Discordant hepatitis C serological testing in Australia and the implications for organ transplant programs

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\textbf{A B S T R A C T}

Background: Discordant and equivocal hepatitis C (HCV) serology testing is problematic for making decisions regarding deceased organ donor (DOD) transplant allocation based on allograft infection status.

Objectives: This study aimed to analyse the prevalence and follow-up testing of discordant HCV tested patients from an Australian population at increased risk of HCV infection, with prevalence modelling for the Australian DOD population.

Study design: De-identified patient discordant HCV serology results (primary chemiluminescent microparticle immunoassay and secondary Bio-Rad MonoLisa HCV Ag/Ab Ultra assay) were retrospectively identified in a general referral laboratory between May 2008 and August 2011. Prior and follow-up serology testing was reviewed. Discordant result prevalence was calculated using Bayes’ theorem for the DOD population using Australian DOD rates and HCV seroprevalence.

Results: The tested population had a 6.6% HCV seroprevalence. The rate of discordant serotesting was 0.54%, with no cases identified as having definite HCV infection at follow-up. Two patients had evidence of definite HCV seropositivity before the index discordant test. Modelling for the Australian DOD population of 337 per year estimated a discordant test prevalence of 1.8 per year.

Conclusions: Discordant HCV serotesting may occur for 1 of 185 patients tested in higher risk populations. The majority of such tests represent falsely reactive tests although a small number may reflect partial seroreversion. Amongst Australian DOD, this represents 1 or 2 discordant cases per year. It is likely that if this discordant sample were from a donor with no blood borne virus risk factors, and was concurrently RNA negative, that HCV infectious risk would be extremely low.

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1. Background

The implementation of screening for blood borne viruses (BBV) in deceased organ donors (DOD) has led to significantly reduced infection transmission. This has been a critical step to ensure patient safety and community confidence in transplantation. However, there exists a potential cost due to possible false positive testing reducing organ availability.\textsuperscript{1} The proposed addition of nucleic acid testing (NAT) to current serological testing of DOD in the United States has recently re-ignited such concerns.\textsuperscript{2} On review however, parallel NAT has been shown to safely extend organ availability as was recently demonstrated in an Australian DOD population at increased risk of BBV.\textsuperscript{3} Further review of BBV testing and the potential for false positive serotesting may provide further opportunities to safely expand DOD rates.

Current hepatitis C (HCV) assessment of intended deceased organ donors (DOD) in Australia involves initial clinical risk screening to identify increased risk of BBV, followed by laboratory screening (Fig. 1). Where clinical screening indicates increased risk, both serological testing and prospective NAT assay are performed before organs are allocated to a recipient. Retrospective NAT is currently performed post transplantation where serology is negative, and prospective NAT with a turnaround time of less than eight hours, when serology is positive.\textsuperscript{4} Testing at the New South Wales (NSW) Australia DOD BBV testing facility for the 19 months from Oct 2009 until April 2011 identified a 2.6% (4 of 156) HCV seroprevalence. This compares with the most recent modelled Australian population HCV seroprevalence of 1.3%, and an Australian blood donor prevalence of 0.013%.\textsuperscript{4,5}
Consistent with current Australian guidelines, HCV serological testing at the NSW DOD testing facility involves the use of a second immunoassay for confirmation of initial immunoassay duplicate reactive or equivocal low level results. The use of secondary HCV antibody screening however can be problematic due to the occurrence of discordant results. Australian hepatitis C testing policy advises that discordant test results be interpreted as non-specifically reactive, or reflective of recent or distant past HCV infection, with recommendations made for follow-up serology and/or RNA testing. In the low BBV risk blood donor population discordant seroconversion has been well described and is referred to as biologic false reactive testing (BFR).7 Rates of BFR testing in an Australian blood donor population tested using similar immunoassays to those of the NSW BBV DOD laboratory are 0.34%.8 Longitudinal studies of Australian blood donors with past BFR sampling demonstrate that no subsequent donations displayed definitive HCV infection, with the majority of these individuals having further BFR test results on subsequent testing.7,8 BFR tests in this population have been associated with factors including the specific set of immunoassays used, patient age, season, and influenza vaccination.8,9 Current Australian practice for a first time blood donor with BFR is to discard the donation.7 There are no established guidelines in Australia for interpreting and managing discordant results for DOD, with potential for loss of intended donations if considered within a seropositive category. Since being established in October 2009, the New South Wales BBV DOD testing facility has not had any discordant HCV seroconversion results in donor samples.

