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Research Article

# Transmitted Donor Immunology Not Infection: Common Persistence of Donor Hepatitis C Antibody Production in Aviremic Lung Transplant Recipients

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**Abstract**

Since 2018 The American Society of Transplant has recommended that Hepatitis C Virus seropositive positive, non-viremic donors (HCVAb+/NAT-) be considered non-infectious and safe for transplantation. This report describes clinical outcomes and HCV serological and virological outcomes following lung transplantation (LTx) utilizing such donors. This retrospective cohort study describes seven HCVAb+/NAT- donors used for bilateral LTx. Donor information was sourced from the national organ donation service and recipient information from the institutional LTx database. Seven deceased donors (three female, median age 53,

range 37-63 years) acquired HCV from injecting drug use (n=4), blood products (n=1), unknown (n=1). Four donors had been previously treated and cleared of HCV (sustained virological response at 24 weeks) before death. Seven recipients (2 female, median age 56, range 51-71 years), with pulmonary fibrosis (n=4), re-do LTx (n=3), were waitlisted a median of 87 days (range 8-362). At post-LTx follow up, six of seven are alive with excellent graft function at a median 1625 days (range 779-2582). No patient developed clinical, biochemical or virological evidence of HCV infection, however five recipients demonstrated persistent HCVAb+ (all consistently NAT-) for a median 414 days (range 332-872) post-LTx. Two patients HCVAb sero-reverted at d731 and d1461. Two other recipients remained HCVAb- at d1621 and d1398 on repeat testing. There was no evidence of HCV transmission to seven LTx recipients from HCVAb+/NAT- life-saving lung donors, however persistent HCV Ab positivity was seen in 5 LTx recipients with two demonstrating remote sero-reversion. We postulate this may be a form of Passenger Lymphocyte Syndrome.

**Keywords**
Lung transplant; Hepatitis C; donor-derived infection

## 1. Introduction

As demand for lung transplantation (LTx) has increased, consideration has been given to expanding the pool of potential donors as a strategy to avoid a high waiting-list mortality rate. Clinical evidence now exists to show that the use of these so-called 'extended (ie non-ideal) criteria' donors does not compromise transplant outcomes [1, 2].

Reflecting this, on a case-by-case basis from December 2013, our institution began transplanting lungs from Hepatitis C virus antibody positive and nucleic acid test negative (HCVAb+/NAT-) lung donors. Our experience, as outlined below, confirmed patient safety in terms of infectious outcomes, despite our recipients themselves also becoming HCVAb+/NAT-. Parallel work by ourselves and others raise the possibility the observed results may be a version of the Passenger Lymphocyte Syndrome (PLS); rather than an actual recipient infection [3-5]. The PLS is an immunological phenomenon defined by the transmission to the recipient of viable donor B lymphocytes. Classically these B cells have been shown to subsequently produce antibodies against ABO antigens on recipient red blood cells, leading to a self-limiting hemolytic anaemia [6], however other examples have been recently identified [3-5].

In 2018 the American Society of Transplant (AST) began formally recommending that (HCVAb+/NAT-) donors be considered non-infectious and therefore safe for clinical transplantation [7]. In 2020 the International Society of Heart and Lung Transplant (ISHLT) has taken this one step further and recommended that HCV NAT positive donors be considered safe, in the context of informed consent and with the proven efficacy of potent, effective and well tolerated direct acting antivirals [7, 8]. These statements focus completely on the simple assumption that the donor organ can transfer live virus to the recipient and don't consider the potential of the transfer of passenger cells or molecules that might mimic infection. The current study aims to detail our LTx clinical

experience with HCVAb+/NAT- donors, with the unique consideration of infectious and non-infectious transfer of donor material to the recipient.

## 2. Methods

This retrospective observational review was conducted at the Alfred Hospital Melbourne, Australia. The Alfred has performed over 1700 LTx and currently undertakes approximately 100 transplants per year. Study participants were identified from the institutional LTx database. From December 2013, with the availability of high throughput HCV NAT testing, our transplant service began considering otherwise good quality lung donation from HCVAb+ and NAT- donors for individuals who were critically ill and able to give informed consent as to the use of a perceived 'increased-risk' donor. Recipients were greater than 18 years of age and each considered extremely high risk for waitlist mortality in the absence of imminent transplant. Recipients provided specific consent to receive HCVab+/NAT- donor lungs. Specific consent was obtained at the time of transplant by a lung transplant specialist. The general principles of viral transmission risk and the use of extended organs had also been a key part of the written consent obtained at the time of listing for LTx. Ethics approval was granted by the Human Research Ethics Committee of Alfred Health and was given on the basis of an audit of existing clinical practice.

