Donor Mannose-Binding Lectin Deficiency Increases the Likelihood of Clinically Significant Infection after Liver Transplantation

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Background. Mannose-binding lectin (MBL) is an important mediator of innate immunity and is synthesized primarily by the liver. Low MBL levels are common, are due primarily to polymorphisms in the gene encoding MBL (MBL2), and are associated with an increased risk of infection, particularly when immunity is compromised. We report a large, retrospective study that examined the association between MBL status and clinically significant infection following orthotopic liver transplantation.

Methods. One hundred two donor-recipient orthotopic liver transplantation pairs were studied. Five polymorphisms in the promoter and coding regions of MBL2 were examined. MBL levels were measured, using the mannan-binding and C4-deposition assays, in serum samples obtained before and after transplantation. Associations between MBL status, as assessed by serum MBL levels and MBL2 genotype, and time to first clinically significant infection (CSI) after transplantation were examined in survival analysis with consideration of competing risks.

Results. The median duration of follow-up after orthotopic liver transplantation was 4 years. Thirty-six percent of recipients developed CSI after transplantation. The presence of MBL2 coding mutations in the donor was significantly associated with CSI in the recipient; the cumulative incidence function of infection was 55% in recipients of deficient livers, compared with 32% for recipients of wild-type livers (P = .002). Infection was not associated with recipient MBL2 genotype. Low MBL levels after orthotopic liver transplantation levels (mannan-binding <1 μg/mL or C4 deposition <0.2 C4 U/μL) were also associated with CSI (cumulative incidence function, 52% vs. 20%, P = .003; and cumulative incidence function, 54% vs. 24%, P = .007, respectively). In multivariate analysis, mutation in the MBL2 coding region of the donor (hazard ratio, 2.8; P = .002) and the use of cytomegalovirus prophylaxis (hazard ratio, 2.6; P = .005) were independently associated with CSI.

Conclusions. Recipients of MBL-deficient livers have almost a 3-fold greater likelihood of developing CSI and may benefit from MBL replacement.

Mannose-binding lectin (MBL) is an important pattern-recognition molecule within the innate immune system [1]. MBL is produced primarily by the liver and binds to bacteria and other pathogens via specific repetitive oligosaccharide moieties on their surfaces [1]. This binding initiates complement-mediated killing via the lectin pathway, as well as promoting opsonization and phagocytosis [2]. Within a population, there is a wide range of serum MBL activity. This is determined largely by genetic polymorphisms within MBL2, the gene encoding MBL [1, 3]. Three missense mutations within the first exon of MBL2 significantly affect MBL function (codon 54 Gly→Asp, allele B [dbSNP ID rs1800450]; codon 57 Gly→Glu, allele C [dbSNP ID rs1800451]; and codon 52 Arg→Cys, allele D [dbSNP
ID rs5030737]) [4]. Alleles with these coding mutations are collectively designated O, and the wild-type, sufficient allele is designated A. These common coding variants impair the usual oligomerization of the monomeric MBL triple helix into functional higher-order multimers and may also enhance its degradation by metalloproteinases [5, 6]. Further variability of serum MBL levels result from other MBL2 polymorphisms, primarily within the promoter (position −550 G/C alleles H and L [dbSNP ID rs11003125] and position −221 G/C alleles X and Y [dbSNP ID rs7096206]), as well as the 5’ untranslated regions (position +4 C/T alleles P and Q [dbSNP ID rs7095891]) of the gene.

Almost one-half of white individuals are relatively deficient in serum MBL, conferred through MBL2 coding mutation heterozygosity (A/O) or homozygosity (O/O), in ~35% and ~8% of that population, respectively [7, 8]. Within healthy populations, however, serum MBL activity is determined by the entire MBL2 haplotype, and patients with the LXAXA haplotype have relatively low MBL activity, whereas those with the HYAD haplotype may demonstrate nearly normal function [7, 8].

MBL deficiency has been associated with the increased prevalence and specific manifestations of a wide range of common disorders [4, 9–11]. MBL deficiency is most relevant, however, as a risk factor for infection, particularly when immunity is already compromised through immunological immaturity, comorbidity, or medical therapy [12–18].

