Issues of HIV, HBV and HCV transmission from eye donation in Australia.

*Prevalence, Incidence and Residual Risk in screening and testing regimes.*

A report from the Eye Bank Association of Australia and New Zealand.

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Dr Graeme A Pollock. MPH, PhD.

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HIV, Hepatitis B and Hepatitis C in Corneal Transplantation.

**HIV 1 and 2**

*Transplantation*

HIV has never been reported to be transmitted via transplantation of corneas, sclera or any other ocular tissue.

HIV 1 has been documented to be in tears\(^1\) and corneal buttons\(^2,3,4,5,6\) but only a small percentage of donors with antibody HIV have detectable genome in the cornea\(^7\). The potential for transmission of HIV via corneal transplantation is considered to be lower than that of percutaneous transmission and is likely to be more analogous to transmission through mucous membrane contact\(^8\).

The cornea is avascular tissue -

- The incidence of seroconversion after exposure to HIV-positive blood is 0.3% after a percutaneous exposure (e.g. needle-stick injury) and 0.09% after mucous membrane exposure. The risk of seroconversion after exposure to other tissues or fluids, while not quantified, is felt to be considerably lower\(^8\). In comparison, transmission approaches 100% through blood transfusion.

HIV has not been transmitted via corneal transplantation –

- There are three reports in the literature detailing nine patients who have received corneas from HIV-positive donors\(^9,10,11\). None of the corneal recipients seroconverted or became ill, although all other organ and tissue recipients from these donors seroconverted.

**Hepatitis C**

*Transplantation*

Hepatitis C virus (HCV) has never been reported to be transmitted via transplantation of corneas, sclera or any other ocular tissue.

Polymerase chain reaction assays indicate that only 20-26% of seropositive cornea donors have viral RNA in their serum, and initial attempts to detect viral RNA in the cornea were unsuccessful\(^12,13\). However more recently there was a report of HCV RNA detection in 24% of corneas obtained from seropositive donors\(^14\). However, the potential for transmission of HCV via corneal transplantation is considered to be lower than that of percutaneous transmission and, like HIV, is likely to be more analogous to transmission through mucous membrane contact\(^8\).

The cornea is avascular tissue -

- The risk of contracting hepatitis C after exposure to HCV-positive blood is 1.8% after a percutaneous exposure (e.g. needle-stick injury) and is considered rare after a mucous membrane exposure\(^8\).

HCV has not been transmitted via corneal transplantation –

- Six recipients of corneas from three HCV-seropositive donors (at least two of whom had viral RNA in their serum) did not convert after transplantation\(^15\).
Hepatitis B

Transmission of hepatitis B virus (HBV) has been documented in two recipients from two separate donors \(^{16}\). Since serologic screening for HBV was introduced (in the late 1980’s) there have been no reported cases of transmission. No transmission has been reported from scleral transplantation or any other ocular tissue.

The cornea is avascular tissue -

- In one study less than 10% of HBsAg-seropositive donors had detectable HBsAg in their corneas \(^{29}\), and in a similar study no viral genome was detected \(^{17}\). The chance of a cornea being infectious prior to the appearance of sAg in the blood is considered to be very small \(^{17}\).

HBV has been reported to be transmitted twice by corneal transplantation from two donors.

- Recipients of one cornea from each donor developed clinical and serological evidence of HBV infection 2 months and 14 weeks after penetrating keratoplasty. The recipient of the fellow cornea from one donor died from a CVA 4 months after surgery without undergoing serologic testing. The recipient of the fellow cornea from the other donor never developed clinical characteristics of hepatitis but tested positive for prior exposure to HBV 2 years after penetrating keratoplasty \(^{16}\).

- These cases occurred in 1984 and 1985, before screening for HBV was required. Since then there have been no cases of hepatitis B transmission.

Screening of Donors

Screening for HIV-1 and 2 antibodies, HCV antibodies and HBsAg are required prior to the release of eye tissue for surgical use \(^{18}\).

