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# Intestinal Bacteremia After Liver Transplantation Is a Risk Factor for Recurrence of Primary Sclerosing Cholangitis

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**Background.** Primary sclerosing cholangitis (PSC) is a chronic progressive pathological process, related to inflammatory bowel disease and subsequent bacterial translocation. Liver transplantation (LT) is the only curative therapy, but outcomes are compromised by recurrence of PSC (rPSC). The aim of the study was to investigate a potential link between intestinal bacteremia, fucosyltransferase-2 (FUT2), and rPSC after LT. **Methods.** LT recipients with PSC (n = 81) or without PSC (n = 271) were analyzed for clinical outcomes and positive bacterial blood cultures. A link between bacteremia and the genetic variant of the *FUT2* gene was investigated. **Results.** The incidence of inflammatory bowel disease was significantly higher in PSC recipients but not associated with rPSC. Bacteremia occurred in 31% of PSC recipients. The incidence of rPSC was 37% and was significantly more common in patients with intestinal bacteremia versus no bacteremia (82% versus 30%; $P$ = 0.003). The nonsecretor polymorphism of the *FUT2* gene was identified as a genetic risk factor for both intestinal bacteremia and rPSC. Combined *FUT2* genotype and intestinal bacteremia in recipients resulted in the highest risk for rPSC (hazard ratio, 15.3; $P < 0.001$). **Conclusions.** Thus, in this article, we showed that bacterial translocation is associated with rPSC after LT and related to the FUT2 nonsecretor status.

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## INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic, fibrosing bile duct disease that can result in end-stage biliary cirrhosis for which liver transplantation (LT) is the only curative therapy.[1,2] In patients suffering from PSC, chronic and mild inflammation of the biliary tree leads to progressive concentric, "onion skin," fibrosis around the bile ducts.[3] This is characterized by strictures interchanged with dilatations throughout the biliary tract on cholangiography.[4]

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*The authors would like to state that some overlap with a Ph.D. thesis that was published in 2016 (C.J.Verhoeven, "Biomarkers to assess graft quality in liver transplantation," ISBN: 978-94-6169-879-7) is present in this submitted article. As it is, to a certain extent, an adaptation of a chapter in this Ph.D. thesis, overlap is inevitable.*

*The data, analytic methods, and study materials will be made available upon reasonable request.*

*Supplemental visual abstract; http://links.lww.com/TP/C717.*

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The pathogenesis of PSC is regarded as multifactorial, including environmental factors, genetic predisposition, compromised biliary and intestinal defense mechanisms, and immunologic dysregulation. There is a close association between PSC and inflammatory bowel diseases (IBD, 60%–80% of all patients), with ulcerative colitis (UC) as the most frequently classified bowel affection.[5,6] Although the relationship between bowel and bile duct manifestations of PSC is unclear, the leaky gut hypothesis has gained more interest in recent years.[7–9] Bowel disease and disruption of bowel permeability may eventually lead to microbial infection of bile, subsequently causing cholangiocytes to activate a response that leads to inflammation and fibrosis within the liver.[10]

Although not yet shown in patients, disturbance of the intestinal barrier function in animal models has been associated with the development of dysbiosis (imbalanced gut bacteria), which predisposes to colitis.[11] In addition to a possible role in bowel disease, dysbiosis, and subsequently altered permeability, was also identified as a cause of inflammatory liver injury in the mouse.[12] Recent literature implicates that in patients with PSC, increased gut permeability and bacterial translocation are pathophysiological key features.[13] Several biomarkers, such as interferon γ-related biomarkers, accumulation of abnormal high endotoxins in biliary epithelial cells, and structural intestinal damage biomarkers, were associated with worse outcomes of PSC patients and correlate with transplant-free survival.[9,14–16]

