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ORIGINAL ARTICLE

Utilization of hepatitis C antibody-positive livers: genotype dominance is virally determined

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Summary

Because of the unrelenting donor shortage, utilization of all potential liver donors is essential. However, when utilizing marginal donors it is critical to precisely characterize the risks, inform recipients of those risks, and allocate these higher risk organs to appropriate candidates. Towards this goal, we need to determine the safety and potential consequences, if any, of utilizing hepatitis C (HCV) antibody-positive donors in HCV infected recipients. To further characterize HCV antibody-positive donors, we analyzed prospectively collected serum samples from HCV antibody-positive donors transplanted into HCV RNA-positive recipients from 5/1993 to 10/2008 for HCV viral load (Roche Cobas AmpliPrep/Cobas Taqman HCV Assay) and genotype (Siemens Versant 2.0 LiPA HCV 5' UTR/Core Assay). Seventeen of 32 (53%) HCV antibody-positive donors were RNA negative. Fifteen patients received an HCV RNA-positive donor and nine donor–recipient pairs had different genotypes or subtypes for analysis. When genotype 1 competed with a non-1 genotype, it was found in 5/6 recipients. In 2/3 cases of mismatched genotype 1 subtypes, genotype 1a dominated. Kaplan–Meier analysis of patient and graft survival and fibrosis progression did not reveal differences between patients who received an HCV antibody-positive donor that was viremic or aviremic. In conclusion, approximately half of HCV antibody-positive donors were aviremic. Viral dominance in viremic donor–recipient pairs seems virally determined.

Introduction

Hepatitis C (HCV) antibody-positive donors have been used to expand the donor pool for HCV-infected transplant candidates for almost two decades. Several previous publications have reported that donor HCV antibody status did not change post-OLT patient or graft survival [1–4]. However, the presence and level of viral replication in antibody-positive donors was only assessed in one of these studies: Ballarian *et al.* previously showed that 57% of their HCV antibody-positive donors were HCV RNA negative [3]. Seroprevalence studies in the United States have shown that approximately 80% of anti-HCV-positive individuals have active viral infection [5], so the observation of Ballarian likely reflects the rigid selection criteria for potential organ donors. However, these earlier finding require confirmation. If a significant number of HCV antibody-positive donors are HCV RNA negative, then previous studies documenting no change in recipient outcomes with HCV antibody-positive donors may have been diluted with a significant portion of noninfectious donors. As a result, it seems prudent to perform another analysis of HCV RNA status of HCV antibody-positive donors.

Not only may HCV RNA status of a donor impact outcome, but their genotype may affect the recipient's chance to eradicate virus with antiviral therapy after transplant as viral genotype and even subtypes within genotypes may affect treatment response [6–8]. Non-1 genotype infected patients have consistently had superior viral eradication

rates (sustained virologic response rates; SVR) compared with genotype 1 infected patients. The recent availability of HCV-specific protease inhibitors (PIs) has dramatically increased SVR in nontransplant patients; however, currently available PIs are only FDA approved for use in genotype 1 infected patients [9–12].

These genotypic differences in SVR rates make it imperative to understand the viral interactions that occur when an HCV viremic patient receives an HCV viremic donor organ. Past publications evaluated whether the donor or recipient genotype dominated after an HCV RNA-positive organ was transplanted into an HCV RNA-positive recipient without consistent findings. Since there was no clear dominance of donor or recipient genotype in previous studies, we hypothesized that viral factors such as genotype and possibly viral load determine dominance instead of the origin (donor or recipient) of the virus.

Methods

The Baylor Simmons Transplant Institute Biorepository was started in 1985, and prospectively collects serum and lymphocytes that are stored at −80 °C of donors and pre-OLT and post-OLT recipients. In addition, we maintain a prospective liver transplant research database, which contains clinical, demographic, protocol biopsy, and event data on all OLT patients since the program's inception. Institutional review board approval was granted prior to the initiation of this evaluation of prospectively gathered material and information.

All patients were included in this evaluation if they underwent a primary OLT without another organ between 5/1993 and 10/2008 if the recipient was HCV RNA positive and received a HCV antibody-positive donor and a donor serum sample, a pre-OLT recipient serum sample, and a post-OLT recipient serum sample were available for analysis. Pre-OLT samples were taken from recipients at the time they were called for surgery. Year 1 post-OLT serum samples were analyzed on all patients except patient 7 whose serum sample was from day 87 post-OLT because a year 1 sample was not available.

