



vCJD INFECTION RISKS OF BONE PRODUCTS: A COMPARATIVE ASSESSMENT

ISSUE

1. The National Blood Service (NBS) together with the Department of Health's Economics and Operational Research (EOR) division have produced a draft risk assessment to examine the risk of transmission of vCJD via implantation of bone. A previous version of this assessment was considered by the committee in November 2003. This draft has been revised to account for member's comments.

Background

2. This risk assessment follows on from previous work by EOR on the risks of transmitting vCJD via donated blood, hospital surgery and dentistry, all of which have been endorsed by SEAC. It also results from a continuing programme of collaboration with NBS, in which EOR provide analysis of issues around the safety and supply of blood and tissues.
3. The main use of bone is in revision hip procedures (where an artificial hip is replaced after having worn out or become loose). Bone is sourced from either single femoral heads (from living patients undergoing primary hip replacement) or from cadaveric donors. Bone products may be sourced from the NBS or other bone banks. Alternatively bone in the form of individual femoral heads may be taken and pooled in theatre at the time of operation.
4. The assessment compares the risks associated with different bone products i.e. processed or unprocessed, pooled or unpooled, under different scenarios of vCJD infectivity. At the November meeting, the committee agreed that as uncertainties around infectivity were unlikely to be resolved soon, they endorsed the need to consider as many scenarios as possible with respect to the risks from the different bone products.
5. There are major uncertainties as to whether a donor incubating vCJD does carry infectivity in bone tissue, marrow or blood. The draft risk assessment has been carried with the precautionary assumption that if the donor is infected with vCJD, infectivity is present in blood, bone marrow and/or bone.

6. Processing of bone removes a large proportion of the blood and marrow thus reducing this possible source of infectivity (Lomas *et al* 2000)¹. However the processes most effective in removing blood and marrow require pooling of several donations if living donations are used (though not for cadaveric donations, where larger quantities can be obtained per donor).

Advice sought from the committee

7. Members are asked to advise on the comparative risks of the different bone products with regard to vCJD transmission given the scientific uncertainties of the level of infectivity present and the effect of processing (including γ -irradiation).

List of accompanying material

- [Annex 1](#) An NBS introduction to the EOR bone risk assessment paper January 2004.
 - [Annex 2](#) Revised Risk Assessment: “vCJD infection risks of bone products: a comparative assessment”
8. The latest draft of the EOR paper (Annex 2, version 2.6) has been amended to include advice received from members and Professor Tim Chambers². Members should refer to paragraphs 5-25 of the SEAC 80 reserved business minutes to obtain a record of the committee’s previous discussion on this item.
 9. The main amendments to the EOR paper include:
 - Paragraph 2.10. If infectivity is proportionate to the monocyte nucleated cell (MNC) content of tissue, then the infectivity of marrow could be 100-1000 fold per unit volume greater than blood.
 - Paragraph 2.11. In the absence of any experimental data on the distribution of infectivity of vCJD, it remains plausible that bone itself may carry infectivity as osteoblasts (bone forming cells) are of the same cell lineage as macrophages, which have shown to exhibit PrP^{sc}.
 - Paragraph 3.2. Clearance factors of 98% and 99% for infectivity associated with blood and marrow from single washed/centrifuged heads and morcellised heads respectively. These are indicative figures rather than firmly established.
 - Alternative calculations of risk of vCJD infection per unit of bone implanted have been carried out for a 1-log reduction in infectivity following irradiation based on data published by Miekka *et al* (2003)³.

¹ Lomas R, Drummond O, Kearney JN. Processing of whole femoral head allografts: A method for improving clinical efficacy and safety. *Cell and Tissue Banking* 1: 193-200,2000.

² Head of histopathology and a bone biology expert from St George’s Medical School

³ Miekka SI, Forng RY, Rohwer RG, MacAuley C, Stafford RE, Flack SL, MacPhee M, Kent RS, Drohan WN. Inactivation of viral and prion pathogens by γ -irradiation under conditions that maintain the integrity of human albumin. *Vox Sang.* 2003 Jan; 84(1): 36-44.

- Section 4. Additional section on the scenarios with infectivity in bone. Additional emphasis that the presence of significant infectivity in bone would remove the advantage of blood/marrow removal.
 - Research issues. Provides an update on DH-funded research projects involved with assessing the infectivity of vCJD tissues and highlights the limitations arising from test sensitivity.
 - Paragraphs 5.3-5.4. Addresses the possibility of cross-contamination of donations from machinery used for morcellisation and pooling of bone.
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- [Annex 3](#) Paper by Lomas *et al* (2000) on processing of whole femoral head allografts



NBS TISSUE SERVICES

An NBS Introduction to the EOR Bone Risk Assessment Paper October 2003

INTRODUCTION

Tissue banking has grown within the National Blood Service (NBS) since the early 90's, utilising the donor selection, donation testing and quality system expertise already established for blood. The growth has resulted from both the development of tissue banking services by individual blood centres in response to local clinical demand and the decision by a number of well established banks to become part of the NBS. Tissue Banking in Scotland is confined to the SNBTS, which also provides a multi-tissue bank service.

NBS Tissue Services is now the largest multi-tissue banking organisation in the UK. However it is not a monopoly supplier and there are a small number of other cadaveric tissue banks and a larger number of surgical donation programmes run from within hospitals (latest figures collated for the BATB (British Association of Tissue Banks) indicate over 3000 femoral heads (FH) collected independently). NBS is a significant leader in the development of national and international standards, policies and regulation in the field. The policies and procedures used in non-NBS banks are not described here.

Bone for impaction grafting is human derived and no alternatives to this are currently considered effective or are used in the UK for this indication. The clinical need for bone is the main driver for tissue banking in this country.

The accompanying risk assessment is designed to compare the relative risks of different bone products used for a single indication, from the point of the theoretical risk of vCJD. Another risk assessment was undertaken by NBS TS to consider risks of bone products including viral and other issues. There has been concern in professional circles about the benefit and disadvantages of pooling of bone in relation to tracking and dilution of infectivity and to the numbers of potential recipients of a pool.