Several studies have shown that PSC could be triggered by environmental factors in a genetically susceptible host.[17–19] Genome-wide association studies identified various single-nucleotide polymorphisms (SNPs) associated with the development of PSC.[20,21] In particular, a mutation in the fucosyltransferase-2 (*FUT2*) gene accelerates the disease course of PSC and reduces the transplantation-free survival of these patients.[22,23] The wild type allele of the *FUT2* gene contains a G at position 48703417 of chr. 19 (human reference genome version GRCh38), while an A at the same position results in a mutated (truncated and nonfunctional) allele. Thus, donors or recipients homozygous for the mutated allele (=AA) were considered to be FUT2 nonsecretors, whereas donors or recipients with either a GA or GG genotype were considered FUT2 secretors.[22] This rs601338 (G>A) polymorphism is present with an average frequency of 32%, ranging from almost 0% (South-East Asia) to as much as 50% in the African population, thereby affecting ~10% worldwide according to the Hardy–Weinberg equilibrium.[22,24] Individuals with the AA-genotype generate only truncated, dysfunctional FUT2 proteins on the surface of epithelial cells throughout their body. These so-called FUT2 nonsecretors have a perturbed composition of the cellular glycocalyx and are, therefore, less resistant against the passage of bacteria and other pathogens through the intestinal epithelium to extraintestinal sites like the liver and bile ducts via the portal vein.[23,25] In addition, an FUT2 nonsecretor status has an effect on both the composition and function of the colonic microbiota, leading to an increased risk of biliary infections in PSC patients.[26–28] Due to a predisposition for bacterial translocation in *FUT2* nonsecretors and the link between PSC and *FUT2* SNPs, we hypothesized that patients with underlying PSC are prone to develop PSC recurrence after transplantation.[18,27] A recent analysis of the European Liver Transplant Registry showed a rate of recurrence of PSC (rPSC) of 16.7% after a median follow-up of 5.0 y with a negative impact on both graft and patient survival.[29] It is, however, still not completely elucidated if the gut barrier dysfunction is related to the recurrence of PSC.

Bacterial translocation in IBD is speculated to be one of the driving forces of PSC, and it has been hypothesized that bacteria (or other microbes), carried into the liver via the portal vein, may induce local inflammatory responses and affect graft outcome and survival.[13,27,30–32]

The aim of this study was to investigate a potential link between intestinal bacteremia and the development of recurrent PSC after LT. In addition, we studied the role of the FUT2 status for bacterial translocation and the onset of recurrent PSC.

## MATERIALS AND METHODS

### Study Design

Between November 1989 and December 2011, totally 721 adult patients (age ≥18 y) underwent LT at the Erasmus Medical Center Rotterdam, The Netherlands. Partial or split LTs were excluded because of their small numbers and the unique surgical procedure with subsequent complications.

LT recipients with or without PSC were analyzed for clinical outcomes and positive bacterial blood cultures. In case of suspected bacteremia, aerobic and anaerobic bottles were cultured for up to 5 d (BD BACTEC FX Blood Culture System). Positive blood cultures were inoculated onto Columbia blood agar with 5% sheep blood, McConkey Chocolate and Brucella blood agar (BD diagnostics, Breda, The Netherlands).

A link between bacteremia and genetic variant rs601338 in the *FUT2* gene of the recipients or the donors was investigated. The primary outcome measure of this study was to compare the incidence and time to diagnosis of recurrence PSC in recipients following LT based on bacterial species isolated from blood and FUT2 status. Clinical data were collected from electronic medical records and patient charts. The Medical Ethical Committee of the Erasmus MC approved the use of donor materials, and all patients provided written informed consent for the use of clinical information for medical research.

### Sample Collection and Genotyping

Donor DNA was obtained from spleen biopsies, sampled routinely together with the liver, after graft procurement. Recipient DNA was derived from peripheral blood collected from patients at the time of hospital admission just prior to surgery. DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, Wisconsin, USA), according to the manufacturers' instructions and stored at +4°C until further use. SNP genotyping for rs601338 was performed by LGC Genomics (Teddington, UK), using the polymerase chain reaction-based KASP (Kompetitive Allele Specific PCR) genotyping technology. The sequences of SNP-specific

primers are 5′-GGAGGTGGTGGTAGAAGGTCT-3′ and 5′-GGAGGTGGTGGTAGAAGGTCC-3′ and that of the common primer is 5′-GAACGACTGGATGGAGGAGGAATA-3′.

### Statistical Analysis

Statistical analysis was performed using SPSS statistics 25 (SPSS Inc., Chicago, Illinois, USA). Results of continuous data are expressed as medians (interquartile range [IQR]) unless stated otherwise. Analyses on both ungrouped as well as grouped *FUT2* genotypes were performed. Group comparisons were performed using the nonparametric Mann–Whitney *U* tests for continuous data and 2-sided $\chi^2$ test for categorical data. Associations with the time to diagnosis of PSC recurrence were conducted by Kaplan–Meier analyses and log-rank tests. Prediction analyses were performed through backward stepwise Cox proportional hazards regression and expressed as the hazard ratio (HR). *P*-values <0.05 were considered statistically significant.