More than two-thirds of patients who undergo orthotopic liver transplantation (OLT) develop an infection within the first year after transplantation, and infection is the leading cause of morbidity and mortality in these patients [19]. In 2005, Bouwman et al. [20] reported a small study that found an association between low-producing donor (hepatic) MBL2 genotypes and increased rates of clinically significant infection (CSI) after OLT.

Plasma-derived and recombinant human MBL are now available for MBL replacement, and a phase I/II study is currently recruiting recipients of MBL-deficient liver transplantation (A/O or O/O donor livers) for a post-OLT trial of recombinant human MBL replacement [21]. It is important to confirm these initial promising results, however, before proceeding with larger clinical trials.

In the present study, we tested the hypothesis that MBL deficiency increases the risk of CSI after OLT, and we determined the most clinically relevant assessment of MBL status in the context of liver transplantation.

**Patients and Methods**

**Patients.** One hundred thirty-four consecutive OLT recipients who received transplantation before December 2005 were retrospectively identified through the Victorian Liver Transplant Unit (Melbourne, Australia). Patients not followed up at the Victorian Liver Transplant Unit’s resident institution (Austin Hospital, Melbourne; 17 patients) or for whom DNA was not available from both donor and recipient (15 patients), were excluded from the study. This resulted in a final sample of 102 liver transplants involving 101 recipients. The study fulfilled the requirements of the Declaration of Helsinki and was approved by the Austin Health Research and Ethics Committee (institutional review board).

**Samples and clinical data.** Routine clinical data were collected, including recipient age, sex, indication for transplantation, cytomegalovirus (CMV) status of donor and recipient, model for end-stage liver disease (MELD) score [22], and peri-transplant management. Cases of CSI were identified from the hospital case notes and pathology records by an infectious diseases physician (D.F.J.) blind to the MBL results. Posttransplant CSI was defined as an invasive infection occurring after liver transplantation that was associated with clinical symptoms and signs of infection that was confirmed by the microbiological demonstration of the causative organism from a sterile site. Pneumonia was considered to be a CSI when diagnosed on the basis of typical clinical signs and symptoms accompanied by chest radiographic evidence of lobar, bronchopneumonic, or nodular consolidation or interstitial pneumonitis, even in the absence of an identified causative organism. Such cases of microbiologically unconfirmed pneumonia were included in the definition of CSI because of the clinical importance of pneumonia following OLT but the difficulty of isolating a causative organism [23]. Recipient and donor DNA for MBL2 genotyping was obtained from the Victorian Immunogenetics and Transplantation Service. For the majority of transplant recipients, serum samples for detection of circulating MBL levels were collected before transplantation as well as at multiple times after transplantation. The serum samples were stored in aliquots at −80°C, and repeat freeze-thaw events were avoided. Graft rejection was defined as histopathologically confirmed rejection from liver biopsy obtained after OLT, irrespective of severity or subsequent treatment [24].

**Assays.** Serum MBL activity was quantified by the mannan-binding assay (μg/mL) and the C4-deposition assay (C4 U/μL), as described elsewhere [7]. Pretransplant serum MBL levels were reported as a mean of MBL and C4 deposition from all available serum samples obtained in the immediate pretransplant period. The post-OLT MBL and C4-deposition measurements were reported as a mean of all post-OLT serum samples that were available from each patient. Time-specific, post-OLT serum MBL activity results were calculated as the mean of all samples available from each specific post-OLT time interval (mean of serum results taken 0–7 days, 7–100 days, 100–365 days, and >1 year after transplantation). The MBL2 polymorphisms were genotyped using the PCR and sequence-specific primers described elsewhere [25].