The theoretical risks of UK derived bone products also needs to be considered in the context of the age of the recipient population, which in the case of impaction grafting is predominantly elderly, although the age range is wide. The risks to this population of reduced mobility should also be noted. This may be exacerbated if bone is in short supply and thereby increasing the waiting time for revision joint surgery. In addition, there is the possibility which needs consideration is sourcing donor bone from countries where vCJD is not

known in the population. This might be particularly important for young recipients who have a long life expectancy and who have not been exposed to dietary risks associated with vCJD. Unfortunately there is no register of bone impaction procedures so the number of recipients remains undefined. An approximation might be in thousands per annum in contrast to millions of recipients of blood components per annum.

Lastly, the risk assessment is based on the presumption that the bone marrow, which is removed by processing, has the same infectivity as blood. However, light microscopy of bone marrow often shows lymphoid follicles. No vCJD infectivity studies of bone marrow have been reported and the basis for the presumption is therefore speculative. Expert advice on this subject of infectivity is sought for the scenarios that have been used in the vCJD risk assessment.

DONATION PROGRAMMES

NBS Tissue Services runs two major donation programmes, run by a team of about 20 highly trained senior nurses.

- **Surgical bone donation**

Femoral heads are removed during primary hip replacement and banked for future use by other patients. This programme requires close co-operation with over 70 collaborating hospitals. Around 4 - 5,000 donations are collected by the NBS each year.

- **Cadaveric tissue donation**

Bone, tendons, skin and heart valves are retrieved from donors after death (see later section for clinical uses). NBS tissue retrieval teams go to the mortuary to carry out the retrieval

TISSUE PROCESSING AND CLINICAL USE

A proportion of femoral heads is issued for use without processing, as long as the results of bacteriology testing are negative. The remaining surgical bone donations, and all cadaveric bone donations, are processed and/or sterilised before issue. Processing involves washing and shaping or washing and grinding followed by either freeze-drying or deep freezing, with exposure in the final packaging to either ethylene oxide or gamma irradiation. Ground thoroughly washed bone is the major processed product used in revision surgery, the shaped grafts being used less frequently. An internal NBS report of the validation performed in September 2000, of Leucodepletion of bone led to a revised washing protocol for ground bone. The study examined washing efficiency for ground bone, which involved assessment of haemoglobin, protein and DNA in washing eluates (CJ Hunt, S Poniatowski, J Staniforth, NBS). Processing of femoral heads involves the pooling of 17 femoral heads together. Pooling of cadaveric bone donations is not undertaken because there is sufficient bone in a single cadaveric bone donation for it to be feasible

to process from one donor at a time. Surgeons using individual femoral heads pool bone in theatre at the time of operation. Theatre pooling techniques vary between individual centre preferences and may or may not include a washing step. Washing efficiency for centrifuged whole femoral heads was also undertaken. (Lomas P, Drummond O, Kearney JN (2000) Processing of whole femoral head allografts. A method for improving clinical efficacy and safety. Cell and Tissue Banking 1: 193-200.) This method is under further development in Scotland, SNBTS, TS, and is not yet operational.

The great majority of bone is used during joint revision surgery. The development of impaction grafting techniques has caused significant growth in the clinical demand for unprocessed femoral heads, which are morcellised by the surgical team in theatre, and also for processed ground bone produced in the Tissue Services facilities (Figure 2).

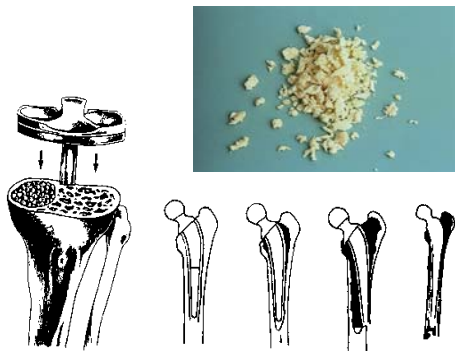


Figure 2: Bone impaction grafting in revision joint surgery

BONE PROCESSED PER ANNUM BY TISSUE SERVICES

Bone donations are processed in the following proportions:

- 1500 unprocessed surgical FH which surgeons process in theatre.
- 800 irradiated but otherwise unprocessed surgical FH which surgeons process in theatres.
- 1000 surgical FH are processed in pools.
- 200 cadaveric bone donations processed to produce 1500 surgical FH equivalents.

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3rd October -2003

vCJD INFECTION RISKS OF BONE PRODUCTS: A COMPARATIVE ASSESSMENT

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Department of Health**

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Summary

This paper considers the potential risk of variant CJD (vCJD) being transmitted from patient to patient through the implantation of donated bone. The absolute risk of this happening cannot be quantified at present. This is because the prevalence of vCJD in the population is unknown, as is the potential infectivity of an implant sourced from a donor incubating the disease. However we can compare the risks that might be associated with alternative bone products.

Most bone is used during the course of revision hip surgery (replacement of an artificial joint that has failed, worn out or come loose). Bone is sourced either from femoral heads taken from living donors during the course of primary hip replacement, or from cadaveric donors. Some femoral heads are used without processing, an approach favoured by many surgeons. Alternatively, bone may be used in the form of processed (frozen or freeze-dried) packs, in which case high proportion of blood and marrow will have been removed. However the full processing of bone from living donors currently requires femoral heads from several donors to be pooled, due to the limited mass of bone in each single head). So a point of particular interest is the balance between the effects of pooling and the advantages of processing.

This is explored across a very wide range of scenarios. Some consider the effect of infectivity being present at different levels in blood and/or marrow: others that of infectivity also being present in bone itself. In many respects, the relative advantages of the products are strongly scenario-dependent.

- Use of unprocessed femoral heads would carry the highest vCJD risk in some scenarios where infectivity is confined to blood and marrow. Each such procedure involves the transfer of a significant quantity of untreated blood / marrow. This contrasts with the precautions now being taken against vCJD transmission via blood donation.
- If infectivity is high, however, the disadvantage of pooling donations becomes the key consideration. This applies if infective doses in blood / marrow are high, and/or there is significant infectivity in bone itself.

These contrasting findings highlight the need for further scientific investigation - especially on the potential infectivity of both marrow and bone - to clarify which scenario may actually prevail. Some experiments are already in train. However the tests currently available have limited sensitivity. So it may be impossible to reduce these uncertainties significantly in the near future, leaving open the full range of scenarios discussed here. Other uncertainties - e.g. on the amount of blood and

marrow left after processing could also be investigated. This would allow the analysis to be refined, but would be unlikely to alter its conclusions.