2. Objectives

This study aimed to determine the background rate of discordant false reactive HCV seroconversion, in order to inform DOD testing and allocation algorithms. A relatively high BBV risk population of community and hospital tested patients was retrospectively reviewed to determine HCV discordant seroconversion prevalence and follow-up. Compared with examining risk of false reactive testing in a low seroprevalence blood donor population, this provides a risk setting that more closely resembles organ donor HCV seroprevalence. Of additional interest were the calculated rates of sample positive NAT in the presence of discordant seroconversion, representing the practice of parallel NAT and seroconversion of DOD. These data were used to model the rates of discordant seroconversion with reference to the DOD population.

3. Study design

Since May 2008 HCV seroconversion in the DOD testing facility has used an automated Abbott Architekt anti-HCV chemiluminescent microparticle immunoassay (CMIA) as the primary immunoassay (Ia1). The secondary assay used for further assessment of positive or equivocal 1a1 testing was the fourth generation enzyme immunoassay (EIA), the Bio-Rad Monolisa HCV Ag/Ab Ultra assay (HCV Ag/Ab) (Ia2) (Table 1). The latter detects both capsid antigen and HCV antibodies and significantly reduces the window period for infection detection compared with assays that only detect antibody.10 When evaluated with confirmatory Chiron RIBA the specificity of the CMIA for blood donors is reported as 99.92% and 99.43% for diagnostic testing.11 The HCV Ag/Ab assay is reported to have a specificity of 99.85% for blood donors, and 100% for diagnostic sampling.12 The sensitivity of the HCV Ag/Ab assay is 100% for chronic HCV infection, and it detects early acute infection significantly earlier when compared with HCV antibody assays.12
The serology testing algorithm used by the NSW DOD testing facility and the general laboratory (South Eastern Area Laboratory Services) servicing the hospital and community population are the same.

The general referral laboratory services a population of approximately 1.6 million, which includes NSW Corrective Services, drug and alcohol facilities, a major tertiary hospital and sexual health clinics. Baseline characteristics of the cohort are incomplete due to variable complete de-identification of samples including gender identifiers.

The general laboratory de-identified database of HCV serotesting of 31,192 patients, and 38,443 tests between May 2008 and August 2011 was retrospectively reviewed. There were 31,311 non-duplicated patient Ia1 test results (the difference of 119 tests was from patients who had different results of Ia1 testing at different times). Discordant serotesting was identified for 136 patients. From this group, prior (dating to January 2000) and follow-up serology (dating to August 2011), and/or NAT testing of cases were reviewed. Follow-up serology and parallel NAT testing of all discordant tested samples was not available due to the retrospective nature of this review.

The patient rate of discordant serotesting from review of the general laboratory database was applied to the DOD population according to Bayes’ theorem, using current Australian DOD rates and HCV seroprevalence, to estimate potential rates of DOD discordant serotesting.

The nomenclature and definitions used in the study are summarised in Table 1.

4. Results

Of the 31,311 patients from the general laboratory database with non-duplicated results, mean age at testing was 40.93 years (SD of 19.66 years). Where gender data was available, 10,607 (44%) of these patients were female and 13,427 (56%) were male. The mean age for those with discordant testing was 47.08 years (SD of 21.32 years) which was significantly different (t test p < 0.05) to the age of the total 31,311 cohort.

Of the 31,311 non-duplicated Ia1 patient test results, 2214 (7%) patients had at least one positive Ia1 assay. The population HCV seroprevalence based on positive Ia1 and Ia2 testing was 6.6% (2063 of 31311) (Fig. 2). The Ia2 results for the 2214 patients were discordant from the Ia1 result in 6.1% (136 of 2214) (Fig. 2). Following these discordant tests, 93 (68%) patients had no identified follow-up serology, although 13% of these were RNA negative at the time or within one month of the discordant result. Thus, 81 patients (60% of those with discordant serotesting) had neither follow-up serology, nor parallel HCV RNA testing identified from this database. Of these 136 patients with discordant serotesting, 35 had at least one further discordant result on follow-up. Negative follow-up serology was demonstrated for 8 of these 136 patients. No cases of discordant serotesting were subsequently found at follow-up to display definitive HCV seropositivity.

Equivocal Ia1 screening results occurred in 66 of 31,311 results (0.2% of all non-duplicated patient Ia1 results). A negative Ia2 assay was demonstrated for 34 of these 66 patients (52%). No definitive HCV infection was demonstrated for the 11 of these 34 patients with available follow up serology. This testing occurred 1–7 months after initial testing.