**Definition of MBL deficiency.** The MBL2 deficiency ge-
notype was classified according to the presence of an MBL2 coding mutation; the A/A genotype was considered to indicate a sufficient MBL2 genotype, and the A/O and O/O genotypes were considered to indicate a deficient MBL2 genotype [20, 21]. Within these 2 groups, there is a range of serum MBL activity; the A/A group contains the relatively deficient LXA/LXA haplotype, whereas the A/O group contains the relatively sufficient HYA/D haplotype [7]. Therefore, we conducted a second, separate analysis in which the MBL2 deficiency genotype was extended to include the HYA/D haplotype but exclude the LXA/LXA haplotype. We also examined associations between CSI and serum MBL activity, both as continuous and as categorical variables. Serum MBL activity was assessed at several thresholds to determine the best definition of post-OLT serum MBL deficiency. Specifically, the association between CSI and serum MBL level was examined at 0.3 μg/mL, 0.4 μg/mL, 0.5 μg/mL, and 1.0 μg/mL, and the association between CSI and C4 deposition was examined at 0.1 C4 U/μL, 0.2 C4 U/μL, and 0.3 C4 U/μL.

Statistical analyses. Associations between categorical variables were analyzed by Pearson’s χ² test. MBL levels had non-normal distributions; therefore, associations between MBL2 haplotypes and levels were analyzed using the Kruskal-Wallis test. The Wilcoxon matched-pairs signed-rank test was used to compare the matched pre- and post-OLT levels (Statas Statistical Software, version 10 [StataCorp]). Associations between categorical variables and time to CSI were examined by estimating the cumulative incidence function (CIF) of CSI with use of Gray’s estimator [26]. Noninfective death and retransplantation were treated as competing risks. Unless otherwise stated, the CIFs are reported for the last available time point of the study. Multivariate analysis of associations with CSI with consideration of competing risks was performed using the method of Fine and Gray [27]. Recipient age, sex, indication for liver transplantation, and serum MBL activity (both as continuous and categorical variables); CMV status of recipient and donor; the administration of CMV prophylaxis or additional immunosuppressive agents; the pre-OLT MELD score; and the presence of donor or recipient MBL2 coding mutations were considered. All variables that had a univariate association with CSI of P < .2 were included in the initial model. Variables with results of P > .1 were sequentially removed, in order of P value (highest to lowest). The collinear continuous and categorical serum MBL activity variables were analyzed together and separately, without affecting the final model. All transplant pairs were genotyped, but only 79 of the recipients had serum samples available for analysis of the post-OLT MBL levels. To provide a balanced comparison between genotyping and serum testing in terms of association with CSI, the first multivariate analysis included only patients with available serum. Donor MBL2 genotype was the only measure of MBL status independently associated with CSI; thus, a second multivariate analysis that included all 102 OLTs was performed to produce the final hazard ratios. Analyses were performed using the cmprsk and CumIncidence packages in R [28]. The association between recipient MBL2 genotype and CSI was considered at 30 and 60 days after OLT, as well as at 7 years after OLT, because of the possibility that recipient MBL status, if important, might be most important in early post-OLT infection. All bivariate analyses were 2 tailed, and the α level for statistical significance was set at .05.

RESULTS

Population and clinical posttransplantation management. This study evaluated 102 liver transplants involving 102 donors and 101 recipients. There was 1 retransplantation, which was considered to be a second, discrete episode. Recipient and donor characteristics are presented in table 1. The median age, sex, MELD scores, and indications for transplantation—hepatitis C virus infection, 24%; alcohol-caused cirrhosis, 13%; hepatocellular carcinoma, 11%; primary sclerosing cholangitis, 11%; hepatitis B virus infection, 7%; metabolic liver disease, 7%; autoimmune hepatitis, 4%; biliary atresia, 4%; primary biliary cirrhosis, 4%; fulminant hepatic failure, 3%; and miscellaneous disease, 12%—were comparable to the average Australasian adult liver transplantation population (table 1) [29]. The median duration of post-OLT follow-up was 4 years (range, 0–8.1 years). In the immediate post-OLT period, all patients routinely received a calcineurin inhibitor, corticosteroid, and a third immunosuppressive agent (mycophenolate mofetil or azathioprine). Ten patients received an additional immunosuppressive agent in the immediate posttransplant period: 9 patients received basiliximab, and 1 patient received muromonab. The CIF of CSI was 50% in these patients, compared with only 34% in those who received standard triple immunosuppressive therapy, but this difference was not statistically significant (P = .29) (table 2).