Clinical and epidemiological data from recipients were obtained from the Hospital's electronic medical records, whilst that of donors were collected from the DonateLife (the national organ donation service) Electronic Data Registry. Patient characteristics and outcomes were analyzed using descriptive statistics using median and range.

Donors underwent HCVAb and NAT testing as per local protocols set out by DonateLife, whilst recipients underwent pre-LTx HCVAb testing as part of a general assessment for LTx suitability, repeated routinely on the day of LTx. Donor HCV testing was performed on the Abbott Architect (Abbott, Weisbaden, Germany, B6C730 Nov 14). Reactive results underwent further testing using the Liaison XL Murex HCV Ab kit (Diasorin, Via Crescentino snc – 13040 Saluggia – Italy, 200/007-927,05 – 2016-09) before results were released to DonateLife. HCV NAT testing was performed at the Australian Red Cross Blood Service using the Procleix Tigris system (Grifols Diagnostic Solutions Inc. Emeryville, CA) on a fully automated NAT platform for blood screening (Procleix Tigris system, Grifols Diagnostic Solutions Inc.) [9].

HCVAb screening on recipients was performed in the Alfred Hospital microbiology laboratory using Abbott Diagnostics Architect Anti-HCV Assay for the HCVAb. In the case of an HCVAb+ assay, sera was spun at 10,000g and repeat tested in duplicate. If 2/3 tests have a serum/cutoff (S/Co) ratio $\geq$ 5.0 they were further tested on the DiaSorin Liaison XL using the LIAISON XL murex HCVAb assay. Sera that have 2/3 S/Co ratios <5.0 were referred to the Victorian Infectious Diseases Reference Laboratory for confirmatory NAT testing with Murex manual HCV and Roche Anti-HCV assays.

Donor/recipient matching and recipient general and immunosuppressant management were along standard institutional protocols for all LTx recipients and have previously been described [2, 10, 11]. Briefly, immunosuppression included tacrolimus (initial target 8-10 ng/ml), azathioprine (initial target 1.5-1.0mg/kg and corticosteroids (initial target 0.8-0.25 mg/kg). All patients had indefinite transplant center follow-up, with a maximum of 3 monthly appointments, blood work (including liver function tests) and spirometry. Definitions of Primary Graft Dysfunction (PGD) and

Chronic Lung Allograft Dysfunction (CLAD) were as per ISHLT Guidelines.

## 3. Results

Since December 2013 seven HCV seronegative LTx recipients received lungs from HCVAb+/NAT- donors. Donor characteristics, donor HCV risk factors and prior therapy are detailed in Table 1. Recipient features and outcomes are detailed in Table 2.

**Table 1** HCVAb positive donor details.

| Case Number | Age | Sex | Lungs | Kidney | Heart | Liver | Donor type | ALT | Bilirubin | Death Category | ? Risk category | ? Known HCV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 53 | M | 2 | 1 | 1 | 1 | DBD | 21 | 63 | Traumatic brain injury | Travelled | no |
| 2 | 46 | F | 2 | 0 | 0 | 0 | DCD | 4213 | 42 | Cerebral ischemia | IVDU ex 20yrs | no |
| 3 | 63 | M | 2 | 2 | 0 | 0 | DBD | 55 | 16 | Intracranial hemorrhage | IVDU ex 40yrs | yes, HCVAb 2006 |
| 4 | 53 | M | 2 | 0 | 0 | 0 | DCD | 122 | 5 | Cerebral ischemia | Asian born | yes, HCVAb 2007, treated 2012 |
| 5 | 62 | F | 2 | 0 | 0 | 0 | DCD | 17 | 4 | Intracranial hemorrhage | Blood 1990s | yes HCVAb ~ 2000 |
| 6 | 37 | F | 2 | 0 | 0 | 1 | DBD | 295 | 3 | Cerebral ischemia | IVDU current | yes HCVAb 2006, treated 2015 |
| 7 | 29 | M | 2 | 0 | 0 | 1 | DBD | 26 | 8 | Cerebral ischemia | IVDU recent | no |

M=male, F=female, DBD = Donation after brain death, DCD = donation after circulatory death, IVDU intravenous drug user.

**Table 2** Recipient details of HCVAb donor lungs.

| Case number | age | sex | LTx indication | waitlist days | PGD | Hospital LOS | CLAD | Currently alive | Post-LTx days alive | Cause of death |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 51 | male | ILD | 8 | 1 | 19 | 1 | no | 1831 | Zoster encephalitis |
| 2 | 54 | female | sarcoid | 362 | 2 | 36 | 1 | yes | 1534 | - |
| 3 | 62 | male | reLTx CLAD | 302 | 0 | 15 | 0 | yes | 1520 | - |
| 4 | 64 | male | ILD | 87 | 0 | 18 | 0 | yes | 1951 | - |
| 5 | 56 | female | reLTx CLAD | 197 | 1 | 24 | 0 | yes | 2582 | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 71 | male | ILD | 27 | 1 | 30 | 1 | yes | 1689 | - |
| 7 | 56 | male | reLTx CLAD | 76 | 1 | 22 | 0 | yes | 779 | - |

ILD = interstitial lung disease, reLTx CLAD = repeat lung transplant (LTx) for Chronic Lung Allograft Dysfunction (CLAD).