In addition, physical examination of the donor for signs of AIDS and hepatitis and a thorough review of the medical and lifestyle history with specific attention to high-risk behavioural criteria are required. Such measures halve the incidence of HIV, HBV and HCV infected individuals entering the potential eye donor population (USA figures), and these intercessions need to be taken into account when risk calculations are performed \(^{19}\).
Prevalence, Incidence and Risk in the Australian Eye Donor Population

EBAANZ member banks have reported on the number of positive serology results (screening tests) returned for markers of HIV, HBV and HCV in Australia and New Zealand for the past 2 years.

While the screening test results as reported have not all been confirmed to be truly reactive the rates of confirmed positive results can be estimated by subtracting the number of false positive results (determined on the basis of specificity analyses of data from the United States). Thus prevalence in the Australian eye donor population can be calculated. This is the method used in the seminal paper by Zou and colleagues\textsuperscript{19}. In addition, figures are available for Australia in relation to incidence rate in the blood donor population for viraemic markers\textsuperscript{20}, and these can be applied using Zou’s method to estimate the an incidence rate among Australian eye donors. In turn, the estimated probability of undetected viraemia or residual risk (the probability that any eye donor was in the viremic window period with an infection that was undetected by screening tests at the time of eye donation) can be calculated using the Model B mathematical modelling equation described by Seed and colleagues\textsuperscript{20}.

The same methodology of residual risk estimate has also recently been performed and published for musculoskeletal tissue donors in Australia\textsuperscript{21}. This enables a direct comparison of residual risk across Australia’s blood, eye and musculoskeletal tissue donor populations.

Calculating Prevalence

From September 2007 through to June 2009 there were 1,856 eye donors in Australia\textsuperscript{22}. In this time, unconfirmed positive serology results reported were HIV - one, HBV - nine and HCV – seven. Applying Zou’s reported and validated figures of percent false positives for each of these results (HIV – 89%, HBV – 68% and HCV- 28%)\textsuperscript{19} the Australian eye donor prevalence rates are listed in Table 1.

Table 1. Australian Eye Donor Prevalence Rates

<table>
<thead>
<tr>
<th></th>
<th>Reported</th>
<th>Estimated Positive†</th>
<th>Number of Donors</th>
<th>Prevalence per 100,000 persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>1</td>
<td>0.11</td>
<td>1856</td>
<td>5.93</td>
</tr>
<tr>
<td>HBsAg</td>
<td>9</td>
<td>2.88</td>
<td>1856</td>
<td>155.17</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>7</td>
<td>5.04</td>
<td>1856</td>
<td>271.55</td>
</tr>
</tbody>
</table>

†fractional values are presented as a result of the estimation of numbers following Zou’s\textsuperscript{19} estimations.

Table 2. Prevalence Rates among different donor populations

<table>
<thead>
<tr>
<th></th>
<th>Australian eye donors</th>
<th>Australian first-time blood donors†</th>
<th>Australian musculoskeletal donors‡</th>
<th>United States tissue donors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>5.93</td>
<td>5.12</td>
<td>64.44</td>
<td>92.58</td>
</tr>
<tr>
<td>HBsAg</td>
<td>155.17</td>
<td>135.97</td>
<td>407.13</td>
<td>228.41</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>271.55</td>
<td>215.29</td>
<td>534.63</td>
<td>1091.16</td>
</tr>
</tbody>
</table>

† Published in Yao et.al.\textsuperscript{21} for 1993-2004, * Published in Zou et.al.\textsuperscript{19} for 2001-2002.

These comparative prevalence rates follow the lower prevalence rates for each marker in the general population of Australia compared to the United States. For example, the prevalence of HIV in the...
United States general population is estimated at around 600/100,000 people compared with Australia 69/100,000 (and New Zealand 10/100,000)\(^2\(^3\)). The exception to the relativity of these rates is the high prevalence rates in the Australian musculoskeletal population when compared to the Australian blood and eye donor populations (and compared to the HBsAg of United States tissue donor population).