Given this, the key practical need is for *robust* options – i.e. those reducing vCJD risk in the widest possible range of scenarios. In this context, the ideal would be to reduce the use of bone – either by reducing the need for revision surgery or through development of some artificial substitute for bone. The possibility of importing bone might also be explored, especially for the small minority of young recipients.

Considering the existing bone products, the most robust options involve removal of blood / marrow *without* pooling of donations.

- In all scenarios, processed bone from a single (necessarily cadaveric) donor is the best (or joint best) option in minimising vCJD risks.
- Washing and centrifuging of individual femoral heads also represents a robust option that may meet the preferences of clinicians, though the removal of blood / marrow appears likely to remain less complete than with the morcellised processed products.

Throughout, this analysis considers only the potential risks from vCJD, not other advantages and disadvantages of alternative products (e.g. minimisation of viral or bacterial risks, or the mechanical properties of the bone). It is therefore intended that the analysis should provide one contribution to wider discussion of these issues. The need to maintain an adequate overall supply of bone to meet surgical need is also a key consideration.

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1. Introduction

Purpose of paper

- 1.1 Variant (or “new variant”) Creutzfeldt-Jakob Disease is a fatal degenerative brain disease, one of a small number of Transmissible Spongiform Encephalopathies (TSEs). While much remains to be learnt, infection appears to be associated with the presence of a deformed prion protein known as PrP^{Sc}. Unlike sporadic or “classical” CJD, which has long been known to affect about 1 in 1 million of the population per year, many of the victims are young. The agent that causes vCJD is presently indistinguishable from that which causes Bovine Spongiform Encephalopathy (BSE) in cattle, and it is now widely accepted that it may have passed into the human population through consumption of BSE-infected bovine tissues. Whatever the origins of primary human infection, the question arises of how great the risks might be of secondary (i.e. person-to-person) transmission of vCJD.
- 1.2 The analysis set out here addresses the theoretical risk of vCJD being transmitted through the implantation of bone tissue. It was originally requested by National Blood Service (NBS) and has benefited from input both from NBS and the Scottish National Blood Transfusion Service (SNBTS).
- 1.3 Bone may be sourced from living or cadaveric donors. Because vCJD may have a long incubation period, it is possible that a donor of either type might have been incubating vCJD without showing symptoms. A potential transmission route therefore arises. The absolute risk of vCJD being transmitted in this way cannot be quantified at present, as both the prevalence of the disease and the potential infectivity of the tissues involved are unknown. However the analysis can clarify the risks *that would exist in different scenarios* consistent with what is presently known. Specifically, it examines the *comparative* risks of vCJD transmission associated with different options for sourcing and processing bone, in particular the potential effects of pooling bone taken from several donors and those of removing blood and marrow prior to use.
- 1.4 This paper considers potential vCJD risks only, not other advantages or disadvantages of different bone products – e.g. mechanical properties, or the elimination of bacterial risks. These would have to be taken into account in developing any recommendations, as would the need to maintain adequate supplies of bone to meet surgical need. This paper aims thus to clarify the issues around vCJD risks, so as to inform wider debate and consultation with suppliers and users of bone products.

Background

- 1.5 Almost all implanted bone is used in the course of revision joint surgery, mainly in replacement of worn-out artificial hips. An accompanying paper prepared by NBS provides some further information on the procedure generally adopted (which involves packing bone around the new artificial joint), and on the number of operations carried out. In this context, it should be

noted that while the patients were generally elderly, they are selected as being fit before they are submitted for this type of operation. If infected with vCJD then, such patients might well live long enough to develop symptoms of the disease unless the secondary incubation period is long. There are also a small number of patients under 20 years (<0.1%) who require joint revision surgery for Juvenile Rheumatoid Arthritis (Still's disease)

- 1.6 Bone can be sourced and processed in various ways, as again detailed in the NBS paper. Individual femoral heads are frequently collected and stored locally by surgeons (typically from patients undergoing primary hip replacements), ground up in the operating theatre at the time of the recipient's operation and used either with no further processing or after undergoing some washing steps and/or gamma-irradiation. Some surgeons express a preference for this approach, which provides local control of supply and (arguably) material with superior mechanical properties. In the context of vCJD however, the disadvantage is that the bone will contain significant quantities of marrow and blood – about 15-20ml per femoral head, according to NBS research. As discussed below, this *may* be a vehicle for transmitting vCJD, if the donor has the disease.
- 1.7 Alternatively, bone may be supplied from a central bank, NBS being the largest – though not a monopoly - supplier for England and North Wales. Though NBS also stores and issues fresh-frozen unprocessed femoral heads in order to meet demand for these, a substantial proportion of the NBS output consists of *processed* bone. This bone may come from a cadaveric donor, in which case a single donor can provide bone equivalent to 8 femoral heads. Alternatively, processed bone may be sourced from living donors undergoing primary hip replacement. Because less bone can be obtained than from a cadaveric donor, donations from several donors (typically 17) are pooled in order to facilitate processing. In either case, the bone is ground up (morcellised), thoroughly washed and then either freeze-dried or frozen. Some bone from surgical donors, and all that from cadaveric donors, is gamma-irradiated after processing. Though the use of pooling has attracted some controversy, processing has the advantage of removing a very large proportion of marrow and blood.
- 1.8 In Scotland, all bone has historically been used in unprocessed form. However SNBTS is currently extending previous NBS work to validate removal of bone and marrow by washing and centrifuging of individual femoral heads. Experiments so far show that this can be achieved to large extent, though processing bone in morcellised form should remove blood and marrow more completely.
- 1.9 Given the variety of bone products available across the UK, it is natural that questions have been raised as to their relative merits. NBS has already carried out a comparative risk assessment concentrating on factors other than vCJD. The present paper should therefore complement that analysis. Specifically, it aims to delineate those scenarios in which the effects of processing would have the predominant effect in reducing risk, and those in which it would be more important to avoid pooling bone from different donors. It is hoped that

this may contribute both to debates about preferred clinical practice and to the prioritisation of further research.

