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IRB Protocol Submission

The Predictive Value of Hepatitis B Core Antibody for Occult Hepatitis B Infection in Transplant Donors

A. Study Purpose and Rationale

Over the past several years, there has been a dramatic increase in orthotopic liver transplant (OLT) activity due to an increased eligibility of donors and the use of "marginal grafts," obtained from older or hepatitis B virus (HBV) infected donors for use in emergencies. The donor pool has grown to include those who are tested Hepatitis B core antibody (anti-HBc) positive (3.8-12.3% prevalence, but may exceed 50% in endemic areas).[2,3,8] While historically like the clearance of Hepatitis B surface antigen (HBsAg) and development of Hepatitis B surface antibody (anti-HBs), the presence of anti-HBc alone and absence of HBsAg was thought to be a marker for cleared or resolved infection, more recent research has shown otherwise.

Anti-HBc+ donors were identified as a potential source of de novo HBV infection (defined as development of HBsAg+ serum) in recipients when transmission continued despite HBsAg screening of blood and organ donors. Reduced but active viremia existed at similar levels in those with anti-HBs and anti-HBc versus anti-HBc alone, suggesting HBsAg negative patients retained low infectivity.[6] HBcAg is most immunogenic component of HBV during infection and can function as cell-independent antigen, resulting in anti-HBc IgM later evolving to IgG and persisting with slowly decreasing titers. Anti-HBc has therefore been noted to be a cost-effective, reliable serological marker of HBV infection.[7]

HBV transmission continued to occur at high rates among anti-HBc+ donors occurs despite clinical and serologic recovery, suggesting latent hepatitis B infection. Prior to discovery of this *de novo* infection of HBV from anti-HBc+ donors in liver transplant recipients (without previous HBV exposure), a similar phenomenon was noted in patients with malignancy who were subjected to immunosuppression. Low levels of HBV DNA in liver and peripheral blood mononuclear cells were demonstrated. However, serum HBV DNA is often not detected in recipients with de novo HBV infections from anti-HBc+ donors. Tissue HBV DNA has only been isolated in very few anti-HBc+ recipients, all of whom have been tissue HBV-DNA positive. This latent tissue DNA, more precisely described as covalently closed circular DNA (cccDNA) in the extrachromosomal space of the hepatocyte nuclei that leads to de novo HBV infection especially among immunosuppressed patients.[7,9] This cccDNA appears to persist and may not shed viral particles, resulting in serum HBV DNA detection in up to 10% in many anti-HBc+ populations.[7] Transmission of de novo HBV infection via graft to recipient was confirmed by comparing the cccDNA sequence in graft tissue to the infected recipient serum. This was done initially using the "s" region of cccDNA[10] and later by comparing entire genome sequence homology.[9]

Given that reported risk of transmission in blood for anti-HBc+, HBsAg- donors ranged from 2.1%-8.6%, routine screening of anti-HBc was mandated federally by 1991 for blood units. Various studies have demonstrated up to 78-94% rate of de novo HBV infections in anti-HBc+ graft recipients.[3,8,10] In 1997, Dickson, et al[3] demonstrated that among 1109 donors in 4 US transplant centers, 18 of 23 recipients (78%) who were anti-HBc+ and HBsAg- developed de novo infection. Of 21 patients who developed de novo HBV infection, 18 (86%) were attributable to anti-HBc+ donors (others thought to be externally acquired). Serum HBV DNA was identified in only 1 of 7 newly infected recipients tested.

