

SEAC 104/2

ESTIMATING THE PREVALENCE OF SUBCLINICAL vCJD

ISSUE

1. The prevalence of subclinical vCJD in the UK is highly uncertain. Current estimates are based on the Hilton *et al* data on abnormal prion protein (PrP^{vCJD}) in stored appendix samples, however the National Anonymous Tonsil Archive (NATA) study is on-going, and a further study of appendices has been commissioned. In addition, a pilot to assess the feasibility of a post mortem study to test spleen tissue has also been commissioned. The NATA data are currently within the confidence intervals of the Hilton data, but there remains a possibility that once completed, the data from these studies might be discrepant.
2. All samples in the NATA survey (over 80,000) have tested negative by EIA. However, one of 10,000 samples re-tested by IHC has given a positive result in one follicle. Extensive further testing of this sample has produced negative results.
3. Prevalence of infective material in subclinical individuals is a key factor determining the risks of secondary vCJD transmission via surgery, or donated blood, tissues or organs. To assess these risks, presence of PrP^{vCJD} has been used as a surrogate indicator of infectivity, though the relationship between the two is not fully established.
4. In addition, risk assessments have assumed that in principle, the same “prevalence” would drive all transmission risks. For example, if the prevalence of sub-clinical infection was “1 in x”, then 1 in every x surgical procedures encountering any lymphoid tissue (e.g. tonsil, appendix or spleen) would meet with infective material. Similarly, 1 in every x blood donations would be infective. At present, this means that assessments are based primarily on the Hilton *et al* appendix results. This approach may not be appropriate if the presence of PrP^{vCJD} varies markedly by site, and possibly over time. Nor would one necessarily expect different “prevalence studies” to be mutually consistent.
5. The Committee is asked to consider the following questions:
 - How many samples do studies need to test in order to have sufficient power to influence estimates for prevalence of PrP^{vCJD} in any given tissue?
 - Should one necessarily expect the prevalence of PrP^{vCJD} in all lymphoid tissues (and in blood) to be similar at any given time?
 - Should “sub-clinical prevalence of vCJD” (more generally) be interpreted in terms of presence of PrP^{vCJD} in *any* tissue)? If so, what evidence would SEAC need to see to enable it to revise its current advice on this?
 - How should the finding of one “positive” sample in the NATA study be interpreted, and what does this mean for the further testing of tonsils?

CURRENT PREVALENCE ESTIMATES

6. Based on SEAC advice, current analyses use estimates of the prevalence of subclinical vCJD based on the survey by Hilton *et al*¹. This study of stored appendix and tonsil tissues removed between 1995 and 1999 found three PrP^{vCJD} positive appendix samples (all from individuals born between 1961 and 1985 - the 1961 to 1985 birth cohort) from analysis of 11,109 appendix samples. No PrP^{vCJD} positive samples were found from analysis of 1 565 tonsil samples. A crude estimate of the prevalence of subclinical vCJD based on these data is 1 subclinical infection per 4,000 of the population in this age cohort. However, the confidence interval is wide (95% CI, 1 in 1,400 to 1 in 20,000), and the prevalence in other age cohorts remains conjectural.
7. A smaller study by Frosh *et al* (2004)² of 2,000 tonsil samples collected between July 2000 and August 2002 found no samples positive for PrP^{vCJD}. About 1,000 samples were from individuals less than nine years of age with the other samples from individuals aged nine years or more at the time of collection.
8. The National Anonymous Tonsil Archive was set up to prospectively collect and analyse 100,000 tonsils to obtain more precise estimates of the prevalence of subclinical vCJD. To date, no PrP^{vCJD} positive samples have been found from testing over 80,000 samples by EIA, including approximately 16,000 samples ,from the 1961 to 1985 birth cohort (the same birth cohort in which Hilton *et al* found three PrP^{vCJD} positive appendix samples).
9. SEAC considered the NATA testing methodology to be robust and noted that it was sufficiently sensitive to detect PrP^{vCJD} at levels between 100 to 1000-fold lower than present in the tonsils of clinical vCJD cases. Whilst the analytical methodology used in NATA is capable of detecting PrP^{vCJD} when present at relatively low concentrations in tonsil tissue, the capability of tonsil testing to identify individuals infected with vCJD is not well understood.
10. Further testing of 10,000 NATA samples from the 1961-1985 birth cohort by IHC has identified one positive sample. The positive finding, in one follicle, was subject to exhaustive additional analysis by Western Blot by the National CJD surveillance Unit, which was negative. Full details of the tests carried out are attached at **Annex A**. Advice on interpretation of these discrepant results is needed.
11. The data from Hilton *et al* (appendix samples only) and findings from NATA are shown in Table 1 (**Annex B**). The data from the two tissue surveys remain formally consistent with each other – even if all NATA samples are interpreted as negative - as the 95% confidence intervals overlap for the birth cohorts for which data are available from both studies. The final column of Table 1 shows the

¹ Hilton *et al*. (2004) Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol.* 203, 733-739.