Information on Bone Products

- 1.10 Some further information about the various products is summarised in Table 1 below. The morcellised frozen and freeze-dried products are each produced in two pack sizes, as shown. The freeze-dried packs are approximately half the weight and volume of the equivalent frozen products. However this is thought to be purely a consequence of water loss, the original volume of both products being the same. When donations from 17 living donors are pooled, enough processed bone is typically produced to make up 12 of the larger size frozen or freeze-dried packs.

Table 1 – Product information provided by NBS (per pack)

Product	volume (cc)	Weight (g)	Number used per procedure
Femoral heads - unprocessed		80	2 or 3
- washed/ centrifuged			2 or 3
Frozen processed bone –	70	32	2
17 Femoral heads or 1 Cadaveric	35	16	4
Freeze-dried processed bone –	35	13	2
17 Femoral heads or 1 Cadaveric	15	5.5	4

- 1.11 From the point of view of potential vCJD risks, the distinction between the frozen and freeze-dried products appears to be immaterial. We therefore consider the following alternatives:
- Unprocessed femoral heads from living donors (with or without gamma irradiation)
 - Single, washed, centrifuged and gamma-irradiated femoral heads from living donors
 - Morcellised frozen or freeze dried, gamma irradiated bone pooled from 17 living donors
 - Morcellised frozen or freeze dried, gamma irradiated bone taken from 1 cadaveric donor
- 1.12 All bone in products (b) – (d) is washed prior to production. With regard to product (a), local practice on washing varies: these heads also may or may not be irradiated. For ease of comparison, we start by considering completely unprocessed bone under (a), used without significant washing or any irradiation. While this categorisation slightly simplifies the variety of products used, the analysis will also allow us to draw some conclusions about bone that undergoes more limited local processing.

2. *Calculating risks per unit used*

Methodology

- 2.1 In considering risks to recipients of bone, we take single femoral heads and the larger packets of the two processed products (70cc frozen or 35cc freeze-dried) to be essentially equivalent “units”. For present purposes, the total usage of units of all types is assumed to be constant: reflecting a given clinical need for revision surgery. The key consideration is then the *risk per unit used*, for a given prevalence of infective donors. It is this that drives the risk to the population of recipients, rather than the number who might be exposed to a given donor.¹ However the latter is of concern for the management of incidents in which a potentially-infective donor has actually been identified: some brief comments on this appear later in the paper.
- 2.2 The prevalence of vCJD is assumed to be the same for living or cadaveric donors.² It is also assumed to be small – e.g. not more than 1 in 100 donors infective. This simplifies the calculations by ensuring that even if several donations are pooled, the chance of any unit containing bone from *more than one* infective donor will be vanishingly small. (For higher prevalences, the model would slightly overstate the number of new infections caused.) **However no specific assumption is made as to how high or low the prevalence of vCJD might be** – the aim being to clarify the relative risks associated with each product.
- 2.3 Clearly, the risks associated with any of the products will depend on the potential dose in an infected unit. Specific scenarios are discussed below. More generally, risks will depend on the assumed “dose response model”, relating the dose received by an individual to his or her chance of contracting the disease.
- 2.4 The present analysis is based on a linear dose-response model. This follows previous work on vCJD infection risks for blood and blood products carried out both by EOR and risk consultants DNV. Doses are calculated in terms of ID₅₀s, one ID₅₀ being the dose sufficient to infect 50% of individuals receiving it. For doses above 2 ID₅₀s, infection is considered to be certain. Below this dose, we use the formula [Chance of infection = ½ * dose received, in ID₅₀s].

¹ To illustrate using an extreme example, suppose that 100 units of some tissue could be sourced from a single donor. Suppose also that there is enough infectivity per unit to infect for certain if the donor is infected, and the prevalence of the disease is 1 in 1,000. Sourcing all 100 units from 1 donor creates a 1/1,000 chance of that donor being infective and infecting all 100 recipients. So expected no. of infections = 1/10. But if the 100 units are sourced from 100 different donors, each of them would have a 1/1000 chance of infecting 1 recipient - so again the expected number of infections would be 100 x 1/1,000 = 1/10. However the pattern of infection "incidents" would be different - in the first case, they would be much rarer but worse when they happened.

² The comparative youth of cadaveric donors might make some difference here. Both living and cadaveric donors have about a 30% chance of transfusion prior to donation. If this increases the chance of having been infected, the prevalence of the disease might also be higher than in the general population. But this would affect both sets of donors (roughly) equally, and would not affect the relative risks of the different bone products.

- 2.5 This model has previously been endorsed by the Spongiform Encephalopathy Advisory Committee (SEAC) as a sufficiently good working assumption unless or until any contrary evidence emerges. However it is rather simplistic in taking infection to become certain at “precisely” 2 ID₅₀s. In addition, the dose received would be expected to affect the incubation period: a patient receiving a dose of (say) 200 ID₅₀s would be more likely to develop the disease before dying of some other cause than one receiving just 2 ID₅₀s. The 2 ID₅₀ threshold, though important, is thus not *all*-important.

Scenarios for infectivity

- 2.6 Clearly, a unit of any of the products considered contains the following in larger or smaller quantities:

- Blood
- Marrow
- Bone

Any of these *may* be capable of transmitting vCJD, but there is no direct evidence on the level of infectivity that might be present.

- 2.7 In the case of blood, TSE transmission has been demonstrated in sheep (Houston *et al*, 2001; Hunter *et al* 2002). In other contexts, precautionary measures – notably leucocyte depletion of components and import of plasma derivatives - have been taken on the basis that the blood of those incubating vCJD *may* be infective. In addition, an incident was reported in December 2003, in which a recipient of blood from a donor incubating vCJD also went on to develop the disease. This adds weight to this concern (though a single incident cannot conclusively demonstrate that blood-borne transmission occurred). Unlike donated blood components, blood transferred along with a femoral head will not have been subject to leucocyte depletion.
- 2.8 An earlier review of published animal models carried out for the Department of Health by risk consultants DNV (DNV 2003) suggested that if blood *is* infective, a reasonable baseline scenario would be a specific infectivity of around 2 ID₅₀ per ml. However great uncertainty around this is acknowledged, as regards both the level of infectivity and the stage of incubation at which it might appear.
- 2.9 Infectivity in human bone marrow has not so far been demonstrated. Current WHO guidance lists both marrow and blood amongst “lower-infectivity tissues” (WHO, 2003) but it may be prudent to assume that marrow is *at least as* infective as blood. One theoretical argument is that potential vCJD infectivity may be proportional to Monocyte Nucleated Cell (MNC) content.
- 2.10 At the SEAC meeting of November 2003, Professor Tim Chambers, a bone biology expert from St George’s Hospital Medical School, commented that the cellular density of marrow is approximately 100-fold (per ml) greater than blood, and this could increase infectivity. Blood was considered a good analogy for bone marrow given that 20-30% of bone marrow were B-cells. One approach to assess the relative risks from blood and marrow would thus

be to estimate the number of marrow cells provided in a bone graft and compare that with those used in a blood transfusion. However without clinical or experimental data on the number of cell types within a bone graft it was difficult to complete this comparison. In addition, the linkage of vCJD infectivity with MNC content appears still to be uncertain.³