**Calculating Estimated Incidence**

Incidence rates are not available for eye donors or tissue donors because this type of donation is a single non-repeatable event (therefore no time period can be assigned). To overcome this Zou and colleagues extrapolated incidence rates from United States blood donors to assign estimated incidence rates among tissue donors\(^1\(^9\). Yao and colleagues made the same extrapolation between Australian musculoskeletal and Australian blood donors\(^2\(^1\). This calculation involves adjusting the rates to reflect the different prevalence rates among the tissue donors and the populations used for comparison (a prevalence ratio). The same prevalence ratio can be applied to the Australian eye donor population to estimate the incidence rates. The prevalence ratio (calculated from Table 2) and calculated incidence ratios for Australian eye donors are presented in Table 3.

**Table 3: Incidence in Australian Eye Donors**

<table>
<thead>
<tr>
<th>Prevalence ratio</th>
<th>Incidence rate in blood donors* (no./100,000 person-years)</th>
<th>Estimated Incidence rate in eye donors (no./100,000 person-years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>1.16</td>
<td>0.35</td>
</tr>
<tr>
<td>HBsAg†</td>
<td>1.14</td>
<td>1.13</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>1.26</td>
<td>2.40</td>
</tr>
</tbody>
</table>

\(†\) transient nature of HBsAg makes estimations difficult. Seed\(^2\(^0\) provides an adjusted incidence figure to account for underestimation

\(\ast\) derived from Yao et.al.\(^2\(^1\) for 2003-2004

**Calculation of residual risk**

The estimated probability of viraemia at the time of donation can be calculated using the Incidence-window period Model B mathematical modelling equation described by Seed and colleagues\(^2\(^0\), which is that also used by Zou\(^1\(^9\) and Yao\(^2\(^1\).

- Assumes that Window Period transmissions represent the major component of the residual risk
- Probably holds true for HIV and HCV, but less so for HBV where chronic infection can be marked by transient HBsAg detection
- \(P = \lambda \times WP\) where
  - \(P\) = probability donor gave infectious donation during window period
  - \(\lambda\) = the incidence
  - \(WP\) = window period (in days)

Results for Australian eye donors using serologic testing methods are presented in Table 4.
Table 4 – Residual risk after serologic testing in Australian Eye Donors

<table>
<thead>
<tr>
<th>Window period† (days)</th>
<th>Estimated Incidence (no./100,000 person-years)</th>
<th>Estimated probability (no./100,000 eye donors)</th>
<th>Odds of infected donor being missed</th>
<th>Expected no. in Australian eye donors (per 1000/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>22</td>
<td>0.35</td>
<td>0.0211</td>
<td>1 in 4,739,336</td>
</tr>
<tr>
<td>HBsAg</td>
<td>59</td>
<td>1.29</td>
<td>0.2085</td>
<td>1 in 479,613</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>70</td>
<td>3.02</td>
<td>0.5792</td>
<td>1 in 172,651</td>
</tr>
</tbody>
</table>

†from Dodd et al.24.

These results compare to the published United States estimates for the Tissue donor population/100,000 donors of HIV 1.815 (1 in 55,096), HBV 2.962 (1 in 33,760) and HCV 2.374 (1 in 42,122), and the Australian musculoskeletal donor population of HIV 0.78 (1 in 128,000), HBV 0.53 (1 in 188,000) and HCV 1.82 (1 in 55,000).

Calculation of residual risk with Nucleic Acid Testing (NAT)
NAT testing for these viral markers reduces the estimated “window-period” and thus reduces the calculated theoretical residual risk (Table 5).

Table 5 – Residual risk after NAT testing in Australian Eye Donors

<table>
<thead>
<tr>
<th>Window period† (days)</th>
<th>Estimated Incidence (no./100,000 person-years)</th>
<th>Estimated probability (no./100,000 eye donors)</th>
<th>Odds of infected donor being missed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>7</td>
<td>0.35</td>
<td>0.0067</td>
</tr>
<tr>
<td>HBsAg</td>
<td>20</td>
<td>1.29</td>
<td>0.0707</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>7</td>
<td>3.02</td>
<td>0.0579</td>
</tr>
</tbody>
</table>

†from Jackson et al.25.

Calculation of Residual Risk of Transmission by Corneal Transplantation

For HIV and HCV where transmission by corneal transplantation has not been reported the residual risk of transmission must be based on theoretical rates of seroconversion, and these need to be based on published rates of seroconversion from similar inoculation scenarios.

