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Original Article

Risk of virus transmission through femoral head allografts: A Belgian appraisal

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OBJECTIVES

Background: To evaluate the incidence and the risk of transmitting a virus through a bone allograft from a living donor.**Material and methods:** A total 7032 femoral heads have been collected from 24 Belgian institutions. The tissue along with the screening blood tests were systematically sent to the bank. Serological screening included: for HIV, a HIV₁₋₂ antibody test; for HBV, a HBS antigen and HBS and Hbcore antibodies; for HCV, a HCV antibody test. Syphilis was also screened with a non-specific and a specific assays. HTLV₁₋₂ screening was recommended but not obligatory.**Results:** From the 7032 femoral heads, 1066 (15.2%) implants were definitively excluded. Hundred forty-six femoral heads, representing 2.1% of all grafts and 13.9% of the excluded ones, were discarded for positive serological testing associated with a risk of disease transmission. There were 2 donors who tested positive for HTLV₁₋₂. The prevalence of HIV in the femoral head donor population was six times lower than in the general one. The prevalence of hepatitis B and C was similar but far higher than HIV. The risk was computed to be 0.54 out of 1×10^5 for HIV and HCV without quarantine or tissue processing. For HBV, the risk was 0.77 out of 1×10^5 .**Conclusion:** Current standards of tissue banking incorporated safety and quality as their main features. This policy is now regulated at the European level. With a multi-step screening-policy, stringent donor selection guidelines, the risk of viral transmission through a tissue is minimized.

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1. Introduction

Tissue banks have the ultimate aim of providing safe and appropriate tissues for surgeons. The main source for bone in Europe is the femoral head from patients undergoing hip arthroplasty. As bone implant can transmit disease, safety remains a concern.¹⁻⁴ Amongst the potential transmittable

diseases, viruses and prions are the most difficult to track. Hepatitis C (HCV) and HIV virus transmission has been well documented.³⁻⁸ Dura mater implants but not bone and related tissues? have caused Creutzfeldt-Jakob disease.^{9,10} Although HIV virus is the most present in the media, Hepatitis B virus (HBV) is the most prevalent and as such carries more risks for transmission.^{7,11} Procedures have been

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developed to ensure the supply of safe bone.^{2,12} These procedures include guidelines on donor selection, tissue quarantine and tissue processing. In an effort to assess the risk, we have evaluated the incidence and the risk of transmitting a virus through a bone allograft from a living donor in Belgium. This paper does not deal with legal aspects or donor standards selection.

2. Material and methods

7032 femoral heads have been collected from 24 Belgian institutions between November 1997 and June 2003. The tissue along with the screening blood tests were systematically sent to the bank where the medical director of tissue bank made the final selection. Serological screening included: for HIV, a HIV₁₋₂ antibody test; for HBV, an HBS antigen and HBS and HB core antibodies; for HCV, an HCV antibody test (AXSYM, ABBOTT, North Chicago, USA). Syphilis was also screened with a non-specific (VDRL, venereal disease research laboratory) and a specific assays (TPHA, Treponema Pallidum hemagglutination assay). HTLV₁₋₂ screening (MUREX HTLV I + II, ABBOTT, Dartford, UK) was recommended but not obligatory. The mean donor age was 68 years and 63% of the donor population were females.

3. Results

From the 7032 femoral heads, 1066 (15.2%) implants were definitively excluded as non-compliant to the European and Belgian standards (Table 1). Hundred forty-six femoral heads, representing 2.1% of all grafts and 13.9% of the excluded ones, were discarded for positive serological testing associated with a risk of disease transmission.

Prevalence of diseases was assessed based on the complete available serological data of 6386 heads (Table 2). There were 2 donors who tested positive for HTLV₁₋₂ assay with borderline value but negative on confirmation tests. The main virus prevalence in the femoral head donor population was compared with their prevalence in the general population (Table 3).¹³⁻¹⁵ The prevalence of HIV in the femoral head donor population was six times lower than in the general one. The prevalence of hepatitis B and C was similar but far higher than HIV.

Twenty-three donors were positive for VDRL or TPHA but none were positive for the screened viruses.

Table 1 – Exclusion (1066/7032).

- Incomplete serology	60.6%
- History of cancer	15.8%
- Infectious agent	13.7%
- Neurological troubles	2.8%
- Auto-immune disease	2.6%
- Various	2.9%
- Consent refusal	0.84%
- Graft defect	0.75%

Table 2 – Prevalence of positive serological screening tests.

HIV ₁₋₂ ab	0.016%
HCV ab	0.86%
HBS ag	0.16%
Isolated HB core ag	0.86%
HB core ab/HBS ab	5.73%
HBV total prevalence	6.75%
Syphilis	0.36%

Reports of HIV and HCV transmission by seronegative organ and tissue donors have highlighted the risk of transmission from a donation during the window period, that is to say, the period between contamination and antibody development (Table 4). By testing more sensitive and early positive markers, and performing molecular biology tests such as polymerase chain reaction (PCR) for HIV and HCV, the window period is reduced to 16 days. The risk for transmitting a virus in the window period in patient screened by serological assays can be calculated by the formula: risk = incidence × window period (Table 5).^{16,17} The risk was computed to be 0.54 out of 1×10^5 for HIV and HCV without quarantine or tissue processing. For HBV, the risk was 0.77 out of 1×10^5 .

If a second line of safety is added such as a 6-month quarantine, the risk decreases by one hundred (logarithmic reduction of 2) with an estimated risk to be one out of 2×10^7 donors for HIV and HCV.