- 2.11 TSE infectivity in bone has not so far been found in animal tests published to date, but these involve only BSE in cattle, which is not considered a good model for vCJD in humans. However SEAC advised (at the same meeting), that in the absence of any experimental data on the distribution of infectivity of vCJD, it remains plausible that bone may carry infectivity. It was pointed out that osteoblasts (bone forming cells) are of the same cell lineage as macrophages, which have been shown to exhibit PrP^{Sc}. SEAC also commented that a review of the epidemiology of CJD had found limited evidence for bone allo- or xeno-grafts in sheep transmitting CJD: however these results were not statistically significant. If infectivity were present in bone, the substantial volume used in an implant meant that this could well outweigh any risk from blood and marrow residues.
- 2.12 A final consideration is the possible effect of Gamma-irradiation on vCJD infectivity. A starting assumption is that there is no significant effect, as reflected in current ACDP/SEAC guidance on this topic. However there are indications from US research of irradiation achieving a significant (1.5-log) reduction in scrapie infectivity in brain homogenate (Miekka *et al*, 2003). This research is based on irradiation at a dose of 50 kGy, as compared with 40 kGy in current NBS processing. We therefore also explore scenarios in which irradiation of Femoral Heads achieves a 1-log reduction in infectivity, whether present in blood, marrow or bone itself.
- 2.13 In summary, it is clear that a wide variety of scenarios need to be considered. Though there are many unknowns, the most fundamental concern the possible levels of vCJD infectivity in blood and marrow, and its possible presence in bone. It is helpful to consider these two factors separately, as they have very different impacts on the relative advantages and disadvantages of processing and pooling. Section 3 considers scenarios in which vCJD infectivity is confined to blood and marrow, Section 4 those in which it is also present in bone.

³ Prof James Ironside [National CJD Surveillance Unit, Edinburgh University] commented on an earlier version of this analysis. He suggested that it does not necessarily follow that if blood is infectious, then bone marrow may be more infectious in proportion to its cellular content. "The cells of the bone marrow may not be able to support location of abnormal PrP, which appears to be confined to follicular dendritic cells in 'organised' lymphoid tissues. Admittedly, since scrapie infectivity has been transmitted by transfusion of buffy coat, there are implications that the cellular fraction of blood may be infectious, but the data for this in humans with variant CJD is lacking so far. I therefore think that it would be prudent to be cautious [and to]... assume a range of possibilities'

3. *Numerical Scenarios for infectivity in blood and marrow*

- 3.1 If infectivity is essentially confined to blood and marrow, a key question is the amount of these left after processing. The calculations that follow are based on the higher NBS estimate of 20 ml in an unprocessed femoral head. Regarding the residue left after processing. Evidence from NBS research shows 97.7% removal of leucocytes (and 96.4% of soluble protein) being achieved by sonification, washing and centrifuging of single femoral heads (Lomas, Drummond, and Kearney, 2000). Unpublished SNBTS research suggests that these processes should be capable of removing 98% of the marrow/blood originally present. Advice from NBS is that more complete removal should be attained when the heads have been morcellised, so that at least 99% removal should be achieved for the NBS processed products.
- 3.2 For illustration, we use clearance factors of 98% and 99% for infectivity associated with blood and marrow from single washed / centrifuged heads and morcellised heads respectively. However these should be seen as indicative figures rather than firmly-established.
- 3.3 The exact composition of this blood / marrow residue is at present unknown (a significant proportion may actually be fat). While further research may clarify the composition of this mixture, this will *not* resolve the uncertainties around the potential infectivity (if any) of each portion. The simplest approach is therefore to consider a range of scenarios for this blood / marrow / fat mix.

Illustrative scenario

- 3.4 As noted above, the DNV review suggests that *if* blood is infective, it may carry 2 ID₅₀ per ml. Here, however, the blood is mixed with marrow – arguably of higher infectivity. For illustration, suppose that the resulting mix has an infectivity of 20 ID₅₀ / ml, or 400 ID₅₀ in total for a residue of 20 ml in an unprocessed femoral head.
- 3.5 In any scenario, the chance of infection is governed by:
- The probability of the unit coming (at least in part) from an infective donor. For unpooled products, this will simply be the prevalence of infective donors (say **p**). For pooled products, it will be **p** x (number of donors in the pool).
 - The dose that would be present in a unit *if* this occurs.
- Units sourced from several donors will be more likely to have some infectivity, but with a smaller dose per unit. The balance between those two factors is key to this part of the analysis.
- 3.6 Table 2(a) summarises these calculations for this infectivity scenario, with gamma-irradiation having no effect on residual infectivity. Table 2(b) gives results for the same scenario, except with gamma-irradiation reducing any infectivity by 1 log (i.e. ten-fold). Calculations underlying both tables are set out more fully in Annex A

3.7 In both tables, the final row shows the estimated chance of a unit of each product infecting the recipient, given a prevalence **p** of infective donors. Any specific scenario for prevalence can be used if required. In the first column, of Table 2(a) for example, if 1 donor in 10,000 is infective, the chance of an unprocessed head infecting its recipient is also 1 in 10,000. If the prevalence is 1 in 100,000, so is the risk of infection per unit of bone implanted. The comparisons between the options are not altered by making **p** greater or smaller: for example the units pooled from 17 Femoral Heads will still carry 2.8 times the risk associated with the other options.

