Title Page:

Recently acquired blood borne virus infections in Australian deceased organ donors: Estimation of the residual risk of unexpected transmission.

Title Character Length: 144 with spaces.**Authorship Page:**

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Clinical Trial Notation:
N/A

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2. Participated in the writing of the paper
3. Participated in the performance of the research
4. Contributed new reagents or analytic tools
5. Participated in data analysis

Disclosure:

The authors declare no conflicts of interest

Funding:

This project was unfunded.

Abbreviations Page

HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
NAT	Nucleic Acid Test
OBI	Occult Hepatitis B Infection

Abstract (Word Count: 246)

Background: Unexpected donor-derived infections of HBV, HCV and HIV are rare but important potential complications of deceased organ transplantation. The prevalence of recently acquired (yield) infections has not been previously described in a national cohort of Australian deceased organ donors. Donor yield infections are of particularly significance, as they can be used to gain insights in the incidence of disease in the donor pool, and in turn estimate the risk of unexpected disease transmission to recipients.

Methods: We conducted a retrospective review of all patients who commenced workup for donation in Australia between 2014-2020. Yield cases were defined by having both unreactive serological screening for current or previous infection, and reactive NAT screening on initial and repeat testing. Incidence was calculated using a yield window estimate, and residual risk utilising the incidence/window period model.

Results:

The review identified only a single yield infection of HBV in 3,724 persons who commenced donation workup. There were no yield cases of HIV or HCV. There were no yield infections in donors with increased viral risk behaviours. The prevalence of HBV, HCV and HIV was 0.06% (0.01-0.22), 0.00% (0-0.11) and 0.00% (0-0.11) respectively. The residual risk of HBV was estimated to be 0.021 % (0.001 - 0.119).

Conclusions:

The prevalence of recently acquired HBV, HCV and HIV in Australians who commence workup for deceased donation is low. This novel application of yield-case-methodology has produced estimates of unexpected disease transmission which are modest, particularly when contrasted with local average waitlist mortality.

Introduction:

Screening potential deceased organ donors for transmissible infections is an important component of routine donor workup. Screening includes both the laboratory testing of specimens from the deceased donor by nucleic acid testing (NAT) and serology, and the eliciting of antecedent behaviours that may have place the deceased at increased risk of recent disease acquisition[1].

The value of behavioural screening has recently been the subject of increased academic attention[2-4]. Very recently acquired infections could potentially be within the NAT window period and therefore not detected, but still result in transmission[5].

Behavioural screening's utility lies in its ability to sub-stratify donors who do not have laboratory evidence of infection, into standard and increased risk groups for recently acquired infection that could potentially be within the NAT window period.

In addition to routine donor screening, national guidelines recommend routine post-transplant screening of recipients of increased viral risk [1]. Whilst unexpected donor derived blood borne virus infections appear rare, sporadic events have occurred[6-8].

By definition, the incidence of donor infections which occur during the NAT window period cannot be measured directly. Traditionally, methods to infer viral incidence in organ donors include using the incidence of unexpected transmission events in transplant recipients[9], and by extrapolating incidence rates from surrogate (non-organ donor) populations at increased risk in the community[10-12].

NAT window period infections, even within increased risk organ donor cohorts, are rare[13-16], and whilst biovigilance auditing of transmission events are an essential quality process for mature transplant sectors, insights into the frequency of transmission events are limited by incomplete screening of transplant recipients[17, 18], and small event sample sizes, even in large jurisdictions[5].

The methodological challenges of high-consequence low-probability transmission events, and single point-in-time pathology testing are shared in the study of residual risk in both deceased organ donors and first-time blood donors[19]. The blood transfusion literature has described methods for identifying recently acquired human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in first time donors with subsequent extrapolation of incidence and residual risk of infection[6, 20, 21]. Blood donor screening typically incorporates both NAT and serological testing. Viral RNA/DNA is the earliest marker of infection which is later followed by the appearance of antibody. Yield-case methodology leverages the differences in window periods for NAT and serology viral markers taken at the same timepoint. Recent infections within the serological window period but detected by NAT are referred to as NAT-yield cases.

The application of NAT-yield case methodology does not appear to have been previously applied to the field of organ donation and transplantation.

In this brief communication, we report our audit of recently acquired infections in patients being worked up for organ donation and describe their prevalence and

donor risk behaviours. We also use methods pioneered in transfusion-transmission risk modelling to estimate the incidence and residual risk of window period infection.