Similarly, among 222 transplanted patients in Japan without previous HBV exposure studied retrospectively b1etween 1990-1995, 16 patients received anti-HBc+ grafts, 15 (93.8%) of whom developed de novo HBV infection confirmed by post-transplant detection of HBsAg+.[10] None of the newly infected recipients had serum HBV DNA detected, but 2 patients who were tested for tissue HBV DNA were found to be positive. However, not all anti-HBc+ graft recipients had tissue DNA testing performed. An additional three anti-HBc+ graft recipients in a prospective arm of the study were found to be serum HBV DNA negative and tissue DNA positive as well with no subsequent de novo infection after passive immunization with intravenous hyperimmune hepatitis B immunoglobulin (HBIG). The only other study where tissue DNA was isolated was Rokuhara, et al[9] where two anti-HBc+ donor specimens were found to be cccDNA+, but only one went on to develop de novo HBV infection off prophylaxis (the other received prophylaxis). Two other anti-HBc+ graft recipients in that study also developed de novo infections, but only one had cccDNA detected in tissue, while the other two recipients had anti-HBc prior to transplant. All the above studies did not isolate DNA from tissue from anti-HBc+ grafts in those without de novo infection.

Donor screening for HBV infection consists only of anti-HBc and HBsAg at the moment. Not all institutions (approximately 31%) transplant grafts from anti-HBc+ donors into naïve patients.[1] Current standard of care at our institution involves providing all recipients of grafts from anti-HBc+ donors with post-transplant antiviral prophylaxis in the form of a nucleoside/nucleotide analogue. This is done for all patients, even though some relation between transmission risk and vaccination status among some recipients has been noted.[1]

As noted in the aforementioned studies, Prieto, et al (2001)[8] also found 15 of 30 anti-HBc+ graft recipients without subsequent HBV de novo infection. The real question is why these immunosuppressed patients did not develop de novo HBV infection despite latent infection. Without tissue DNA, one cannot determine if the positive anti-HBc was truly indicative of latent infection. As a result, our hypothesis is that some patients who are found to be transplanted with anti-HBc+ grafts are actually cccDNA-free and therefore unnecessarily receiving antiviral prophylaxis. There have been no studies characterizing the predictive value of true latent HBV infection among anti-HBc+ donors using tissue HBV-DNA (cccDNA) as confirmation of such occult infection. In other words, the anti-HBc serology used to screen these patients is conferring false positive results. Potential causes of false positive anti-HBc proposed include non-specific reactions associated with competitive anti-HBc enzyme immunoassays (EIAs) and cross-reactivity with interfering serum substances or molecules produced from nonspecific

HBV-activated B-lymphocytes, or perhaps as a result of HBV vaccination in rare cases.[5,7] Various adjustments have been made to assays to reduce cross-reactivity.

If significant false positivity is identified in the anti-HBc assay, there may be treatment and screening-related implications in addition to further understanding transmission patterns of HBV. Unnecessary use of medications can lead to unwanted adverse effects of the medication including a variety of infectious, neuropathic, hematologic, and gastrointestinal side effects with nucleoside analogues, and hypertriglyceridemia and CNS side effects with nucleotide analogues. Unnecessary treatment also entails improper allocation of resources and additional cost. Implentation of cccDNA as a way to streamline treatment would be a next step.

B. Study Design and Statistical Analysis

A retrospective review will be conducted by identifying orthotopic liver transplant donors of all ages found to be anti-HBc+ on routine screening at CUMC. Among anti-HBc+ donors, those with other serum HBV markers indicative of active infection (HBsAg+, HBeAg+) would be excluded from the study. Our primary endpoint is defined as false positive anti-HBc, defined as anti-HBc+ donors with no HBV-DNA isolated from tissue.

Given our single-armed setup, a hypothesized 10% false positive rate of the anti-HBc assay and a fixed 5% occurrence of false positivity are used to define an impact on screening practices. The effect size is therefore 5% or 0.05. A Chi-squared test used to calculate the sample size to demonstrate 80% power at $p < 0.05$ ($\alpha = 0.05$ chance of committing Type I error, or 95% confidence) would yield a necessary sample size of 14. A study powered at 80% would confer a β of 0.20, or 20% chance of committing a Type 2 error. Given the clinical ramifications of false positivity for transplant recipients, one would assume anything above 0% occurrence to be clinically important, but given the theoretical possibility of false negative detection of DNA by PCR, a 5% occurrence was employed. Due to comparison within one group with one proportion being fixed at 5% to define impact, the sample size can be halved from 28 to 14 for adequate power. Therefore, two patients with false positive anti-HBc (as demonstrated by anti-HBc(+) and HBV DNA negative) would be necessary to demonstrate >10% false positivity among anti-HBc+ donors and reject the null hypothesis.