Table 2 (a) – Risk of vCJD infection per unit of bone implanted (illustrative infectivity scenario, with no effect from irradiation)

PRODUCT	Unprocessed FH	Centrifuged FH – 98% removal	Processed - 17 FH 99% removal	Processed – 1 cadaver 99% removal
Dose per infected unit (ID₅₀)	400	8	0.33	5.7
Probability of dose being present	p	p	17p	p
Risk of Infection per unit implanted	p	p	2.8p	p

Table 2 (b): As above, with irradiation having 10-fold effect

PRODUCT	Unprocessed FH	Centrifuged FH – 98% removal	Processed - 17 FH 99% removal	Processed – 1 cadaver 99% removal
Dose per infected unit (ID₅₀)	400 (40 if irradiated)	0.8	0.033	0.57
Probability of dose being present	p	p	17p	p
Risk of Infection per unit implanted	p	0.4p	0.28p	0.28p

3.8 Though only based on one possible scenario for infectivity, these tables illustrate some points of interest. In Table 2(a), we can see that for all options except the pooling of 17 donations, the dose in an infected unit would remain above 2 ID₅₀. So although the dose from an unprocessed femoral head (first column) is by far the greatest, this differential is not fully reflected in the risks of transmission. Units pooled from 17 donors (1 assumed to be infective) would carry the lowest doses. But this benefit is overridden by the increased chance of infectivity being present, giving this product the highest vCJD infection risks per unit implanted.

- 3.9 In Table 2(b), additional benefit is gained from the processed products having been irradiated. This is sufficient to reduce doses below 2 ID₅₀, decreasing the chance of causing infection. Consequently all the processed products are less risky than unprocessed Femoral Heads. Furthermore the pooling of donations is now irrelevant, because at these infectivity levels the increased chance of infectivity being present in a unit is counterbalanced by the decrease in dose.

Alternative Scenarios for Blood/Marrow Infectivity

- 3.10 We have stressed the uncertainties attaching to the potential infectivity of the blood / marrow / fat mix. So we now explore the effects of lowering or raising the infectivity of this mix. Some results are as shown in the next pair of tables. Table 3 (a) generalises the example given in Table 2(a) (i.e. with radiation having no effect on infectivity). Similarly, Table 3(b) generalises the scenario in Table 2(b), with irradiation reducing infectivity by 1 log. (in this case, an extra column is used, to distinguish the irradiated version of otherwise-unprocessed bone). The shaded row within each table represents the original scenario. As before, results show the chance of one unit of each product causing an infection, for a given prevalence of infective donors (**p**).

Table 3(a): Risk of vCJD infection per unit of bone implanted, for different levels of blood/marrow infectivity, and irradiation having no effect

Initial infectivity of blood/marrow (ID ₅₀)		CHANCE OF INFECTION PER UNIT IMPLANTED			
Per ml	For 20ml initial residue per FH	Un-processed	Centrifuged (98% removal)	Processed (99% removal)	
				17 living	1 cadaver
0.01	0.2	0.1p	0.002p	0.0014p	0.0014p
0.1	2	p	0.02p	0.014p	0.014p
1	20	p	0.2p	0.14p	0.14p
10	200	p	p	1.4p	p
20	400	p	p	2.8p	p
50	1000	p	p	7.1p	p
100	2000	p	p	14.2p *	p
≥120	20,000	p	p	17 p *	p

Table 3(b): Risk of vCJD infection per unit of bone implanted, for different levels of blood/marrow infectivity, and irradiation having 1-log effect

Initial infectivity of blood/marrow (ID ₅₀)		CHANCE OF INFECTION PER UNIT IMPLANTED				
Per ml	For 20ml initial residue per FH	Unprocessed		Centrifuged (98% removal)	Processed (99% removal)	
		Non-irradiated	Irradiated		17 living	1 cadaver
0.01	0.2	0.1p	0.01p	0.0002p	0.00014p	0.00014p
0.1	2	p	0.1p	0.002p	0.0014p	0.0014p
1	20	p	p	0.02p	0.014p	0.014p
10	200	p	p	0.2p	0.14p	0.14p
20	400	p	p	0.4p	0.28p	0.28p
50	1000	p	p	p	0.71p	0.71p
100	2000	p	p	p	1.42p *	p
1000	20,000	p	p	p	14.2 p *	p
≥ 1,200		p	p	p	17p *	p

(* indicates risk would be reduced by batch dedication – see below)

3.11 These tables both reinforce the points suggested earlier. In particular:

- For the *lowest* levels of infectivity, the vCJD risks associated with unprocessed femoral heads are significantly greater than for *all* other products.
- *As the assumed levels of infectivity rise*, however, expected infections per unit of unprocessed bone rapidly reach their theoretical maximum in the illustrative scenario (certain infection if the head is infected), so cannot increase further. The differential with the other unpooled products disappears as these also reach this maximum – though it should be noted that the larger doses associated with the unprocessed product could be expected to result in shorter incubation periods post infection.
- Risks from the pooled products increase to a theoretical maximum of 17 **p** (i.e. p * number of donors in pool) per unit used. This occurs when the infectivity in each unit reaches 2 ID₅₀. Given our assumptions about blood/marrow removal, this requires an initial infectivity level of either 120 or 1200 ID₅₀ per ml of blood / marrow depending on whether irradiation has an effect.

- Consequently, the disadvantages of pooling are seen in these high-infectivity scenarios, eventually being sufficient to overcome the benefit of processing.

3.12 The same pattern of results appears in both tables, except that the relative advantages of the different products “switch” at different levels of initial infectivity.

4. *Scenarios with Infectivity in Bone*

4.1 Scenarios considered so far have infectivity confined to blood/marrow. As previously noted, however, bone itself may be capable of carrying infectivity. Scenarios covering this eventuality can be constructed using the same methodology. This can be done in two ways:

- The first is to consider bone infectivity independently of blood and marrow. Given that all the products mostly consist of bone, infectivity at a low level per gram or per ml would be sufficient to cause certain infection. For example, one might take the freeze-dried product to be essentially “pure” bone. One unit weighs approximately 13 g. So a specific infectivity of $(2/13) ID_{50}/g$ – i.e. about $0.15 ID_{50}/g$ - would push the unit infectivity up to the $2 ID_{50}$ limit, even with no contribution from any residual bone or marrow.
- The second approach would be to vary bone infectivity in proportion to that assumed for blood/marrow – e.g. by developing the “cell count” argument noted briefly above.