Another approach is a tissue processing. At our tissue bank, each bone processing included 8 steps of treatment that are able to either remove or inactivate the virus load if present. The capacity of our bone processing to eliminate viruses was assessed by analyzing two critical steps at the Institut Pasteur (Paris). In brief, bone blocks ($2 \times 2 \times 2\text{cm}^3$) were spiked with 5 different representatives of DNA and RNA viruses, with or without envelope. The initial and residual loads after treatment were titrated for obtaining a mean reduction factor. The least cumulated logarithmic reduction factor for the most resistant virus after these two steps was 7.84 (unpublished data).

If tissue processing is performed in a negatively screened donor at donation, the risk is virtually nil as an additional logarithmic factor reduction of 8 is added with a calculated risk less than one out of 2×10^{13} donors.

4. Discussion

Selecting a donor with the lowest risk of disease transmission is one of the major responsibilities of a tissue bank. Donor

Table 3 – Prevalence of main viruses for tissue banking.

Prevalence	Belgian population	Femoral head donor population
HIV ₁₋₂	0.1% ¹³	0.016%
HBV	6.9% ¹⁴	6.75%
HCV	0.87% ¹⁵	0.86%

Table 4 – Window period of main viruses for tissue banking.

HIV _{1–2}	22 days (6–38 days)
HBV	56 days (24–128 days)
HCV	66 days (38–94)

selection, biological screening of the donor and quarantining the tissues are legal requirements and most important. Tissue processing appears to be an alternative to quarantine when this option can be considered.

When selecting a donor two important issues should be taken into account quality of tissues and safety. Guidelines for appropriate donor selection are regularly updated by different associations and legal authorities.^{18,19}

Biological screening of blood is another prominent safety measure to avoid disease transmission. The sensitivity of antibody assay has largely increased with the availability of third-generation assays with a subsequent narrowing of the window period for HIV from 42 to 22 days.^{20,21} The use of either amplification tests such as HIV-DNA polymerase chain reaction or HIV p24 antigen testing will further decrease the window from 22 to 16 days.²¹ PCR can be performed on bone marrow, a good reservoir of lymphocytes presenting the advantage of being easily accessible and less sensitive to hemorrhage in the case of depleted or haemodiluted blood volume.²²

The same holds true for HCV for which a second-generation of antibody detection in 1992 could detect two reactive cases amongst 470 negative sera from previously negatively tested donors with the first-generation anti-HCV assay.⁷ A third generation with better performance is available since 1996.²³

Syphilis testing is even today recommended. It was felt that syphilis testing could be significant as a life-style reflector of the donor and as such to be an indicator of HIV infection. Reviews of blood bank records had shown that about 25% of donors who tested positive for syphilis were also positive for HIV.²⁴ This claim could not be confirmed in this study as none of the syphilis positive donors was positive for HIV or hepatitis. The value of syphilis testing as a surrogate HIV test could not be validated in our experience.

Despite the improved sensitivity of blood testing, there is still an interval between exposure and blood detection. Quarantine of tissue remains the standard protection against

transmission when no additional steps are considered.²⁵ After a 6-month period, the risk of windowing is small in living donor. By that time, 99% of the donors should have seroconverted for HIV.²⁶ In case of organ donor, the procured tissues should be quarantined for at least 3 months until they are back screened by HIV and HCV serological testing of the organ recipients.²⁷ Such measure should avoid transmission of virus from a falsely seronegative donor.²⁸

Tissue processing is another option that may include tissue cleaning, removal of blood and bone marrow by high-pressure washes, immersing in various solvents and thorough rinsing. Most often, processing will be completed by a secondary sterilization by irradiation.

Processed tissues have not so far transmitted HCV or HIV virus from infected donor and appear to offer the best available safety.^{7,28,29}

By eliminating bone marrow and blood, there is no more risk for Rhesus sensitization as described with frozen, non-processed bone.^{30,31} In removing bone marrow from the cancellous bone of the femoral neck, the risk to transplant occult pathological conditions is further decreased.^{32,33} Finally, six-month quarantine requires seeing the aged patient back, a requirement not often met. This failure of recalling patient causes an important loss of bone grafts unless it will be processed.³²

When properly managed, tissue banking keeps the risk of virus transmission to an acceptable minimal level. However, it should be kept in mind that tissue banks screen a limited number of known viruses and that transmission of unknown pathogens still remains possible.

The risk calculation is a probability but still, its significance remains elusive and it is difficult to set the cut off for decision-making. To evaluate the risk, comparison with realistic events is easier. Surgeons utilize sterile material on which the chance to find a microorganism is less than one in a million. In other words, he should use a million times the sterile item to get one contamination. In USA, the risk of dying from a cancer is about 221 in 100,000. The risk associated with death in a road accident is 17 in 100,000 (You have 340 times more chances to die in a traffic road accident when you are in motor-driven vehicle in USA than being HCV contaminated by receiving a Belgian frozen, nonprocessed bone allograft after a 6-month quarantine) and the one related to death from a medical complication during treatment is one in 100,000.³

Current standards of tissue banking incorporated safety and quality as their main features. This policy is now regulated at the European level. With a multi-step screening-policy, stringent donor selection guidelines, the risk of viral transmission through a tissue is minimized in Belgium, being much lower than most other risks associated with surgical procedures and is become nearly virtual with tissue processing.

Table 5 – Risk of transmission in the window period by a femoral head donation screened with serology but without quarantine serology but without quarantine.

	Incidence/100,000	On 100,000 donations
HIV _{1–2}	9 ¹³	0.54
HIV _{1–2} (based on HIV-DNA polymerase chain reaction)	9	0.39
HCV	3 ¹⁵	0.54
HBV	5 ¹⁶	0.77

Conflicts of interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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