² Frosh *et al*. (2004) Analysis of 2000 consecutive UK tonsillectomy specimens for disease-related prion protein. *Lancet* 364, 1260-62.

range of numbers of infected individuals in each age cohort that would be compatible with the data from both surveys. Depending on the interpretation of the "IHC-positive" in the NATA sample, however, the two data sets may at some stage become discrepant.

PREVIOUS SEAC CONSIDERATION

12. SEAC previously discussed this issue at SEAC 100, on 28 April 2008. An updated copy of the paper is attached at **Annex C**. A position statement was published in July 2008, which concluded that:

"Given the biological uncertainties about the timing and rate of accumulation of PrP^{Sc} in human tonsil and appendix tissue, SEAC does not consider that the data from the appendix survey and NATA can be combined to give a single credible estimate of prevalence of subclinical infections for the purposes of risk management considerations. Therefore, it would be prudent to consider that the estimate based on the appendix survey alone provides a reasonable, pragmatic and precautionary working scenario for the prevalence of subclinical infections."³

13. The position statement also recommended that the Hilton data could be refined by commissioning a further study of appendices. Provided appendix samples were collected from the appropriate birth cohorts, in particular the 1941 to 1960 and 1961 to 1985 birth cohorts, and the same analytical methodology as in Hilton *et al* was used to test the samples, the data from a new survey could be combined with the Hilton survey. However, the Committee concluded that the most reliable estimates of the prevalence of subclinical infection could be derived by testing spleen tissue.

NEW TISSUE SURVEYS

14. Based on SEAC's advice, the HPA has started a study of 30,000 archived appendix samples, which will be tested by IHC. The aim is to collect and test 20,000 samples from the 1961 to 1985 birth cohort and 10,000 samples from the 1941 to 1960 birth cohort. Interim findings should be available by April 2011, with full results by December 2012. This study replicates the Hilton methodology. The attached paper - **Annex C** - sets out the rationale for this study.
15. The HPA has also started a pilot study to prospectively collect and analyse samples of spleen obtained from post mortems. The pilot will assess three separate methodologies for obtaining tissue samples, using coroners and NHS bereavement services. A report will be prepared in the summer of 2010, which will be used to inform decisions by the Department of Health on whether it is possible to roll the study out more widely, and on what timescale it might be possible to obtain results. At present, however, the indications are that it will be very difficult to gain access to significant numbers of samples.

³ Position Statement – Prevalence of Sub-clinical variant Creutzfeldt Jakob Disease Infections, August 2008, <http://www.seac.gov.uk/statements/statements.htm>.

PRESENCE OF PrP^{vCJD} IN THE TISSUES BEING STUDIED

Appendix testing

16. PrP^{vCJD} has previously been detected in the appendices of patients in the sub-clinical phase of the disease. PrP^{vCJD} was detected in appendices removed from two vCJD cases 8 months and 2 years prior to the onset of clinical disease^{1,4}. PrP^{vCJD} was not detected in an appendix removed from another vCJD case, 10 years prior to the onset of clinical disease. However in this case the patient may have not yet been infected or else have been in the early stages of infection. In addition, PrP^{vCJD} was not found in the appendix of the case of subclinical vCJD associated with blood transfusion⁵. Three PrP^{vCJD} positive appendix samples were found by Hilton *et al* in the survey of stored appendectomy samples. No clinical cases of vCJD have yet been linked indirectly to these anonymous samples. Therefore, it would appear that PrP^{vCJD} accumulates in appendix tissue some time before the onset of clinical disease (if this develops). However, a more precise timing for PrP^{vCJD} accumulation in appendix tissue during the vCJD incubation period has not been established.
17. Three studies that examined the accumulation of PrP^{vCJD} in appendix tissue from clinical cases of vCJD showed PrP^{vCJD} to be present in 6/6⁶, 0/1⁷ and 1/4⁸ cases. PrP^{vCJD} was not found in the appendix of the case of subclinical vCJD infection associated with blood transfusion of MV genotype.⁹ Thus, it is possible that the tissue accumulation of PrP^{vCJD} may be influenced by prion protein gene codon 129 genotype (although, it is possible that the tissue distribution of PrP^{vCJD} in this case may have been, in part, influenced by the route of transmission). These data suggest that the extent of PrP^{vCJD} accumulation in appendix tissue could be variable.