General referral laboratory database prevalence of discordant HCV serotesting for all non-duplicated Ia1 results was 0.54% (170 of 31311). This rate was applied to the 337 Australian deceased organ donors for the year of 2011 (nationwide DOD HCV seroprevalence of 2%, although different immunoassays are used across Australian states), using the New South Wales DOD BVV testing record of a 2.6% HCV seroprevalence over the 19 months until April 2011. This modelling estimated a rate of discordant testing in the DOD population in Australia of 1.8 per year.

On review of tests from January 2000 onwards, of the 170 patients with discordant results post May 2008, 24 had been previously tested, and 2 of these were identified to have prior definite hepatitis C (Table 2). One of these was co-infected with HIV, and both had evident BBV clinical risk factors noted from the recorded clinic of testing.

5. Discussion

In this Australian subpopulation with 6.6% HCV seroprevalence, a 0.54% prevalence of discordant HCV serology testing was demonstrated. Where a fourth generation immunoassay was used as the secondary screening test, no cases of discordant serotesting demonstrated concurrent NAT assay positivity, or HCV infection on follow-up. On review of discordant serology cases, two cases with BBV clinical risk factors were identified to have occurred following past definitive seropositive, RNA negative HCV. This most likely represents partial seroreversion following HCV infection, a phenomenon described on follow-up of resolved HCV infection such as perinatally acquired HCV. Complete seroreversion (that is seropositive serology becoming seronegative on multiple assays) has been described with immunosuppression associated with HIV, haemodialysis, post allograft transplant, and on

### Table 1

<table>
<thead>
<tr>
<th>Study nomenclature</th>
<th>Definitions</th>
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<tbody>
<tr>
<td>Ia1: Primary immunoassay</td>
<td>Anti-HCV chemiluminescent microparticle immunoassay (Abbott Architect),</td>
</tr>
<tr>
<td>Ia2: Secondary immunoassay</td>
<td>Bio-Rad Microlisa HCV Ag/Ab Ultra assay</td>
</tr>
<tr>
<td>Positive serology</td>
<td>Positive Ia1 AND Ia2 (sample-to-cut-off ratios &gt; +1.1)</td>
</tr>
<tr>
<td>Negative serology</td>
<td>Negative Ia1 (and Negative Ia2 if performed due to particular clinical circumstance) (sample-to-cut-off ratios &lt; 0.9)</td>
</tr>
<tr>
<td>Equivocal immunoassay</td>
<td>Sample-to-cut-off ratio of 0.9–1.09</td>
</tr>
<tr>
<td>Discordant serology</td>
<td>Positive or equivocal Ia1 with negative Ia2</td>
</tr>
<tr>
<td>Definite HCV infection</td>
<td>Positive serology or positive nucleic acid test</td>
</tr>
<tr>
<td>False reactive testing</td>
<td>Discordant serology where follow-up repeat serology remained discordant or became negative</td>
</tr>
<tr>
<td>Seroreversion</td>
<td>Negative serology results following past definite HCV infection</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Test</th>
<th>CMIA</th>
<th>HCV Ag/Ab assay</th>
<th>RNA</th>
<th>Test 2 (months post test 1)</th>
<th>CMIA</th>
<th>HCV Ag/Ab assay</th>
<th>RNA</th>
<th>Test 3 (months post test 2)</th>
<th>CMIA</th>
<th>HCV Ag/Ab assay</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Time 0</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>12 months</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>11 months</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Time 0</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>8 months</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>3 months</td>
<td>Equiv</td>
<td>–</td>
</tr>
</tbody>
</table>

CMIA, primary screening HCV immunoassay (Abbott Architect chemiluminescent microparticle immunoassay); HCV Ag/Ab assay, secondary screening HCV assay (Microlisa Bio-Rad HCV Ag/Ab Ultra assay); + positive; - negative; equiv, equivocal; ND, not done.
follow-up of successfully treated infection. Thus both serodiscordant and seronegative testing can represent false negative testing with respect to recognition of HCV exposure. In the general referral laboratory, serodiscordant results are currently interpreted according to Australian hepatitis C testing policy as non-specifically reactive, or reflective of recent or distant past HCV infection, with repeat testing recommended.