Methods

We conducted a national retrospective electronic clinical chart review of all Australian organ donors who commenced work-up for organ donation between 2014 and 2020. A detailed description has previously been published[22], but in brief it involved a review of the national electronic donor record database. This review was approved by the Melbourne Health Human Research Ethics Committee (QA2019030) and the study was conducted in accordance with the ethical standards laid down in the Declarations of Helsinki and Istanbul.

Potential donors were included independently of whether they proceeded to donation surgery. Each potential donor's record was reviewed for completed pathology screening results for HIV, HBV and HCV infection. Donors were included for analysis if they had paired early (NAT) and late (serology) viral markers for any of the 3 viruses: HBV, HCV and HIV. We excluded patients from analysis if there was previous (serological) evidence of infection. For yield cases, risk factors were extracted from the behavioural risk assessment questionnaire which was administered to family of the potential donor by trained donation nursing staff.

HIV and HCV Methodology

To define recent HIV and HCV infections, we employed a modified version of parameters utilized by Busch and colleagues[6]. Our choice of viral markers was based on local screening practices, including the routine use of individual NAT donor

screening, rather than mini-pool NAT, and anti-HIV-1/2 screening rather than p24Ag detection.

The early and late viral markers utilised were HCV RNA and anti-HCV for HCV, and HIV RNA and anti-HIV1/2 for HIV respectively. Serological median window periods were based on previously published values for the PRISM chemiluminescent immunoassays (Abbott Diagnostics, Wiesbaden-Delkenheim, Germany) [23]. NAT median window periods were based on previously published values for the Procleix Ultrio Plus assay on the fully automated Tigris platform (Grifols Diagnostic Solutions Inc., Emeryville, CA) [23]. Yield window periods were defined by subtracting the median window period estimate of the NAT, from the median window period estimate of the serology test for each disease.

HBV Methodology

The estimation of incidence and residual risk for HBV, using yield-case methods, is more complex than for HIV and HCV. The blood transfusion literature describes a number of methodological approaches to this problem [20, 24]. We have employed the NAT yield / window period ratio model as described by Lelie and colleagues [21]. HBV DNA is used as the early marker and HBsAg as the later marker. After identifying HBV yield cases, anti-HBc positive individuals were excluded. Anti-HBc reactive samples with detectable HBV DNA likely represent occult hepatis B infection (OBI), rather than a true yield case. Intervals used by Lelie were modelled from screening systems using the PRISM system for detecting HBsAg, individual donor NAT on Ultrio and Ultrio Plus assays for detection of HBV-DNA, and an assumed acute OBI rate of 4% [21].

Key time intervals for HBV, HCV and HIV methods are summarised in figure 1.

Yield Case Verification

In contrast to blood donation, national guidelines for the repeat testing of initially reactive samples have not been published for organ donation. Non-reactive samples do not routinely undergo repeat testing. The decision to repeat testing on the same or a newly drawn sample and use of a quantitative or qualitative assay are based on a balance of logistical and clinical constraints.

We manually reviewed the clinical record for all potential NAT yield cases. We assumed a donor as a NAT yield case based on repeat reactivity of the original sample on the same NAT screening assay, or reactivity in a different NAT assay. Cases were excluded from analysis if confirmatory testing was not reactive, or a test result transcription error was identified.

Incidence Rate and Residual Risk Calculations

Incidence was calculated using yield methodology, an adaption of the window-period incidence method [20, 21, 24]. In brief, the total observational period (person-time) was defined as the product of the NAT yield window period and the number of donors. The incidence was defined as the number of yield cases divided by the total observed time. Residual risk was calculated based on the derived incidence rates, and the relevant 50% limit of NAT window periods previously published. The residual risk can be summarised by the following formula:

$$RR = WP_{NAT}/(WP_{Seromarker} - WP_{NAT}) \times \left(N_{Yield\ Cases} \div N_{Organ\ Donors}\right)$$

Prevalence rate and incident rate confidence intervals were based on the 95% exact method from the Poisson distribution. Residual risk confidence intervals were inferred from the mean and bounds of the 95% confidence intervals of the incidence rate. When no yield cases were identified for a specific virus, residual risk was not directly calculated. Instead, a conservative scenario was hypothesised that the "next case" in time would be a NAT yield case. Based on this, and the associated increased in total observation period, incidence rate and residual were re-calculated. Results were then reported as the residual risk odds being "less than" this value (see appendix). In the case of zero yield events, the upper 2.5% CI result from the incidence rate was extrapolated from the incidence rate. Statistics were analysed using Stata IC (15.1, StataCorp LLC, College Station, TX).

Results

Between 2014-2020, 3724 individuals commenced workup for deceased organ donation in Australia. Paired early and late-stage viral markers were available for retrospective yield-case analysis in the vast majority of cases (Figure 2).