Serum was blinded and sent to Medfusion Laboratories for analysis. Serum was analyzed for viral load using Roche Cobas AmpliPrep/Cobas Taqman HCV Assay, which has a lower limit of detection of <43 IU/ml. Genotype was assessed by using Siemens Versant 2.0 LiPA HCV 5' UTR/Core Assay.

The purpose of the study was to determine the percentage of HCV antibody-positive donors with RNA present in serum at OLT. In addition, we wanted to determine the viral characteristics associated with dominance when HCV-infected recipients received an HCV RNA-positive donor.

Kaplan–Meier analysis was performed with log rank testing to determine the difference in graft and patient survival and HCV fibrosis progression. SAS 9.1 was used for statistical analysis with a cutoff for significance of $P < 0.05$.

Results

Thirty-two patients received HCV antibody-positive donors and had serum available for analysis. Donor and recipient characteristics are found in Table 1. Donor livers with a median age of 46 and 7.8 h of cold ischaemia time were transplanted into mostly white male recipients with a median age of 50. All recipients were HCV RNA positive, but 25% also drank alcohol pre-OLT and 31% had hepatocellular carcinoma.

Seventeen of the 32 HCV antibody-positive donors were HCV RNA negative. This indicates an absence of active infection in 53% of donors. Fifteen patients had HCV RNA-positive donors (47%). Six genotype 1a-infected donors were transplanted into genotype 1a-infected recipients leaving nine mismatched genotypes or subtypes for analysis of genotype dominance. The final recipient HCV genotype did not consistently come from either donor ($n = 4$) or recipient ($n = 4$), and in one case resulted in co-infection with both genotypes.

Table 2A shows the three cases where genotype 1a competed with genotype 1b. Genotype 1a became the dominant genotype after transplant in two of three cases; in the case where genotype 1b dominated the viral load was 16-fold higher in the genotype 1b recipient (1 480 000 IU/ml vs 91 800 IU/ml in the genotype 1a donor).

Table 2B shows the six cases where genotype 1 competed with a non-1 genotype. Genotype 1 was found in the recipient in five cases; in four it dominated and in one it co-existed with the other viral genotype. In the one case where a non-1 genotype dominated post-transplant, a genotype 2 infected donor with a viral load of

Table 1. Patient characteristics (median).

Number	32
Recipient age	50
Male sex	75%
Recipient race	
White	82%
Hispanic	9%
AA	9%
HCC	31%
CIT hours	7.8
Donor age	46
Year of OLT	2004

Table 2. Donor and recipient genotypes and viral loads.

	Donor		Recipient			
	Genotype	RNA	Pre-genotype	Pre-RNA	Post-genotype	Post-RNA
A						
1	**1a**	93 300	1b	25 300	1a	10 300 000
2	1b	1 100 000	**1a**	1 040 000	1a	>700 000
3	1a	**91 800**	**1b**	**1 480 000**	1b	18 700 000
B						
4	**1a**	4 140 000	2b	82 400	1a	23 400
5	**1b**	6 630 000	3a	321 000	1b	626 000
6	2b	5 560 000	**1b**	1 730 000	1b	930 000
7	2b	19 700 000	**1b**	20 400	1b	>69 000 000
8	**1b**	12 100 000	**3a**	822 000	1b & 3a	28 400 000
9	**2b**	**8 200 000**	1a	**448**	2	17 200 000

Bold genotypes dominate.

8 200 000 IU/ml was transplanted into a recipient on pegylated interferon and ribavirin with a viral load at transplant of 448 IU/ml.

Although underpowered, Kaplan–Meier analysis did not reveal a difference in either patient or graft survival between patients who received an HCV antibody-positive donor that was viremic or aviremic (data not shown). Similarly, there was no difference in fibrosis progression between recipients of viremic or aviremic grafts (data not shown).

Discussion

As the demand for donor organs continues to grow, we must utilize every possible donor. Previous reports have shown that HCV antibody-positive donors may be used safely in HCV RNA-positive recipients without untoward effects on the recipient [1–4]. Some of these donors are HCV antibody-positive; however, not all HCV antibody-positive donors are HCV RNA-positive. HCV antibody positivity without detectable virus usually indicates prior infection that has resolved, but can occasionally be a false positive test. Either way that donor is not infectious.