Blood transfusion has a rate of transmission from HCV Ab/RNA blood components of approximately 80%. For HIV Ab+ it exceeds 90% but is less than 100%.26 For corneal transplantation (an avascular and bloodless transplant) the likelihood of transmission is significantly less than that of percutaneous transmission with infected blood, which for HCV is approximately 1.8%, for HIV 0.3% and HBV 6-30%.27 (These rates are likely to be overestimates as, in the absence of actual transmission, the rates from mucous membrane transmission are thought to be more applicable to corneal transplantation). Taking into account a transplant rate in Australia of approximately 1.6 corneal transplants per eye donor, and the residual risk calculations after...
serology testing one can calculate the residual risk of transmission from corneal transplantation. These are presented in Table 6.

Table 6: Residual risk of transmission after serology testing for Australian corneal transplants

<table>
<thead>
<tr>
<th></th>
<th>Estimated probability of an infected donor (no./100,000 eye donors)</th>
<th>Theoretical rate of transmission in corneal transplantation (% inoculated)</th>
<th>Probability of transmission† (no./100,000 eye donors)</th>
<th>Residual risk of transmission after serology testing</th>
<th>Expected transmission in Australian eye donors (@1000/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>0.0211</td>
<td>0.3</td>
<td>0.0001013</td>
<td>1 in 987,361,769</td>
<td>1 every 987,361 yrs</td>
</tr>
<tr>
<td>HBV</td>
<td>0.2085</td>
<td>3-60</td>
<td>0.010 – 0.125</td>
<td>1 in 799,360 - 9,992,006</td>
<td>1 every 799 - 9,992 yrs</td>
</tr>
<tr>
<td>HCV</td>
<td>0.5792</td>
<td>1.8</td>
<td>0.0167</td>
<td>1 in 5,994,858</td>
<td>1 every 5,994 years</td>
</tr>
</tbody>
</table>

† This takes into account approximately 1.6 corneal transplants from each Australian eye donor.

Cost of NAT testing

An analysis of the costs and reduced theoretical risks in undertaking NAT testing of Australian eye donors is presented in Table 7. These figures assume A$50 for each test. This is the approximate cost for non-urgent batched testing of donor serum. It does not take into account the additional expenses of transport from donor site to testing laboratory, or the additional costs involved in providing “at-call” testing of donor serum. They do also not take into account the cost of serology (ELISA) testing that must be performed in conjunction with NAT testing. The figures also assume that the residual risk after NAT testing is zero (the actual calculated residual risks are listed in Table 5) – thus the calculated costs are the additional costs of detecting one donor (and one transmission) by NAT testing that would not have been detected by serology testing.

Table 7: Additional costs of NAT testing in Australia

<table>
<thead>
<tr>
<th></th>
<th>Cost per test</th>
<th>Cost of detecting one infected donor</th>
<th>Cost of preventing one transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV</td>
<td>$50</td>
<td>$237 million</td>
<td>$4.93 billion</td>
</tr>
<tr>
<td>HBsAg</td>
<td>$50</td>
<td>$24 million</td>
<td>† $80 million</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>$50</td>
<td>$8.6 million</td>
<td>$300 million</td>
</tr>
<tr>
<td>Total</td>
<td>$150</td>
<td>$269.6 million</td>
<td>$5.3 billion</td>
</tr>
</tbody>
</table>

† assumes 30% transmission rate
**Current status of NAT testing in Australia for Eye Donation and Transplantation**

Validated and licensed NAT testing for HIV, HBV and HCV is currently not available Australia-wide for the testing of eye donor serum.

Unlike serological testing of serum, NAT testing of cadaveric samples in Australia is not a routine and readily available clinical test. Routine turn-around times of 24-hours are unavailable. It is not available outside of the eastern States of Australia.