Postoperatively, all patients received triple antibiotic prophylaxis with a glycopeptide, a third-generation cephalosporin or carbapenem, and Pneumocystis jiroveci prophylaxis with either trimethoprim-sulphamethoxazole (81 patients) or inhaled pentamidine (21 patients). CMV-seronegative recipients of seropositive grafts, as well as any patients who received muromonab, routinely received CMV prophylaxis with oral ganciclovir or valganciclovir (standard dose, 1 g thrice daily and 900 mg daily, respectively) for 3 months.

MBL genotype and serum activity. One hundred two donor-recipient pairs were genotyped for MBL2, 75 had stored serum samples available for measuring pre-OLT serum MBL activity, 79 had serum samples available for measuring post-OLT MBL activity, and 66 had both pre- and post-OLT samples available for testing. The frequency of MBL2 coding mutations

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was not significantly different between donors and recipients and was comparable to the healthy population (A/A vs. A/O and O/O, 62% vs. 38% for recipients and 61% vs. 39% for donors; \( P = .9 \)) [7].

Post-OLT serum MBL activity was determined by donor (hepatic) \( MBL2 \) genotype for both functional MBL and C4-deposition levels (\( P = .001 \)) (figures 1 and 2) but not the recipient \( MBL2 \) haplotype (functional MBL level, \( P = .8 \); C4 deposition, \( P = .9 \)). Consequently, MBL-deficient (A/O or O/O) recipients of wild-type (A/A) livers had a marked increase in serum MBL levels after transplantation, a functional MBL level (median of the pre- and post-OLT means) of 0.43 \( \mu g/mL \) versus 3.3 \( \mu g/mL \) \((P < .001)\), and a C4-deposition level of 0.08 C4 U/\( \mu L \) versus 0.72 C4 U/\( \mu L \) \((P = .001)\). A/A recipients of A/O or O/O livers had a significant posttransplant decrease in serum MBL levels (functional MBL level, 2.7 \( \mu g/mL \) vs. 0.42 \( \mu g/mL \) \((P < .001)\) and C4 deposition, 0.71 C4 U/\( \mu L \) vs. 0.13 C4 U/\( \mu L \) \((P = .002)\)) (figure A1; online only). To characterize the transition from recipient to donor MBL status for all MBL-deficient recipients of wild-type grafts, results of post-OLT serum testing were stratified by time. There was a rapid increase, within days, in (donor liver–derived) post-OLT MBL levels, which were then relatively stable beyond 1 year (figure 3). In addition, there were 3 wild-type MBL recipients who received livers from donors carrying \( MBL2 \) coding mutations who had pre- and post-OLT serum samples available within the first 30 posttransplant days. All were MBL deficient, as determined by C4 deposition or MBL levels on days 11, 15, and 27 after OLT. These results indicate that donor \( MBL2 \) coding genotype and resultant hepatic MBL synthesis is the principal determinant of circulating MBL activity after OLT.

**CSI.** Thirty-seven transplantations (36.3%) involved patients who developed 64 episodes of CSI during the follow-up period. The median time to diagnosis of first infection was 27 days after OLT (range, 1 day to 3.6 years). Pneumonia was the most commonly diagnosed CSI and accounted for 37% of events, followed by bacteremia (33%) and peritonitis (14%). CMV-related disease caused 11% of events, and fungal disease caused ∼5% of events. The causative organisms were typical of those found after OLT (table A1; online only) [19].