Details regarding definitions, perioperative care, antimicrobial prophylaxis, and immunosuppression can be found in the Supplemental Material (**Definitions S1, SDC,** http://links.lww.com/TP/C716).

## RESULTS

### Donor and Recipient Characteristics

During the study period (1989–2011), 721 adult LTs were performed at the Erasmus Medical Center, Rotterdam. All transplantation procedures were ABO-compatible. After exclusion of cases of hepatic artery thrombosis (n = 26), primary nonfunction (n = 6), and split LTs (n = 3), recipient DNA of 352 LT procedures was available for final analysis, including 325 primary LTs and 27 re-LTs. Genomic DNA was available of 254 donors, consisting of 225 donation after brain death and 29 donation after circulatory death grafts (Figure 1).

Donor, recipient, and procedural variables are listed in Table 1. The mean duration of follow-up of the entire study cohort was 5.8 y (minimum 3.0 mo to maximum 20.2 y). In all primary LTs, viral hepatitis was the main indication for LT (28.4%), followed by PSC (23%). Within the PSC recipients group, rPSC, as defined by the criteria described in the supporting information, was diagnosed in 30 patients (37.0%) with a median time to diagnosis of 8 mo (minimum 90 d to maximum 14.6 y).

Significantly more duct-to-duct biliary anastomosis were performed in the group of non-PSC patients (91%) versus the PSC group of patients (27%). On the contrary, Roux-en-Y biliary anastomosis, which are performed historically more often in PSC patients, are applied in 69% of our cohort of PSC patients versus 9% in the non-PSC patients group. Regarding to the impact of the type of biliary anastomosis and evidence of bacteremia in PSC patient s group, we did not receive any significantly difference (*P* = 0.28, data not shown). Of the 271 non-PSC recipients, 36 developed nonanastomotic biliary strictures (NAS) (13.3%), with a median time to diagnosis of 13 mo (minimum 102 d to maximum 10.2 y) after transplantation (Table 1).

