remove the PMCA inhibitors known to be present in human plasma. The pellets obtained following NaCl precipitation were resuspended directly in PMCA substrate, prepared using human platelets, and subjected to four rounds of sPMCA. The fourth round sPMCA products were then screened for PrP^{Sc} by CDI without prior proteinase K digestion. All test samples were screened twice in both *PRNP* codon 129 methionine homozygous (*PRNP*-129MM) and valine homozygous (*PRNP*-129VV) platelet substrates alongside suitable +ve controls (plasma spiked with a 10^{-6} dilution of a 10% (w/v) vCJD brain homogenate for *PRNP*-129MM substrate and plasma spiked with a 10^{-6} dilution of a 10% (w/v) VV2 sCJD brain homogenate for *PRNP*-129VV substrate). Using CDI cut-off values calculated as the mean CDI D/N ratio plus 3 standard deviations for all 250 plasma samples, none tested positive when screened in *PRNP*-129VV substrate. Whereas, one plasma sample (PL45) was a strong repeat reactive when screened in *PRNP*-129MM substrate. PL45 was from a *PRNP*-129MM donor. A sample of PL45, –ve control plasma and +ve control plasma sample (plasma spiked with a 10^{-6} dilution of a 10% (w/v) vCJD brain homogenate) were rescreened in a new batch of *PRNP*-129MM platelet substrate at the NCJDSU (different operator and set of equipment). The fourth round sPMCA products obtained from both PL45 and +ve control plasma were both positive when screened by CDI without prior proteinase K digestion. However, when screened by CDI following proteinase K digestion only the +ve control plasma sample produced a positive signal. This would suggest that the product amplified from PL45 is completely proteinase K sensitive. Aliquots of the fourth round sPMCA products obtained from the –ve control plasma, +ve control plasma and PL45 were inoculated in tg650 mice (transgenic mice over expressing human *PRNP*-129MM PrP^{C}). To date a number of mice in each group have died of natural (non-prion disease related causes) and the spleens of these mice were screened for the presence of abnormal disease associated PrP^{res} by Western blotting following limited proteinase K digestion. For the mice inoculated with the fourth round sPMCA products obtained from a) –ve control plasma 0 out of 2 mice contained PrP^{res} in their spleens, b) +ve control plasma 2 out of 2 mice contained PrP^{res} in their spleens and c) PL45 3 out of 4 mice contained PrP^{res} in their spleens. To date none of the inoculated mice are displaying any signs of clinical disease.
In summary we have reproducibly amplified a CDI reactive, proteinase K sensitive product from a normal human plasma sample which upon i.c. inoculation into tg650 mice induces the accumulation of proteinase K resistant PrP^{res} in spleens of these mice.