Recipients were on the waiting-list for a median of 87 days (range 8-362) prior to LTx. All were clinically deteriorating due to progression of their underlying disease, with a high likelihood of waitlist mortality. Six of the seven recipients are currently alive a median of 1689 days (range 779-2582) post-LTx, living independently with excellent graft function. No case had significant PGD and no treatment for acute rejection has been required. 3 cases have early CLAD (stage 1). One recipient died day 1831 post-transplant secondary to cerebral herpes zoster infection unrelated to HCV (Table 2).

Recipients were all tested and confirmed to be HCVAb negative on 2 separate occasions immediately pre-LTx. No recipient demonstrated clinical or biochemical evidence of hepatitis post-LTx (defined as any ALT abnormality) nor were there any unexplained clinical infective syndromes. As these transplants were not undertaken as part of a protocolized trial, recipients underwent post-LTx HCV testing at the discretion of treating clinicians at time points described in Figure 1.

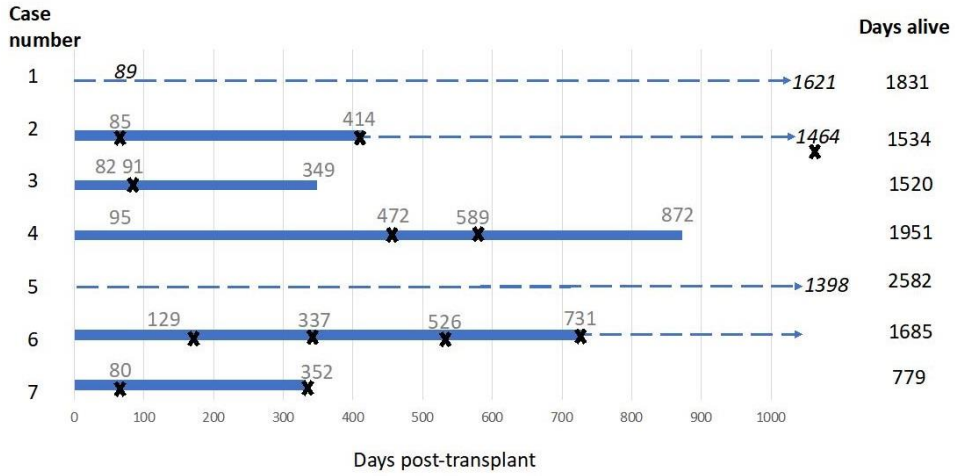


**Figure 1** A timeline of HCVAb and HCV NAT results in the 7 cases post-LTx. The solid line indicates positive serology with the testing days post-LTx for positive cases marked in grey italics above the line. Cases where serology was always HCVAb negative or changed to become negative are marked in black without italics. The crosses indicate the times where HCV NAT results were noted- all were negative at every measurement.

Five recipients demonstrated HCVAb positivity without viremia (all were HCV NAT- at all timepoints). Seropositivity has persisted a median 414 days (range 349-872) post-LTx. Two patients (recipient 2 and 6) have HCV sero-reverted at d731 and d1461 after multiple positive tests (Figure 1). Two recipients (1 and 5) had no HCVAb positivity. The donor for recipient 1 also donated a heart and a single kidney also without the recipients becoming either HCVAb or HCV NAT positive. Importantly, by contrast, the donor for recipient 3 donated 2 kidneys at a separate institution and mirroring our experience, both recipients also developed HCVAb positivity without viremia.

## 4. Discussion

We report on a series of successful LTx outcomes from HCVAb+/ NAT- donors. We have observed long-lasting HCV seroconversion (persisting > 1year after transplant) in 5 of 7 cases without evidence of viremia or allograft dysfunction. There is no clinical evidence of HCV transmission in this donor group, in keeping with observations made in other recent series, who also consider the apparent

donor-derived humoral transfer of HCV immunity [12-15]. Interestingly, two of the five patients with persistent seropositivity demonstrate late sero-reversion at 2- and 4-years post-LTx.