In this review of 6 years of national organ donation practice only 1 yield case was identified.

Of the 3,507 patients without evidence of previous exposure to HBV, only one patient developed an HBV yield infection over a cumulative period of greater than 70,000 person-days.

Of the 3,115 patients without previous evidence of exposure to HCV, no patients developed an HCV yield infection over a cumulative period of greater than 197,000 person-days.

Of the 3,246 patients without evidence of previous exposure to HIV, no patients developed a yield infection over a cumulative period of greater than 52,000 person-days.

The incidence rate in this case series for HBV, HCV and HIV was 0.52, 0.00 and 0.00 per 100 person-years respectively.

We estimate the risk of unexpected disease transmission from deceased organ donors in Australia to be low, with the residual risk of HBV being 1 in 4,664 (95% CI: 1 in 18,447 - 1 in 849)

A conservative assessment of risk, utilising the upper 95% CI, generated the risk of unexpected HCV infection to be 1 in 20,568, and 1 in 2,401 for HIV.

The single yield infection occurred in donor without a history of increased risk behaviours. In patients without serological evidence of current or previous infection, NAT testing had a false positive rate of 0.065%, 0.064% and 0.31% for HBV, HCV and HIV respectively.

Discussion

This retrospective chart analysis identified a very low prevalence of recently acquired blood-borne viral infections in Australian patients who commenced workup for deceased organ donation.

We believe this is the first study to utilise NAT yield cases to infer incidence rates of blood borne virus in organ donors and may have benefits over other methods previously utilised. Additionally, the incidence rates of HIV, HCV and HBV in the general Australian organ donor pool has not been previously estimated.

Residual risk quantifies the risk of newly acquired infection within an assay's window period. For individual-donor NAT assays, this period is measured in days up to a few weeks, depending on the blood-borne virus and assay used. It is remarkable that in the time immediately preceding this window period, we only detected 1 new HBV infection in greater than the equivalent of 70,000 days of observation. Additionally, over extensive periods of observation we were unable to detect any newly acquired cases of HCV or HIV.

Within the Australian context, the incidence rate of blood borne viruses has only been estimated for increased viral risk organ donor cohorts[6]. This is the first paper to estimate the incidence for the Australian general organ donor population. The incidence rates published for deceased tissue donors (which in practice derive largely from standard viral risk organ donors), are similar, sitting within our reported confidence intervals[6].

Human derived biologicals that are transplanted/transfused in Australia include blood, tissues from living donors, tissues from deceased donors, and organs from deceased donors. Our data, together with previously published studies suggest an increasing gradient of residual risk across this spectrum, as reporting of increased risk behaviours transitions from self-reporting to a third-party, and exclusion of donors with increased behaviours no longer becomes mandatory (see appendix) [19, 25, 26].

We report extremely modest risks of unexpected disease transmission from deceased organ donors. By contrast, the risk of death for the average Australian awaiting an organ transplant varies between 1 in 16 for persons awaiting a heart transplant and 1 in 287 for persons awaiting a renal transplant (See appendix).

The application of the NAT-yield method to estimating residual risk in deceased organ donors may have benefits over previously described methods. NAT yield methodology derives estimates of recent viral infection incidence from the same population for which the residual risk is being estimated. This avoids the presumption that incidence rates or incidence: prevalence ratios would be equivalent between organ donor and non-organ donor cohorts. We have previously shown that, for some risk behaviours, the prevalence of infection can vary significantly between "matched" community and donor cohorts [22], and Rothman has argued that extrapolation of the incidence: prevalence ratio is fraught with a number of population equivalency assumptions.

The behavioural risk assessment questionnaire is administered to identify individuals at increased risk of NAT window period infection. Case review showed that despite having a detailed medical-social inventory undertaken by trained donation nursing staff, the potential donor with recently acquired HBV did not have any increased viral risk behaviours identified.

These findings are consistent with previous local and international studies that have highlighted the relatively poor performance of increased viral risk designation in predicting both prior exposure to blood-borne viral disease, as well as current active infection in donors[22, 27].

Limitations

There are a number of limitations when interpreting our findings. Despite a national review of 6 years of clinical practice, we identified only 1 NAT yield case.

Our results reflect contemporary practices within Australia. Should future practice change and result in a greater number of increased viral risk individuals progressing to BBV screening during deceased organ donation workup, the overall incidence of yield cases, and hence the probability of unexpected infections in recipients, may increase.