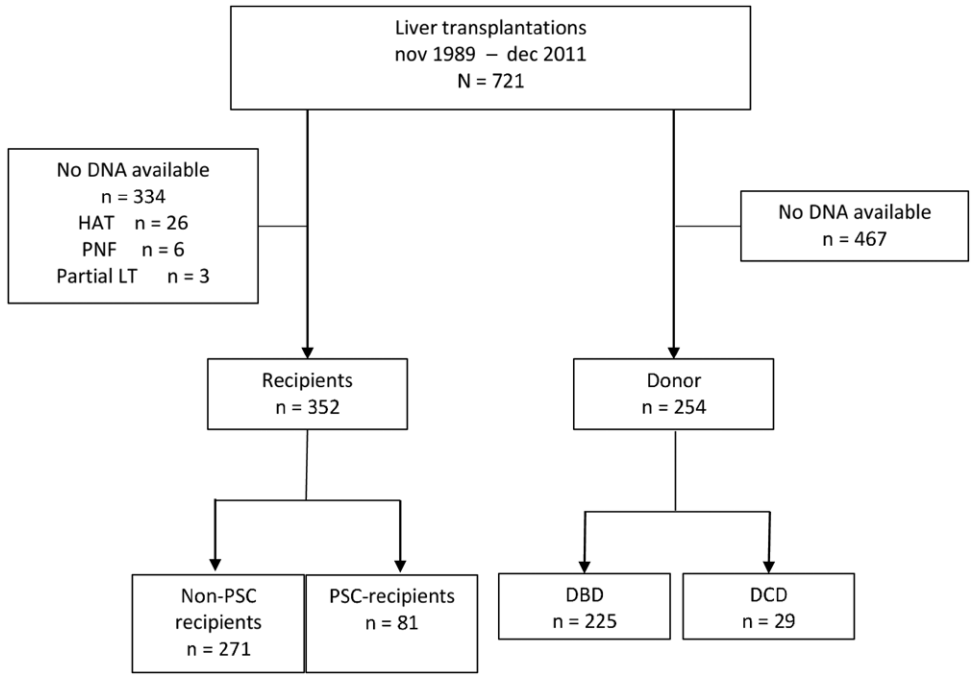


**FIGURE 1.** Study design. Between November 1989 and December 2011, a total of 721 adult patients (age >18 y) underwent liver transplantation (LT) at the Erasmus University Medical Center Rotterdam, The Netherlands. Available DNA samples from donors and recipients were retrospectively genotyped for the *FUT2* mutation rs601338. Clinical data were collected from electronic medical records and patient charts. The primary outcome measure of this study was to compare the incidence and time to diagnosis of recurrence of primary sclerosing cholangitis (rPSC) in recipients following LT based on FUT2 status. Secondary outcome measures consisted of the incidence of nonanastomotic biliary strictures (NAS) in non-PSC patients and the incidence, time of diagnosis, and species of bacteremia. DBD, donation after brain death; DCD, donation after circulatory death.

**TABLE 1.**

**Patient characteristics for PSC recipients and non-PSC recipients**

| | PSC recipients | | | Non-PSC recipients | | |
|---|---|---|---|---|---|---|
| | No rPSC | rPSC | | No NAS | NAS | |
| | N = 51 | N = 30 | *P* | N = 235 | N = 36 | *P* |
| Donor characteristics | | | | | | |
| Age, y | 46 (36–72) | 44 (34–58) | 0.742 | 46 (33–54) | 47 (35–55) | 0.453 |
| Sex | | | 0.917 | | | 0.364 |
| Men (%) | 30 (59) | 18 (60) | | 105 (45) | 19 (53) | |
| Women (%) | 21 (41) | 12 (40) | | 130 (55) | 17 (47) | |
| Graft type | | | 0.249 | | | **0.041** |
| DBD (%) | 48 (94) | 26 (87) | | 215 (91) | 29 (81) | |
| DCD (%) | 3 (6) | 4 (13) | | 20 (9) | 7 (19) | |
| Preservation solution | | | 0.091 | | | 0.091 |
| UW (%) | 41 (80) | 19 (63) | | 202 (86) | 27 (75) | |
| HTK (%) | 10 (20) | 11 (37) | | 33 (14) | 9 (25) | |
| Cold ischemia, minutes | 481 (243–766) | 490 (237–758) | 0.384 | 491 (283–995) | 497 (370–736) | 0.649 |
| Second warm ischemia, minutes | 13 (0–73) | 15 (0–7) | 0.992 | 19 (0–83) | 22 (0–70) | 0.292 |