Tonsil testing

18. PrP^{vCJD} is consistently detected in the tonsil tissue of clinical vCJD cases^{10,11,12,13,14}, including a case presumed to have been infected via blood

⁴ Hilton *et al.* (1998) Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. *Lancet.* 352, 703-704.

⁵ Peden *et al.* (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet.* 364, 527-529.

⁶ Head *et al.* (2004) Peripheral tissue involvement in sporadic, iatrogenic and variant Creutzfeldt-Jakob disease. *Am. J. Pathol.* 164, 143-153.

⁷ Wadsworth *et al.* (2001) Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet.* 358, 171-180.

⁸ Joiner *et al.* (2002) Irregular presence of abnormal prion protein in appendix in variant Creutzfeldt-Jakob disease. *J. Neurol. Neurosurg. Psychiatry.* 73, 597-598.

⁹ Peden *et al.* (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet.* 364, 527-529.

¹⁰ Hill *et al.* (1999) Investigation of variant Creutzfeldt-Jakob disease and other human prion disease with tonsil biopsy samples. *Lancet.* 353, 183-189.

¹¹ Bruce *et al.* (2001) Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. *Lancet.* 358, 208-209.

¹² Wadsworth *et al.* (2001) Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet.* 358, 171-180.

transfusion¹⁵. PrP^{vCJD} has been found in one sample from the NATA study, but was not found in the tonsils of the case of subclinical vCJD associated with blood transfusion⁷. Thus, the timing of PrP^{vCJD} accumulation in human tonsil tissue during the vCJD incubation is unknown.

19. Data from published experimental studies in animals that examined PrP^{TSE} accumulation in tonsil tissue during the preclinical stage of the incubation period of TSEs following oral inoculation are summarised in Table 2 (**Annex D**). Additional unpublished data will be provided about PrP^{vCJD} accumulation in inguinal lymph nodes of cynomolgus macaques and PrP^{BSE} accumulation in lingual tonsils of mice. Although the data are limited, these studies will clearly show that PrP^{TSE} accumulates in tonsil tissue during the subclinical stage of the incubation period of BSE in sheep, mice and lemurs, of classical scrapie in sheep and of CWD in deer. Although the precise timing of PrP^{TSE} accumulation cannot be derived from these studies, it appears from these data that PrP^{TSE} accumulation may not be a late event in the incubation period of these TSEs in these species.
20. The pathogenesis of BSE and classical scrapie in sheep and BSE in non-human primates is generally considered to be a reasonable model for the pathogenesis of vCJD in humans. Thus, it may be reasonable to assume that PrP^{vCJD} also accumulates in tonsils some time prior to the onset of clinical vCJD in humans.

Spleen Testing

21. PrP^{vCJD} is consistently detected in the spleen tissue of clinical vCJD cases^{16,17}. In addition, there have also been two previous cases in which spleen tissue has tested positive for the presence of PrP^{Res} in asymptomatic patients.
22. The first case was a patient who had received a blood transfusion from a blood donor who later died of vCJD, which was examined by Peden *et al* (2004). The patient was asymptomatic for vCJD and died of an unrelated cause. Testing of tissues at post mortem showed that the spleen was positive for the presence of PrP^{Res} by Western Blot, PET blot and IHC, and a cervical lymph node tested positive by IHC. Testing of brain samples was negative. This patient was MV at

¹³ Head *et al.* (2004) Peripheral tissue involvement in sporadic, iatrogenic and variant Creutzfeldt-Jakob disease. *Am. J. Pathol.* 164, 143-153.

¹⁴ Ritchie *et al.* (2004) Advances in the detection of prion protein in peripheral tissues of variant Creutzfeldt-Jakob disease patients using paraffin-embedded tissue blotting. *Neuropathol. Appl. Neurobiol.* 30, 360-368.

¹⁵ Wroe *et al.* (2006) Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. *Lancet.* 368, 2061-2067.

¹⁶ Bruce m E, McConnell I, Will RG, Ironside JW, Detection of variant Creutzfeldt Jakob Disease in Extraneural Tissues, [Lancet](#). 2001 Jul 21;358(9277):208-9.

¹⁷ Wadsworth JD, Joiner S, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, Collinge J, Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay, *Lancet*. 2001 Jul 21;358(9277):171-80.

codon 129.¹⁸ In this case, initial examination of frozen spleen tissue by conventional western blot analysis for PrP^{Res} was negative. However, high sensitivity western blot analysis showed 2 out of 4 initial samples positive for PrP^{Res}, but the intensity of the signal was variable.