Due to the rarity of NAT yield cases, infrequency of deceased organ donation and size of the Australian population, our estimates of incidence and residual risk have

broad confidence intervals. Indeed, for HCV and HIV a residual risk could not be directly calculated. Instead, the most conservative confidence bound must be interpreted. Nevertheless, clinicians should find some reassurance in the rarity of these events, as they likely intrinsically mirror the rarity of window period infections. Determination of residual risk in individual behavioural risk subgroups was not undertaken but would be worthy of future investigation, particularly in larger jurisdictions.

Whilst NAT yield-case methodology has been shown to produce equivalent results to other methods of estimating the residual risk of window period HIV and HCV infection in blood donors[6, 28], it is thought to systematically over-estimate the incidence of HBV infection by up to a factor of 2[21, 29]. In a recently published review, overestimation was thought to predominantly derive from the misclassification of cases as early window period infections, when in fact they represent more established infections with less typical serological patterns and/or viral loads[21]. For HBV, key window period intervals were based on specific NAT and serology assays, and incorporated assumptions about the prevalence of OBI. Within Australia, a number of different platforms are used to screen prospective organ donors for HBV and the prevalence of OBI is unknown.

Additionally, not all window period infections in donors result in transmission and clinical infection in recipients. Pathogen factors, donor-organ, ex-vivo management and immunological status of donors and recipients all likely modulate the probability of unexpected transmission events.

We identified potential NAT yield cases by performing qualitative NAT screening, and individually audited the clinical record for each case. Whilst NAT reactive cases routinely underwent repeat testing, it was not always from a second drawn sample, tested on a different assay or performed in an accredited reference laboratory. Whilst these criteria may be required for the formal diagnosis of viral infection in the community or as part of blood donor follow-up protocols, they are not always logistically feasible in screening organ donors. Donor clinical instability, recipient time-critical need, and donor family requirements during end-of-life care do not always permit extensive exploration of an initially reactive result.

Conclusion

In summary, this report demonstrates the novel use of yield case methodology in the estimation of the incidence of blood borne viruses in deceased organ donors. There are theoretical reasons why this method may be an additionally useful method of estimating residual risk of infection. Recently acquired infections in Australians who commenced workup for deceased organ donation were rare and were not associated with known increased risk behaviours. The extrapolated risk of unexpected disease transmission was low, particularly when juxtaposed with other risks associated with end-stage organ failure.

Acknowledgements

The authors would like to thank the following organ donation professionals from the DonateLife Agency Network who assisted with the case reviews of potential yield cases: Rohit D'Costa, Lee Wood, Tina Coco, Leo Nunnink, Guowen Hu, Stewart Moodie. Michael O'Leary. Australian governments fund Australian Red Cross Lifeblood for the provision of blood, blood products and services to the Australian community.

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Figure Legend Page

Figure 1: Window periods of early and late viral markers of infection

* HBV early marker was HBV DNA as determined by Grifols/Hologic HIV-1/HCV/HBV Procleix Ultrio Plus assay. Late marker was HBsAg on the Abbott PRISM (Abbott Diagnostics, Wiesbaden/-Delkenheim, Germany) Chemiluminescent Immunoassay system. Time intervals based on Lelie and colleagues 2020[21].
† HCV early marker was HCV RNA as determined by Grifols/Hologic HIV-1/HCV/HBV Procleix Ultrio Plus assay. Late marker was Anti-HCV on the Abbott PRISM (Abbott Diagnostics, Wiesbaden-Delkenheim, Germany) Chemiluminescent Immunoassay system. Window period as per Australian Red Cross Lifeblood[23].
‡ HIV early marker was HIV1 RNA as determined by Grifols/Hologic HIV-1/HCV/HBV Procleix Ultrio Plus assay. Late marker was Anti-HIV-1/2 on the Abbott PRISM (Abbott Diagnostics, Wiesbaden/-Delkenheim, Germany) Chemiluminescent Immunoassay system. Window period as per Australian Red Cross Lifeblood[23].

Figure 2: Study flow and key results

*Case 1: Repeat testing, same specimen, same assay: Qualitative. Result HBV DNA negative, Conclusion false positive.
Case 2: Transcription error. Anti-HBc +ve: Non-Yield Case.
† Case 2: Data transcription error. Anti-HCV +ve. Conclusion: Non yield case.
† Case 3: Repeat testing, same specimen, different assay: Quantitative NAT. Viral load undetectable. Conclusion: false positive.
‡ Case 4: Repeat testing, same specimen, differing assay: Quantitative NAT. Viral load undetectable. Conclusion: false positive.
§Case 5: Repeat testing, same specimen, same assay: COBAS MPX, Result: Positive. Conclusion: True positive yield Case