C. Study Procedure

Frozen donor specimens from these anti-HBc positive donors would have tissue purified of their DNA for detection using polymerase chain reaction (PCR). Based on existing research, those with cccDNA (isolated from tissue) were concomitantly anti-HBc+ 100% of the time, whereas serum DNA was often not detected in cases of "latent" HBV infection in anti-HBc+ donors.

The number of "false positive" specimens without cccDNA isolated would be compiled. Given that our study is powered to detect 10% false positive rate, 2 specimens of 14 enrolled anti-HBc+ grafts would have to yield no cccDNA.

The duration of the study, will depend solely upon locating the frozen donor specimens and running PCR on all specimens.

No informed consent will be necessary as the intervention relevant to the study was performed previously, and no patients will be harmed as a result of the study of graft specimens.

D. *Study Drugs*

No drugs will be employed in this study.

E. *Medical Devices*

No medical devices will be employed in this study.

F. *Study Questionnaires*

No study questionnaires will be employed in this study.

G. *Study Subjects*

Inclusion criteria: donors of all ages, anti-HBc positive donors at CUMC.

Exclusion criteria: donors who demonstrate positive serology for active infection or recently resolving active infection (HBsAg+, HBeAg+, anti-HBe+).

These criteria are meant to mirror the usual donor screening procedures.

H. *Recruitment of Subjects*

Given that this is a retrospective review, graft specimens and donor serologies and characteristics were compiled in a database at the time of transplantation and no active recruitment will take place.

I. *Confidentiality of Study*

Data previously collected regard patient and donor characteristics are stored in a secure database with patients coded and without identifying information. The database is stored in a computer that can only be accessed only by investigators in the hospital.

J. *Potential Conflicts of Interest*

There are no conflicts of interest to report.

K. *Location of the Study*

This is a single-center study at CUMC, and all data review and laboratory testing of specimens will be conducted on CUMC premises under the auspices of the Department of Digestive and Liver Diseases.

L. *Potential Risks*

There are no risks to patient or donor as graft specimens will be analyzed separately, and intervention is conducted in neither patient nor donor.

M. *Potential Benefits*

There is no immediate benefit for the patient or donor, but results may impact current post-transplant patients by altering the duration or need for antiviral treatment in those found to have false positive anti-HBc. In addition, this may benefit future transplant recipients by perhaps identifying a potential need for confirmatory cccDNA testing. This

may benefit society by reducing those suffering from side effects of antiviral treatment unnecessarily. A reduction in unnecessary treatment may also translate to a way to prevent excess spending of healthcare resources.

N. *Alternative Therapies*

No experimental therapies are employed in this study.

O. *Compensation to subjects*

There will be no compensation for subjects enrolled in this retrospective study.

P. *Cost to subjects*

No additional cost will be incurred by the donors whose specimens are studied.

Q. *Minors as research subjects*

Minors will not be directly involved in the study. Minors who are liver transplant recipients will be identified solely for the purposes of identifying donor information and locating donor specimens.

R. *Radiation*

No radiation or radioactive substances will be utilized in this study.

S. *Next steps*

Firstly, as previously mentioned, many patients may be unnecessarily receiving antiviral prophylaxis should the results of this study demonstrate a significant number of false-positive anti-HBc. Patients found to be cccDNA negative can further be randomized to continuing antiviral treatment versus discontinuing treatment. A comparison of de novo infection in these settings would then be done. Other comparisons would include rates of adverse effects as well as HBV-related and all cause mortality.

Secondly, it may seem improbable to conduct routine screening of all donor grafts, but screening all donor grafts may confer added benefit to transplant patients to confirm the presence of HBV DNA in tissue.

Thirdly, one could investigate whether certain donors are prone to false positive anti-HBc screening (such as those with other viral co-infections, age, other co-morbidities). This can be done by a more thorough analysis of the characteristics of these donors' histories and biochemical profiles.

References

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