Applying the discordant serotesting prevalence results calculated in this study to the DOD population, it would be expected that at least one case of discordant serotesting would occur in Australia per year. Serodiscordant cases in the absence of BBV clinical risk factors are likely to be non-infectious given our findings of lack of concurrent RNA positivity or follow-up definite HCV infection, which is consistent with published follow-up of blood donor populations. The ability of Australian BBV DOD testing services to provide prospective parallel NAT testing of samples is critical in reducing infectious risk, particularly as the influences that may cause false negative serology and NAT assays differ. In addition, use of the NAT assay may reduce HCV transmission risk from DOD in those with partial seroreversion. Risk of infection however, can continue even with a negative NAT assay, with one published report of possible HCV transmission from an HCV seropositive, RNA negative source. All other cases of documented transmission were subsequently identified to have been associated with an HCV RNA positive donor. Future application of highly sensitive PCR methods, including whole blood screening to detect peripheral blood mononuclear cell HCV reservoirs may even further clarify potential infectious state of donor organs. Liver transplants from seropositive DOD currently are at relatively higher risk of HCV transmission. This is due to occult HCV infection, defined as the presence of HCV-RNA in the liver in the presence of negative results for anti-HCV, and negative serum HCV-RNA tested using conventional assays. However, there continues to be a need to balance risk of infection with donor organ need at the community and individual patient level.

At present, positive donor HCV serology in Australia mandates that these organs be considered only for HCV seropositive, RNA positive recipients following specific informed consent (Nested Fig. 1a). Studies to date demonstrate that compared with seronegative organ donations, these recipients have no difference in graft or infection associated outcomes, even where transmission of donor HCV strain is documented. Such potential recipients remain difficult to identify in practice, resulting in extremely small numbers of such recipients to date in Australia. It is likely that future direct-acting antiviral drug regimens, with the potential for higher sustained virologic response rates (reviewed in ), will further diminish these potential recipient numbers. From 2006 to 2010, there were two occasions in Australia where intended HCV seropositive donors could not proceed to donation due to inability to identify any suitable HCV positive recipients. In 2010, an Australian registry was established to facilitate identification and equitable allocation of kidneys from HCV positive donors, although in November 2011 (over a year after being established) there were no potential recipients listed. Therefore at that time in Australia, both an HCV seropositive kidney, and a serodiscordant tested kidney considered in the seropositive category would have been discarded. For the organ from a serodiscordant DOD, our study has demonstrated a potential for very low residual infection transmission risk, especially where concurrent NAT is negative. There is also likelihood that future application of highly sensitive PCR methods will further minimise residual risk to recipients of such organs.

We acknowledge that this retrospective study is limited by the de-identified cohort that has been used. Therefore, demographic description, including identification of full BBV risk status analogous to that used in deceased donor clinical risk screening, is not possible. There is also limitation due to incomplete patient follow-up and lack of parallel, or follow-up, NAT testing for all serodiscordant samples. This is despite result interpretation for the referral laboratory recommending that referring practitioners arrange follow-up testing. Nonetheless, this large cohort with
relative high BBV risk (as defined by a 6.6% HCV seroprevalence), has provided a key opportunity to assess the potential for definite infection with discordant serostenting. As distinct to publications describing discordant serostenting of blood donors, the study presented herein provides a worst-case scenario population for modelling potential infectivity of serodiscordant samples with reference to the critical issue of deceased donor transplantation risk.7,8 Direct application for other laboratories and DOD programmes of the findings reported herein should be dependent on the combination of immunoassays used for DOD HCV testing. In particular it is critical to link the sensitivity of the second immunoassay to risk of definite infection in serodiscordant samples.

6. Conclusions

This study aimed to provide data for further discussion on the use of organs for transplant that might otherwise be discarded due to potential HCV infection risk. The data has demonstrated that all HCV serodiscordant tested samples tested in a general referral laboratory in NSW Australia, where follow-up was available, were consistent with false reactive testing, apart from two cases that had demonstrable prior definite HCV infection with evident BBV clinical risk factors. Thus, if an organ from an HCV serodiscordant DOD was otherwise to be discarded, we propose that this donor be urgently tested using NAT assay, and if NAT assay negative, the organ offered to a waitlisted patient accepting of a negligible but unquantified potential residual risk. Such discussion with potential recipients could be incorporated into preclinical clinical dialogue, in order that the patient has adequate notice of such an eventuality, and can make an informed decision.

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No funding was obtained for this study.

Competing interests

None declared.

Ethical approval

Ethics approval was not required for this study due to the de-identified nature of all accessed data.

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