4.2 In practice, however, the difference between these approaches is academic. Put simply, **the presence of significant infectivity in bone would remove the advantage of blood/marrow removal**. All products sourced from a single donor would have a per-unit infection risk of **p** (the donor prevalence). Units produced by pooling 17 donations would carry an infection probability of **17p**. In other words, this scenario would be equivalent to that on the bottom row of Table 3(a) or 3(b), where **avoidance of pooling** becomes the key consideration in reducing vCJD risk.

5. *Further Comments*

Pooling of donations

5.1 To recap, we have shown that the effect of pooling several donations depends on the level of infectivity. If this is low enough for the doses received never to exceed $2 ID_{50}$, pooling is in principle irrelevant to the expected number of infections for a given donor prevalence. *Without pooling*, any infective donation will go to only one recipient. *With pooling*, several recipients would be exposed, but each would receive a proportionately smaller dose. With a linear dose-response model, these effects cancel out. Pooling will affect the

distribution of risks amongst recipients, but not the eventual number of infections to be expected.

- 5.2 At the other extreme, if infectivity is so high that even after processing, the dose from one infected donation in a pool would exceed $2ID_{50}$, the risk of infection is multiplied by the number of donors in the pool. This is demonstrated in the last row of Table 3, in which the pooled product would carry 17 times the per-unit risk of any unpooled alternative. The same applies if bone itself were to carry significant infectivity.

Cross-contamination of donations

- 5.3 In principle, a further disadvantage of processing donations is the potential for cross-contamination, in the sense of remnants of bone from one donor making their way into subsequent units (for example by remaining in machinery used to process successive donations). This could increase the number of recipients exposed to an infected donor. The potential for cross-contamination may however be greater where processing is carried out locally.
- 5.4 In the case of processing by NBS, our advice is that all devices used to process successive donations are thoroughly cleaned and autoclaved (to 134°C) between donations, this being done to approved standards in Central Sterile Services departments. Instruments used to harvest and process tissues are designated single-use where possible, and otherwise are subject to decontamination using ultrasound (where possible), mechanical washing and autoclave. While this cannot be said to reduce cross-contamination to zero, any risk from this source appears to be a second-order issue compared to that from the mass of material intentionally implanted.

5.5 Batch dedication

- 5.6 Batch dedication seeks to minimise exposure to any one infected donor by ensuring that where possible, contributions from the same donors go to the same recipients. It is generally impossible to use two Femoral Heads from a given donor in the same revision operation. Even if a donor were to give both his or her femoral heads (in itself rare), the two would normally have been removed in separate operations - typically some years apart. However an alternative form of batch dedication may be feasible for the pooled products, so that any given recipient would only receive bone from one *pool*. Each packet of processed bone contains either 1 or $\frac{1}{2}$ a femoral head equivalent and each revision hip operation uses 2-3 of these equivalents. Ensuring that each recipient received all packs from a single batch would limit the spread of exposure (A similar strategy is already used in the preparation of paediatric red cell packs for infants.)
- 5.7 In lower-infectivity scenarios (i.e. with potential doses remaining below $2ID_{50}$), batch dedication will affect only the spread of the risk, not the expected number of infections. For example, if all units were given in pairs from the same pool rather than from different pools, half as many individuals would be exposed to twice the risk of vCJD infection. Risks per procedure would remain in proportion to the number of units used - e.g. using two would units

double the risk to the individual patient - with or without batch dedication. However, batch dedication would still have some advantages for the management of any incidents involving a specific donor found to have the disease. It would simplify the task of tracing where any “implicated” donation had gone, and minimise the number of recipients who might have to be contacted to guard against further onward vCJD transmission.

- 5.8 However in high-infectivity scenarios - e.g. the cells marked * in Tables 3 (a) and (b), or if bone carries significant infectivity - the dose received from 2 infected units would exceed 2 ID₅₀. Batch dedication of units would then be more strongly advantageous, in limiting the possible number of infections to 17 *p per procedure* (rather than per unit).
- 5.9 A related issue is the possible use of “half-units” (i.e. the smaller packs of either frozen or freeze-dried bone). Again in the high-infectivity scenarios, the vCJD risk attaching to the use of two small packs would exceed that for one large pack, unless the former were both sourced from the same pool.

Research Issues

- 5.10 The contrasting results found in different scenarios highlight the need to investigate the potential infectivity of both marrow and bone further, preferably through experiments using human (rather than other animal) tissues. Experiments on marrow are already in train within the DH-funded research programme, using samples taken post mortem from known vCJD-infected patients. Results should be available during the course of the coming year. Experiments are also being designed to investigate the infectivity of bone itself, subject to availability of samples.
- 5.11 However the limitations of current experimental methods are also key. While a positive result (i.e. demonstrating infectivity on marrow or bone) would be significant, the sensitivity of the tests means that negative results would still leave open the full range of scenarios discussed here. Given the quantities of marrow and bone involved, infectivity below the level of detection could still be enough to give the recipient a high dose. Though this analysis should be reviewed in the light of any new findings, it seems unlikely that the uncertainties around infectivity can be reduced in the near future.

Caveats

- 5.12 This analysis clearly has its limitations, even leaving aside the uncertainties around infectivity of bone / marrow / blood. The simple “piecewise linear” dose-response model treats infection as certain for any dose at or over 2 ID₅₀. From analogy with other disease models, it appears more plausible that certainty of infection would be approached gradually. (In other words, with effective certainty only reached at higher doses)
- 5.13 In addition, the possible effect of higher doses in shortening the incubation period of the disease once infection has taken place has been noted, but not formally analysed. This is important, in making those infected with higher

doses more likely to suffer vCJD symptoms rather than dying of some other cause first.

- 5.14 Finally, we have treated the need for revision surgery, and hence the total demand for bone, as fixed. However such surgery is only necessitated by the wearing-out or failure of the original joint replacement. While one cannot expect any item to have an indefinite working life, continued efforts to ensure the quality and longevity of artificial hips etc will clearly be beneficial. The theoretical risk of vCJD transmission is but one reason for keeping avoidable surgery to a minimum.