23. The second case was a Haemophilia patient who had died without showing any symptoms of vCJD or any other neurological disease. A positive result was found in a single sample of spleen following testing of 4 different samples of the spleen by high sensitivity western blot analysis. A further 20 samples of the spleen all tested negative. The patient was MV at codon 129. The single positive result was confirmed on repeat testing by western blot analysis and densitometry of that particular sample. The National CJD Surveillance Unit audited the sampling and testing process, and considered this single positive result to be a true positive. The PrP^{Res} glycosylation is characteristic of vCJD.
24. The full range of tissues from this patient that were tested is as follows:

Fixed Tissues	Result (immunohistochemistry/PET blot)
Brain	Negative
Heart	Negative
Liver	Negative
Blood vessel	Negative
Appendix	Negative
Spleen	Negative
Lymph node	Negative

Frozen Tissues	Result (Western blot analysis)
Frontal Lobe	4/4 negative
Occipital Lobe	4/4 negative
Cerebellum	4/4 negative
Lymph node	4/4 negative
Spleen *	1/24 positive 23/24 negative

25. The finding of PrP^{Res} in only one of 24 samples from the spleen of this patient is not surprising. In the clinical vCJD cases examined, the spleen contained a lower concentration of PrP^{Res} per unit volume compared with the tonsil or lymph nodes due to the lower number per unit volume of follicle centres (tissue structure where the infectivity is present) in the spleen compared with the other organs. The spleen autolyses rapidly after death and significant atrophy occurs during life such that fewer splenic follicle centres may be present later in life. Given all these factors, heterogeneous distribution of PrP^{Res} should not be unexpected. A heterogeneous distribution of PrP^{Res} was also found in the spleen of the case investigated by Peden *et al* (2004) and also in vCJD cases due to dietary exposure to BSE. It is not known whether the involvement of the spleen is

¹⁸ Alexander H.Peden, Mark W. Head, Diane L. Ritchie, Jeanne E. Bell, James W. Ironside, Preclinical vCJD after Blood Transfusion in a PRNP codon 129 Heterozygous Patient, *Lancet* 2004; 264, pp 527-529.

influenced by the route of transmission. This has implications for the proposed post-mortem study.

26. At SEAC 102, the Chair concluded that careful consideration is needed on the number of samples collected and the sampling and testing protocol. In addition, focused investigations of patients considered to be 'at risk of vCJD' such as those that had received contaminated batches of plasma products would be informative. Members also suggested that an analysis using different assumed sensitivities for the testing approach could provide an indication of the number of spleen samples that would need to be tested by the post mortem tissue archive to prove or disprove estimations of the worst case prevalence of subclinical vCJD, and this will be done in spleen collection is assessed as practicable.

POTENTIAL INFLUENCE OF GENOTYPE ON PRP^{vCJD} ACCUMULATION

27. Two out of the three PrP^{vCJD} positive appendix samples identified by Hilton *et al* were from individuals of the VV genotype¹⁹. As around 10% of the population carry this genotype, this finding may indicate that PrP^{vCJD} may accumulate earlier in the incubation period, and/or faster, in the appendix of infected individuals of the VV genotype compared with the MM or MV genotypes.
28. The finding of sub-clinical infection in the spleens of two patients of the MV genotype suggests that such individuals may have a protracted onset of clinical symptoms or may remain asymptomatic. However, this could also have been influenced by the route of transmission in these two cases, which is considered to have been blood and blood products.

ADVICE SOUGHT FROM SEAC

29. The NATA and Hilton data may become discrepant if the NATA study continues to find no further positive samples. Whilst SEAC has previously stated that data from the NATA and Hilton studies cannot be combined to produce a single estimate of prevalence, this still leaves the issue of how to reconcile datasets of different tissues which might provide different estimates of prevalence. DH is commissioning additional studies of appendix and spleen tissue, but uncertainties remain about the distribution and timing of infectivity in different tissues in the sub-clinical stage of the disease. Sub-clinical vCJD has previously been found in samples appendix and spleen tissue, but not tonsil. However, the precise timing of the emergence of infectivity in any of these tissues remains unknown. This raises questions about how to interpret the data from the various prevalence studies that are either underway, or have been commissioned.

¹⁹ Ironside *et al.* (2006) Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study. *BMJ*. 332, 1186-1188.

30. The Committee is asked to provide advice on:

- How many samples do studies need to test in order to have sufficient power to influence estimates for prevalence of PrP^{vCJD} in any given tissue?
- Should one necessarily expect the prevalence of PrP^{vCJD} in all lymphoid tissues (and in blood) to be similar at any given time?
- Should “sub-clinical prevalence of vCJD” (more generally) be interpreted in terms of presence of PrP^{vCJD} in *any* tissue)? If so, what evidence would SEAC need to see to enable it to revise its current advice on this?
- How should the finding of one “positive” sample in the NATA study be interpreted, and what does this mean for the further testing of tonsils?