Rates of seroconversion from HCV seropositive, non-viremic donors vary between 14-44% [12-14]. Patel et al described 14 recipients of HCVAb+/NAT- heart transplant, 3 of which were found to HCV seroconvert after transplant [14]. While none had detected viremia, all had persistent but declining antibody titres and eventually sero-reverted by day 200, 256 and 139 respectively. Argarwar et al. described 2 kidney recipients who received a kidney each from the same HCVAb+/NAT- donor [12]. Both recipients went on to HCV seroconvert with persistent antibody detectable for over 300 days without documentation of either viral transmission or biochemical hepatitis. We also observed donor-derived HCV seropositivity transmitted from a donor and transmitted to our LTx Case 3 and the both kidney recipients. With several series now suggesting a single donor can seemingly transmit seropositivity to multiple recipients this is strongly supportive of a donor-derived origin.

Recognizing a lack of immediate post-transplant HCV testing in our series, we cannot definitely exclude early viremia with HCV clearance as a theoretical biological explanation for HCV seropositivity. However, donor derived HCV is typically associated with early and persistent viremia and accelerated HCV-related hepatic disease in the recipient [16]. Cases of spontaneous viral clearance and seroconversion have been reported, but are exceptional, occurring many years after transplant and in association with a reduction in immunosuppression [16-18]. In a series of kidney transplants from HCVAb+/NAT- donors described by de Vera et al., HCV NAT remained negative in all cases at 30 days post-transplant while 14 (44%) HCV had seroconverted [13]. In contrast, in a trial utilizing HCV NAT+ donors, Porrett et al. demonstrated HCVAb+ of the IgG subtype (ie not IgM) within days of transplant in 45% of clinically uninfected recipients. The common persistence of kidney recipient HCV positivity past 30 days, with 2 heart transplant recipients past 100 days (ie beyond the half-life of IgG itself) led the authors to speculate that HCV immunity (likely via a cellular source) is more commonly transferred from donor to recipient than previously appreciated.

The concept of the transfer of cells, cell fractions and molecules is increasingly recognized as a common feature of organ transplantation [3-5, 19]. PLS is seen as an active form of this and has been seen as donor B cell production of antibodies against recipient antigens, typically against ABO antigens on red blood cells, leading to hemolytic anemia [6]. This situation is reported to have an incidence of upwards of 70% in thoracic transplants and can persist out beyond 6 months post-LTx [20]. In related work, our team has shown the transfer of long-lasting donor cells producing third-party anti-HLA antibodies to all of 6 organ recipients. In this study the extent of transfer in proportion to the mass of associated lymphoid tissue in each organ- with the lung having the greatest lymphoid load [3]. Koshizuka et al. have also shown that donor-derived plasma cells transmitted in a lung allograft are capable of producing long lasting anti-cytomegalovirus antibodies [5]. Rezahosseini et al. have extended this concept further with a recent paper noting that at 1 year post organ transplant 57% of seronegative recipients show seroconversion for at least one of measles, mumps or rubella viruses without clinical infection [19]. Put together these PLS case series make it quite likely that the current study is yet another PLS example- and does not represent recipient HCV infection perse.

The study findings may have important f*uture study implications* beyond redefining what constitutes a useable HCVAb positive lung donor. Firstly, if alloimmunization can indeed be induced by intra-graft lymphoid tissue or passive transfer of viable donor memory B cells, then this might

provide a mechanism to achieve specific recipient immunization. Secondly, this phenomenon may explain the recognition of other seemingly unexplained non-specific antibodies (eg, non-donor specific anti-HLA antibodies) in the serum of a recipient early post-solid organ transplant [21]. Thirdly, it may explain the lack of a clinically robust recipient immune response in specific post-transplant at-risk populations eg CMV or EBV donor-positive/recipient-negative mismatches, where on occasion significant disease occurs in the setting of apparent immunity.

A limitation on the strength of the current study conclusions is its small numbers and the lack of specific HCV NAT testing before 90 days post-LTx, as this would have allowed for absolute exclusion of HCV viremia as an explanation for the HCV seropositivity.

In summary, the cases described in this report do not represent viral transmission and clearance, but rather, an as yet incompletely characterized immunological phenomenon, most likely representing long acting donor cells continuing to produce donor HCVAb in the recipient sera or bone marrow. Molecular phenotyping, targeting the kinetics of donor-derived cells and antibodies in the recipient circulation needs to be undertaken in order to characterize the mechanisms leading to this persistent seropositivity.

## Author Contributions

Olivia C Smibert wrote the manuscript with input from all authors, with particular support from Greg I Snell.

Joseph S Doyle, Adam WJ Jenney, David Pilcher, Miranda A Paraskeva and Glen P Westall provided critical feedback and helped shape the research, analysis and final version of the manuscript.

Greg I Snell conceived of the presented project, encouraged and supervised the presentation of findings made in the manuscript made by Olivia C Smibert, and was in charge of overall direction and planning of the project.

## Competing Interests

The authors have declared that no competing interests exist.

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