| Recipient characteristics | | | | | | |
| Age, y | 46 (39–58) | 46 (32–49) | 0.805 | 50 (41–58) | 51 (36–59) | 0.642 |
| Sex | | | 0.781 | | | 0.557 |
| Men (%) | 35 (69) | 21 (70) | | 138 (59) | 23 (64) | |
| Women (%) | 13 (31) | 9 (30) | | 97 (41) | 13 (36) | |
| Lab-MELD score (SD) | 22.9 (7.4) | 22.2 (7.1) | 0.832 | 23.7 (8.1) | 24.8 (8.1) | 0.849 |
| Inflammatory bowl diseases | | | 0.984 | | | 0.655 |
| No IBD (%) | 12 (24) | 7 (23) | | 231 (98) | 35 (97) | |
| Crohn's disease (%) | 11 (22) | 2 (7) | | 3 (1) | 0 (0) | |
| Ulcerative colitis (%) | 28 (55) | 21 (70) | | 1 (0) | 1 (3) | |
| Colectomy prior to LT (%) | 7 (14) | 2 (7) | 0.329 | 2 (1) | 1 (3) | 0.304 |
| Primary LT (indication) | | | | | | |
| PSC (%) | 51 (100) | 30 (100) | – | – | – | – |
| Viral hepatitis (B and C) (%) | 2 (4) | 1 (3) | 0.561 | 90 (38) | 7 (19) | **0.028** |
| Alcoholic (%) | 0 (0) | 0 (0) | – | 50 (21) | 9 (25) | 0.614 |
| Cryptogenic (%) | 0 (0) | 0 (0) | – | 29 (12) | 2 (6) | 0.234 |
| PBC (%) | 0 (0) | 0 (0) | – | 22 (9) | 4 (11) | 0.740 |
| ALF (%) | 0 (0) | 0 (0) | – | 19 (8) | 6 (17) | 0.098 |
| AIH (%) | 1 (2) | 0 (0) | – | 16 (7) | 3 (8) | 0.739 |
| Other (%) | 0 (0) | 0 (0) | – | 38 (16) | 3 (8) | 0.222 |
| Biliary anastomosis | | | 0.189 | | | **<0.001** |
| Duct-to-duct anastomosis (%) | 11 (22) | 11 (37) | | 195 (83) | 27 (75) | |
| Roux-en-Y anastomosis (%) | 37 (73) | 19 (63) | | 13 (6) | 9 (25) | |
| Postoperative characteristics | | | | | | |
| Postoperative interventions | | | **0.003** | | | **<0.001** |
| ERCP (%) | 3 (6) | 6 (20) | | 54 (23) | 18 (50) | |
| PTC (%) | 4 (8) | 7 (23) | | 10 (4) | 3 (8) | |
| Graft failure ending in re-LT (%) | 1 (2) | 10 (33) | **<0.001** | 9 (4) | 27 (75) | **<0.001** |
| Median graft survival, y (SE) | 16.6 (3.4) | 10.6 (2.2) | **0.017** | 15.0 (2.7) | 10.4 (1.6) | **0.004** |
| Median recipient survival, y (SE) | 17.4 (2.2) | 14.2 (1.9) | 0.438 | 19.9 (2.1) | 19.3 (1.4) | 0.811 |