Patients who received livers with \( MBL2 \) coding mutations had a higher rate of CSI (CIF, 55%) than those receiving wild-type livers (CIF, 32%; \( P = .002 \)) (table 2 and figure 4). Post-OLT serum MBL deficiency, defined by MBL <1 \( \mu g/mL \) and C4 deposition <0.2 C4 U/\( \mu L \), compared with other thresholds, had the best association with CSI (CIF, 52% vs. 20% \((P = .003)\) and 54% vs. 24% \((P = .007)\), respectively) (table 2 and figure 4). These values were used as MBL thresholds in earlier studies [30–32]. Of the 79 patients who had post-OLT serum available for testing, 33 (42%) had an MBL <1 \( \mu g/mL \) and 24 (30%) had C4 deposition <0.2 C4 U/\( \mu L \). Overall, 34 patients (43%) were characterized as being deficient by at least 1 of these serum criteria. These results are very similar to those of other study populations [7, 17].

Recipient \( MBL2 \) coding mutations were not associated with CSI overall (CIF, 44% vs. 40%; \( P = .34 \)) (table 2), nor was it associated with early CSI at either 30 or 60 days after OLT (\( P = .4 \) and \( P = .8 \), respectively). The extended definition of the donor \( MBL2 \) deficiency genotype, which reclassified HYA/D haplotype as sufficient and the LXA/LXA haplotype as deficient, was significantly associated with CSI (CIF of 51% vs. 35%; \( P = .015 \)) but did not improve the classification by coding-region mutation alone. Only 4 patients received an O/O liver, but they did not appear to have a greater CIF of CSI than those receiving A/O livers (5-year CIF of CSI, 58% with an A/O liver vs. 25% with an O/O liver; table A2; online only). It is acknowledged, however, that this study was not sufficiently powered to allow a meaningful comparison of CSI for all \( MBL2 \) haplotypes.

CMV seropositivity of the recipient, but not the donor, was associated with a lower CIF of CSI (31% vs. 48%; \( P = .04 \)). The administration of CMV prophylaxis was associated with a

**Table 1. Patient characteristics stratified by donor \( MBL2 \) coding genotype.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Donor genotype</th>
<th>( A/A )</th>
<th>( A/O ) or ( O/O )</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) of subjects</td>
<td></td>
<td>62 (60.8)</td>
<td>40 (39.2)</td>
<td>102</td>
</tr>
<tr>
<td>Female:male ratio</td>
<td></td>
<td>1:1.3</td>
<td>1:3.0</td>
<td>1:1.8</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td></td>
<td>49.9 (18.2–63.6)</td>
<td>51 (12.3–66.2)</td>
<td>50.2 (12.3–66.2)</td>
</tr>
<tr>
<td>CMV positive, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipient</td>
<td></td>
<td>49 (79)</td>
<td>28 (70)</td>
<td>77 (75.5)</td>
</tr>
<tr>
<td>Donor</td>
<td></td>
<td>32 (51.6)</td>
<td>19 (47.5)</td>
<td>51 (50)</td>
</tr>
<tr>
<td>Rejection, no. (%)</td>
<td></td>
<td>34 (54.8)</td>
<td>23 (57.5)</td>
<td>57 (55.9)</td>
</tr>
<tr>
<td>Mean MELD score (range)</td>
<td></td>
<td>19.8 (1–48)</td>
<td>18.2 (1–37)</td>
<td>19.2 (1–48)</td>
</tr>
<tr>
<td>CSI, no. (%)</td>
<td></td>
<td>15 (24.2)</td>
<td>22 (55)</td>
<td>37 (36.3)</td>
</tr>
</tbody>
</table>