Unlike donated tissues, donated corneas are extremely “time-sensitive”; much like whole organs. The Australian Corneal Graft Registry 2007 report shows that approximately 70% of all corneal transplants are performed between 0-4 days after the death of the donor. Almost 10% are performed within 24 hours. Less than 3.5% are performed at 7 days or more. This is because with increased storage times surgical handling is more difficult, the post-operative course is extended, and transplant outcomes are less successful. Corneal transplantation involving hypothermic preservation ideally requires the flexibility to transplant corneas from within 24 hours after death of the donor. Such routine turn-around times are available for serologic testing of HIV, HBV and HCV across Australia but not for NAT testing.

*Logistic difficulties in access to NAT testing within the time-frames required transplantation reduces the number of safe and viable corneas available for transplant in Australia.*

Additional problems relate to the test itself and the serum sample requirements –

- Some laboratories indicate the need for up to 20ml of blood to perform the three NAT tests. One laboratory indicates at least 2ml of serum per test is required. The availability of serum samples from eye donors can be extremely restrictive.
  - If plasma dilution has taken place a pre-dilution ante-mortem sample taken within 7 days prior to death is required. Often it is difficult to obtain enough sample for completion of serological requirements.
  - The amount of blood taken directly from a deceased person can be very limited and the amount of serum derived from it very small but usually within the limits of serology testing requirements.

*The unavailability of enough serum from eye donors for mandatory NAT testing reduces the number of corneas that become available for transplantation.*

- Heparin and other common inhibitors interfere with NAT testing. Samples are usually required in an EDTA tube.
  - Given the demographics of eye donors there are a significant number of patients that have inhibitors in their blood stream at the time of death.
  - Restricting blood samples to an EDTA tube further restricts access to ante-mortem samples if they are required.

- Time frames for collection, separation of plasma, and storage and transport requirements severely restrict the availability of suitable samples for NAT testing.
  - Plasma for HIV NAT needs to be separated within 2 hours of collection, for hepatitis NAT within 5 hours (although some laboratories allow 48 hours). Given the distances and times involved in some donations compliance to these time-frames is not always possible.
Plasma or serum can be stored for up to 72 hours at 2-8°C but must be frozen if storage is to be beyond 72 hours. Again this restricts the availability of ante-mortem samples if required for testing and substantially increases transport costs for samples.

Issues related to serum/plasma sampling for NAT testing reduces the number of safe and viable corneas available for transplant in Australia.

Conclusions

Overall, current measures used to evaluate eye donors in Australia and New Zealand are effective. Benchmarking with anti-HIV, HBsAg, anti-HCV prevalence data from Australian blood donors confirms that prevalence rates in eye donors are low and are comparable to blood donor rates. In addition, the risk of transmission of these viral diseases from an eye donor is lower than that from a blood donor. This is in contrast to the low but significantly higher risk (compared to eye donors) among Australian musculoskeletal tissue donors. Additional benchmarking against United States equivalents also confirms that Australian corneal donation and transplantation is among the safest of any type of donation in the world.

Implementing NAT to screen individual eye donors is estimated to reduce the residual risk of a donor being vireamic within the “window-period”. However, this calculated reduction from an already low level of risk comes at a cost. NAT is more complex, time consuming and expensive than serological testing. The cost-effectiveness of nucleic acid testing may not compare favourably with that of other health preventative measures such as the evidence-based application of stringent donor exclusion criteria or the employment of increased numbers of senior experienced professional staff assessing donors. This is certainly the experience with HIV and HCV NAT donor screening in the United States where its cost effectiveness has been assessed as poor. In this study the costs involved in identifying one donor, or preventing one transmission of disease cannot be justified by any public health criteria. Responsible risk management suggests that rather than continuing to focus with marginal benefit on already comparatively low risks of viral transmission there needs to be a re-focus on increasing donor rates to enable more patients to benefit from what are already demonstrably safe corneal transplants.

In summary

- In the Australian and New Zealand context mandatory NAT testing will provide no reduction in risk, either in relation to detection of eye donors with viraemia or in regard to the transmission of viruses through corneal transplantation.
- The low prevalence of the disease in the Australian and New Zealand eye donor population makes the risk/benefit ratio of loss of tissue as a result of NAT testing unacceptable.
- The significant increasing costs related to tests that have not been scientifically validated to significantly reduce risk cannot be justified.

In conclusion, mandatory NAT of the Australian eye donor population cannot be supported at this time.
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