Our results show 53% of our HCV antibody-positive donors are HCV RNA-negative. However, recipients of HCV-antibody positive, RNA-negative grafts were not retested to ensure genotype stability, which represents a limitation of our study. Our percentage of HCV-RNA positivity is similar to Ballarian *et al.* previous results showing 57% of their HCV antibody-positive donors were HCV RNA-negative [3]. Since this is a departure from the expected natural history of HCV, it likely represents surgeon selection bias whereby more viremic donors are excluded from donation based on inflammation and fibrosis.

Since our study population is small, it seems prudent to perform a separate prospective multicenter analysis of HCV fibrosis progression and graft survival in recipients of HCV RNA-positive donors. Fortunately, our data show graft and patient survival and fibrosis progression are unchanged in recipients of viremic and aviremic donors.

Furthermore, since half of HCV antibody-positive donors are RNA negative it seems prudent to only consider nucleic acid testing (NAT) HCV antibody-positive donors instead of what was recently proposed – to NAT test all donors. Although, HCV NAT-positive and negative liver grafts should be used this would allow further prospective study of outcomes and viral interactions during transplantation. The HCV antibody test, like the HIV antibody test is 99.9% sensitive [13]. Therefore, NAT testing all donors for HCV will only lead to increased costs and rates of false positives and unnecessary decreased access to liver transplant for those without HCV [14]. Although NAT testing HCV antibody-positive donors would increase costs for those donors, negative patients (>50%) could then safely have their lungs, hearts, kidneys and pancreases used thereby increasing donor supply. Therefore, this small cost to increase donor supply seems cost-effective. If access to the testing could not be achieved after hours and on weekends in some areas, those donors would need to be assumed to be viremic.

Testing donor viral load and genotype prospectively should be done as part of a multicenter trial to further understand viral interactions and dominance; however, time constraints will never allow this to occur prior to donor organ allocation.

Regardless of whether the predominant viral genotype originates in the donor or recipient, HCV viremia post-OLT leads to more rapidly progressive fibrosis post-OLT than pre-OLT. The results of treatment of genotype 1 HCV infection after liver transplantation have been disappointing [15]. SVR rates to treatment are higher in patients infected with non-1 genotypes, and for those in whom the donor and recipient both have a C/C IL28B

genetic polymorphism [16,17]. Therefore, patients infected with non-1 genotypes would be disadvantaged if they are given a genotype 1 infected graft and genotype 1 dominated. Our analysis also shows that the majority of time when genotype 1 is present in a donor–recipient pair, it dominates. Vargas also demonstrated this in four of four cases [18]. However, viral levels may also play a role, although our ability to draw conclusions about this is limited because of a lack of serial samples to confirm each patient's level of viremia.

We have finally entered a new era of HCV therapy with the introduction of direct acting antivirals (DAA). Unfortunately, these medications are costly, have additional side effects, have marked drug–drug interactions [19,20], and many are genotype specific [9–12]. Therefore it seems prudent to only give genotype 1 infected patients a HCV antibody-positive donor. However, as more agents are released onto the market that improve cure rates even further, it will be imperative to further understand the viral interactions that occur during transplantation to appropriately triage these organs to those who will benefit from them without adversely affecting their chances for viral eradication post-transplant.

In conclusion, we found 53% of HCV antibody-positive donors are HCV RNA-negative, indicating an absence of active infection. Although no difference in patient or graft survival or fibrosis progression was found in patients receiving an HCV RNA-positive versus negative donor further large scale study is warranted. In addition, viral factors including genotype and possibly viral load likely determine the dominant HCV-viral species post-transplant. In the new era of DAA where genotype and even subtype have an impact on the risk, cost, and curability of HCV-infection, it seems prudent to only allocate HCV antibody-positive donors to genotype 1 infected recipients at this time.

Authorship

JGO: designed research/study, analyzed data, wrote the paper. MAN: performed research/study, collected data. JFT: designed research/study, analyzed data. GLD: designed research/study, analyzed data, wrote the paper. GBK: designed research/study, analyzed data, wrote the paper.

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