**NOTE.** CMV, cytomegalovirus; CSI, clinically significant posttransplant infection; MELD, model for end-stage liver disease.
Table 2. Association between patient characteristics and clinically significant infection (CSI) on univariate analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cumulative incidence function of CSI, %</th>
<th>Hazard ratio of CSI</th>
<th>Univariate $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dichotomous</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBL2 coding mutation vs. A/A genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor</td>
<td>55 vs. 32</td>
<td>...</td>
<td>.002</td>
</tr>
<tr>
<td>Recipient</td>
<td>44 vs. 40</td>
<td>...</td>
<td>.34</td>
</tr>
<tr>
<td>Recipient sex, female vs. male</td>
<td>41 vs. 39</td>
<td>...</td>
<td>.56</td>
</tr>
<tr>
<td>CMV status, positive vs. negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor</td>
<td>57 vs. 31</td>
<td>...</td>
<td>.32</td>
</tr>
<tr>
<td>Recipient</td>
<td>44 vs. 40</td>
<td>...</td>
<td>.04</td>
</tr>
<tr>
<td>CMV prophylaxis, yes vs. no</td>
<td>72 vs. 30</td>
<td>...</td>
<td>.005</td>
</tr>
<tr>
<td>Received CMV prophylaxis and an MBL-deficient liver vs. no prophylaxis and a wild-type liver</td>
<td>83 vs. 22</td>
<td>...</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Post-OLT C4 deposition assay, $\geq$0.2 vs. $&lt;$0.2 C4 U/μL</td>
<td>24 vs. 54</td>
<td>...</td>
<td>.007</td>
</tr>
<tr>
<td>Post-OLT functional MBL level, $\geq$1 vs. $&lt;$1 mg/mL</td>
<td>20 vs. 52</td>
<td>...</td>
<td>.003</td>
</tr>
<tr>
<td>Rejection, yes vs. no</td>
<td>40 vs. 40</td>
<td>...</td>
<td>.43</td>
</tr>
<tr>
<td>Additional immunosuppression, yes vs. no</td>
<td>50 vs. 34</td>
<td>...</td>
<td>.29</td>
</tr>
<tr>
<td><strong>Continuous and ordinal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipient age, by decade intervals</td>
<td>...</td>
<td>0.73</td>
<td>.048</td>
</tr>
<tr>
<td>Post-OLT C4-deposition assay, by C4 U/μL</td>
<td>...</td>
<td>0.35</td>
<td>.076</td>
</tr>
<tr>
<td>Post-OLT functional MBL level, μg/mL</td>
<td>...</td>
<td>0.71</td>
<td>.027</td>
</tr>
<tr>
<td>MELD score</td>
<td>...</td>
<td>1.0</td>
<td>.27</td>
</tr>
</tbody>
</table>

NOTE. MBL, mannose-binding lectin; MELD, model for end-stage liver disease; OLT, orthotopic liver transplantation.

The significant univariate association for donor MBL2 genotype was maintained on multivariate analysis. The initial multivariate model included only the 79 patients for whom post-OLT serum MBL measurements were available. In this model, donor MBL2 coding mutation (hazard ratio, 3.7; $P = .002$) and CMV prophylaxis (hazard ratio, 3.2; $P = .006$), but not serum MBL levels, were independently associated with CSI. Because serum MBL levels were not independently associated with CSI, the multivariate analysis was repeated to include data from all 102 transplants. In this final model, donor MBL2 coding mutation (hazard ratio, 2.8; $P = .002$) and CMV prophylaxis (hazard ratio, 2.6; $P = .005$) were again the only factors that were independently associated with CSI (table 3).

Figure 1. Association between recipient and donor MBL2 coding genotype and pre- and post-orthotopic liver transplantation (OLT) serum mannose-binding lectin (MBL) levels. A, Functional MBL level. B, C4-deposition assay. The pre- and post-OLT serum levels are similar when the recipient and donor genotypes are the same. There are greater differences between pre- and post-OLT levels after MBL2 genotype-discordant transplantation, with donor genotype determining post-OLT MBL levels.
Figure 2. A, Functional mannose-binding lectin (MBL) level. B, C4-deposition assay. Donor-derived MBL2 haplotype determines the post–orthotopic liver transplantation (OLT) circulating MBL levels. There is a significant difference in MBL levels stratified by donor MBL2 genotypes ($P < .001$) for both functional MBL and C4 deposition.

Figure 3. Box-plot graph showing the time profile of serum mannose-binding lectin (MBL) levels. A, Functional MBL level. B, C4-deposition assay. MBL levels are shown pre–orthotopic liver transplantation (OLT) and at 4 post-OLT time points in MBL-deficient recipients of wild-type grafts.