6. *Conclusions*

- 6.1 This paper has compared the risks of the various bone products across a wide range of scenarios. Pooling donations is never advantageous from the point of view of vCJD risks, so a point of particular interest is the balance between its disadvantages and the advantages of processing in removing blood and marrow. As we have seen, this balance depends on the presumed infectivity initially present, and whether it is confined to blood and marrow. The efficacy of removal is also subject to some uncertainty (though this is unlikely to affect the overall conclusions set out here).
- 6.2 From the point of view of vCJD risks, use of unprocessed femoral heads is the least preferable option in many scenarios. Such procedures involve the transfer of a significant quantity of untreated blood / marrow. It may be difficult to reconcile this with the precautions now being taken against vCJD transmission via blood donation. The continued use of unprocessed heads may however be justifiable on other grounds – e.g. superior mechanical properties – lying beyond the scope of this analysis. This would require further discussion.
- 6.3 In the highest-risk scenarios, however, the disadvantage of pooling donations becomes the key consideration.
- 6.4 As argued above, it is unlikely that further research will allow us to narrow the range of possible scenarios in the near future. With uncertainty liable to persist, the key practical need is thus for *robust* options – i.e. those reducing vCJD risk in the widest possible range of scenarios. In this context, the ideal would be to reduce the use of bone – either by reducing the need for revision surgery or through development of some artificial substitute for bone. Importing bone from donor populations less at risk from vCJD might also be considered, particularly for the small minority of young recipients.
- 6.5 Considering the existing products, the most robust options involve removal of blood / marrow from donated bone *without* pooling of donations.
- 6.6 In all scenarios, processed bone from a single cadaveric donor is the best (or joint best) option in minimising vCJD risks. (A further potential advantage of cadaveric donation is that it may become feasible to screen such donors for vCJD using tests that would be impracticable for living donors.) Washing and centrifuging of individual femoral heads also represents a robust option that may meet the preferences of clinicians, though the removal of blood / marrow

appears likely to remain less complete than with the morcellised processed products.

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Annex A: Details of Calculations

The following pair of boxes show risk calculations for the initial illustrative scenario in the main text, i.e. infectivity confined to blood and marrow, at an overall level of **20 ID₅₀ per ml**. In Box A, gamma-irradiation is assumed to have no effect, whereas in Box B it is assumed to reduce infectivity by a factor of 10 (1 log)

Box A: Calculations for illustrative scenario, with gamma-irradiation having no effect

(a) Unprocessed Femoral Heads

The blood and marrow in an infected head would contain a dose of 400 ID₅₀ (more than sufficient to infect for certain), this occurring with probability **p**. So chance of infection = $1 * p$

(b) Washed / Centrifuged Heads

Following removal of 98% of blood / marrow, an infected head would contain a residual dose of 8 ID₅₀, still enough for certain infection (though the dose is much smaller than in (a)). This would again occur with probability **p**. So chance of infection = **p**

(c) Pooling from 17 living donors

Suppose each pool of 17 is used to make 12 units of processed bone. If one donation is infected, it will contain 400 ID₅₀ before processing. Assuming 99% is removed and the residue split equally between the 12 units, each will contain a dose of $(400/12 * 1/100) = 0.33 \text{ ID}_{50}$

The chance of any 1 of the 17 heads being infected is $17 p$.

So the chance of infection per unit used = $\frac{1}{2} * 0.33 * 17 p = 2.8 p$

(d) Pool from single cadaveric donor

On average, the bone obtained from each cadaveric donor is approximately 2/3 the mass of that in a pool from 17 living donors. (This is reflected in the fact that 8 units are obtained, as compared with 12.) We assume that the percentage of marrow/ blood is similar to that in femoral heads from living donors, and that the processing has the same effect as regards the residue left. So if the donation is infective, it would initially contain $(400 * 2/3 * 17) = 4533 \text{ ID}_{50}$.

If processing removes 99% of this, each of the 8 units obtained would carry a dose of $(4533 * 1/100 * 1/8) = 5.67 \text{ ID}_{50}$, still enough to infect for certain.

There is a probability **p** of the donation being infected

So the chance of infection per unit used = **p**.

Note:

Calculations assume that the loss of material in converting 17 heads to 12 packets implies no loss of infectivity: this may be overly-pessimistic. Similarly for processing of cadaveric donations. A more detailed examination of this point might be possible, but would be of limited practical value given gross uncertainties around potential infectivity of the materials.

Box B: Calculations in illustrative scenario, with gamma –irradiation having 1-log effect

(a) Unprocessed Femoral Heads

Each infected head would contain a dose of 400 ID₅₀, this occurring with probability **p**. So chance of infection = $1 * p$

Note that gamma-irradiation alone would leave a residual dose of 40 ID₅₀, still enough for certain infection.

(b) Washed / Centrifuged Heads

Following removal of 98% of blood / marrow and with infectivity reduced 10-fold by irradiation, an infected head would contain a residual dose of 0.8 ID₅₀, entailing a 0.4 risk of infection. This would again occur with probability **p**. So the chance of infection = $0.4 p$

(c) Pooling from 17 living donors

Suppose each pool of 17 is used to make 12 units of processed bone. If one donation is infected, it will contain 400 ID₅₀ before processing. Assuming 99% is removed and the residue irradiated and split equally between the 12 units, each will contain a dose of $(400/12 * 1/100 * 1/10) = 0.033 \text{ ID}_{50}$.

The chance of any 1 of the 17 heads being infected is $17 p$.

So the chance of infection per unit used = $\frac{1}{2} * 0.033 * 17 p = 0.28 p$

(d) Pool from single cadaveric donor

With the same assumptions as in Box 1(a), the blood / marrow in an infective donation would initially contain $(400 * \frac{2}{3} * 17) = 4533 \text{ ID}_{50}$.

If processing removes 99% of this, and irradiation gives a ten-fold reduction in infectivity each of the 8 units obtained would carry a dose of $(4533 * \frac{1}{100} * \frac{1}{10} * \frac{1}{8}) = 0.57 \text{ ID}_{50}$.

There is a probability **p** of the donation being infected

So chance of infection per unit = $\frac{1}{2} * 0.57 * p = 0.28p$



Lomas R, Drummond O, and Kearney JN. Processing of whole femoral head allografts: A method for improving clinical efficacy and safety. *Cell and Tissue Banking*. 2000; 1:193-200.