DISCUSSION

This study confirmed the importance of donor MBL status in the pathogenesis of post-OLT infection. Most notably, MBL2 coding mutation in the donor, reflecting impaired MBL synthesis by the transplanted liver, was significantly associated with CSI. Recipients of livers harboring such mutations (A/O or O/O) had almost a 3-fold greater likelihood of developing a post-OLT CSI (table 3).

Donor MBL2 genotype was the strongest determinant of posttransplant circulating MBL levels, as measured by either functional MBL (mannan-binding) level or the C4-deposition assay. In turn, impaired post-OLT serum MBL activity was associated with post-OLT CSI. However, on multivariate analysis, there was no independent association between post-OLT serum MBL activity and CSI. If all patients in our study had early post-OLT serum available for testing, it is possible that MBL level might have been comparable to donor MBL2 genotyping in its association with CSI. From our results, however, donor MBL2 coding genotype was the best predictor of post-OLT infection. Donor MBL2 genotyping could be easily integrated into clinical practice.

Although these results support the principal findings from a smaller study [20], our study genotyped more than twice the number of donors and >4-fold the number of recipients, which enabled us to demonstrate through multivariate analysis, that donor but not recipient MBL deficiency was associated with CSI. Moreover, we were able to demonstrate a similar association between posttransplant circulating MBL activity and post-OLT infection. Despite initial concerns with regard to the small number of recipients genotyped in the study by Bouwman et al. [20], we reached the same conclusion, that recipient MBL2
status was not relevant to either post-OLT MBL serum activity or risk of infection.

Our study is particularly timely, given the current potential of immunoprophylaxis in OLT. There is currently a phase I/II study recruiting recipients of MBL2 coding mutation–containing livers for a trial of 8 weekly infusions of recombinant human MBL. This follows an earlier successful phase I trial as well as case reports of MBL replacement [33, 34]. Our results support the selection criteria used in this trial—that is, recipients of A/O or O/O livers. Although recipients of MBL2 deficiency genotype grafts seem the most likely to benefit from MBL immunoprophylaxis, any potential advantage may not necessarily be limited to the donor-deficient subset. Approximately two-thirds of recipients of MBL2 coding mutation–containing livers who developed CSI did so within 8 weeks after transplantation, which provides some rationale for the trial’s duration.

The present study confirms that, at least in a post-OLT context, extrahepatic MBL2 status appears to be irrelevant with respect to frequency of CSI or circulating MBL activity. It is quite conceivable, however, that extrahepatic MBL production does occur and has clinically important effects, perhaps in the setting of more extreme immunosuppression, such as after allogeneic hematopoietic stem cell transplantation [35, 36], or even in the pathogenesis of noninfectious diseases, such as autoimmunity.

Although not a primary end point of this study, recipients of CMV prophylaxis had a 2.6-fold greater likelihood of developing post-OLT CSI, compared with those who did not receive CMV prophylaxis. This could reflect adverse clinical factors or enhanced immunosuppression as an indication for CMV prophylaxis in these patients, particularly the off-protocol use of prophylaxis, or may relate to prophylaxis-associated neu-

### Table 3. Association between patient characteristics and clinically significant infection (CSI) on multivariate analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>Multivariate P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor MBL2 coding mutation</td>
<td>2.8</td>
<td>.002</td>
</tr>
<tr>
<td>CMV prophylaxis</td>
<td>2.6</td>
<td>.005</td>
</tr>
</tbody>
</table>

NOTE. Donor MBL2 coding mutation and cytomegalovirus (CMV) prophylaxis were the only 2 factors independently associated with an increased likelihood of post-orthotopic liver transplantation (OLT) CSI on multivariate analysis. Initially, only the 79 patients with known post-OLT serum levels were included, but this table reports the multivariate analysis for the entire study (102 transplants). CMV, cytomegalovirus.
tropenia. The very high incidence of CSI in those who received an MBL-deficient liver as well as CMV prophylaxis suggests that these patients may be an important subset to investigate in future studies.

Donor MBL2 deficiency genotype and the resultant reduction in serum MBL activity are important risk factors for clinically significant infection after OLT. Our findings support trials of MBL-replacement therapy in recipients of MBL-deficient livers.

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