AIH, autoimmune hepatitis; ALF, acute liver failure; DBD, donation after brain death; DCD, donation after circulatory death; ERCP, endoscopic retrograde cholangiopancreaticography; HTK, histidine–tryptophan–ketoglutarate solution; IBD, inflammatory bowel disease; Lab-MELD, model for end-stage liver disease; LT, liver transplantation; NAS, nonanastomotic biliary strictures; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; PTC, percutaneous transhepatic cholangiography; rPSC, recurrence of PSC; UW, University Wisconsin solution.
*P*-values <0.05 are considered statistically significant and are presented in bold.

The main difference between NAS and PSC recurrence was the confirmed diagnosis of PSC as the indication for LT.

## Relation Between IBD and PSC

Previous studies showed a link between PSC and IBD.[5,19,32] This led to the hypothesis that increased bacterial translocation could be involved in the pathophysiology of rPSC. The relative incidence of Crohn's disease (CD) and UC in all LT patients is shown in Figure 2A.

The number of patients transplanted due to PSC that also presented CD or UC was significantly higher compared to the number in non-PSC transplant patients

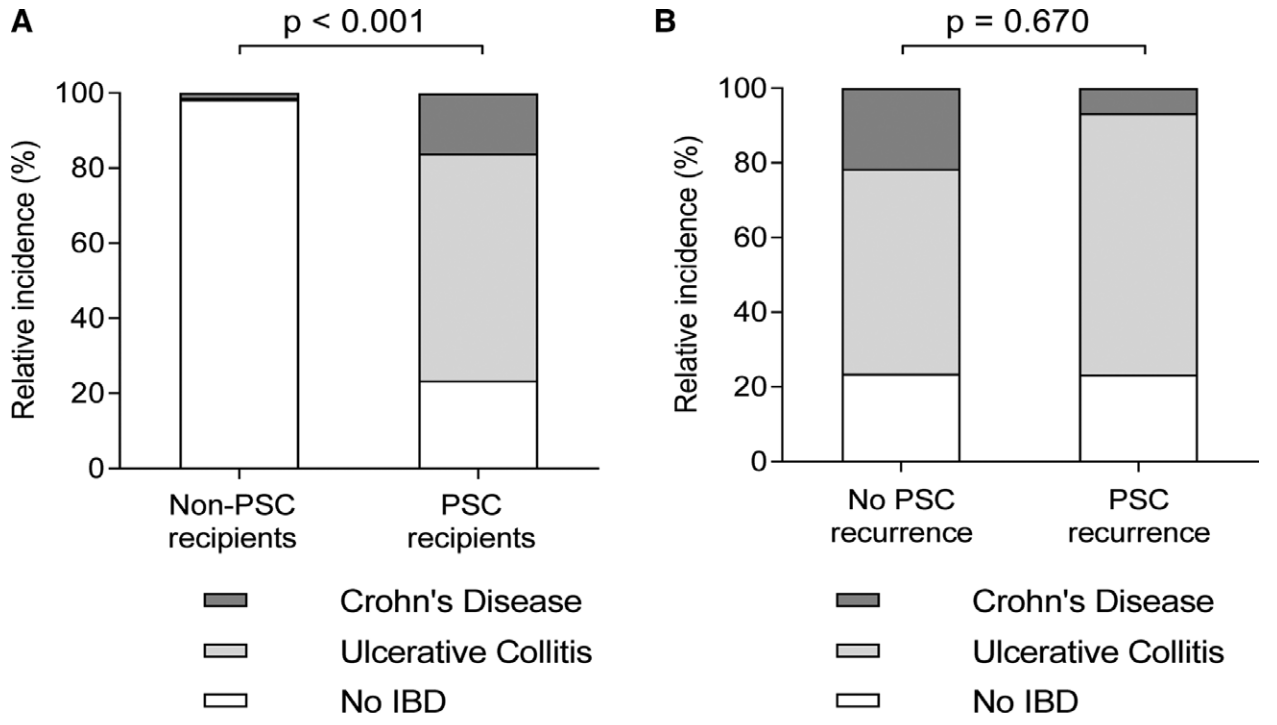


**FIGURE 2.** Inflammatory bowel disease (IBD) is associated with primary sclerosing cholangitis (PSC) but not PSC recurrence (rPSC) after liver transplantation (LT). A, Relative incidence of IBD in patients that were transplanted due to PSC (PSC recipients) or due to other pathologies (non-PSC patients). In particular, ulcerative colitis was associated with PSC, occurring in 60.5% of PSC patients ($P < 0.001$). B, Comparable incidence of PSC recurrence between PSC recipients with or without coexisting ulcerative colitis ($P = 0.670$).

($P < 0.001$) in our LT cohort. In particular, UC was very common among PSC recipients (60.5%) but was almost absent in non-PSC recipients (0.7%; $P < 0.001$; Figure 2A). Thirteen patients in the PSC group are diagnosed with CD (2 patients in the rPSC group and 11 in the non-rPSC group). Of these 13 patients, 3 had Crohn's in their terminal ileum location (TI) (1 and 2 in the rPSC group and non-rPSC group, respectively) and 10 patients had Crohn's colitis. Inflammatory colitis rather than small bowel Crohn's (TI) is associated with the recurrence of PSC (21 inflammatory colitis versus 1 TI).

The onset of PSC recurrence, however, was not significantly different between patients that were diagnosed with IBD when compared with patients that had not developed IBD prior to LT (Figure 2B; $P = 0.670$).

## Relation Between Bacteremia and Recurrence of PSC

In the PSC recipients group (n = 81), 20 patients with PSC recurrence (66.7%) and 28 patients from the nonrecurrence group (54.9%) were tested for bacteremia. All blood cultures were taken on a clinical indication as determined by the responsible physician. The median (IQR) of the C-reactive protein and white blood cell count in patients with bacteremia from the rPSC group were 54 mg/L (34–196) and $13.2 \times 10^9$/L (10.1–15.6), respectively. The median (IQR) of the C-reactive protein and white blood cell count in the rPSC group without bacteremia was 91 mg/L (45.7–127.2) and 11.6 × 109/L (11.1–12.5), respectively (data not shown).

As mentioned earlier, 20 out of the 30 patients in the rPSC group were tested for bacteremia. As shown in Table 2, 10 of them presented with positive cultures. All

**TABLE 2.**

**Patients blood cultures outcomes**

| | PSC recipients | | | Non-PSC recipients | | |
|---|---|---|---|---|---|---|
| | No rPSC | rPSC | | No NAS | NAS | |
| | N = 51 | N = 30 | *P* | N = 235 | N = 36 | *P* |
| Patients with blood cultures taken (%) | 28 (55) | 20 (67) | 0.354 | 152 (65) | 27 (75) | 0.260 |
| Blood cultures outcome | | | **0.027** | | | **<0.001** |
| Patients with only negative blood cultures (%) | 23 (82) | 10 (50) | | 123 (81) | 13 (48) | |
| Patients with positive blood cultures (%) | 5 (18) | 10 (50) | | 29 (19) | 14 (52) | |
| Patients with positive blood cultures for intestinal bacteria (%) | 2 (7) | 9 (45) | **0.004** | 14 (9) | 8 (30) | **0.003** |
| Number of negative cultures (SEM) | 9.1 ± 2.3 | 16.7 ± 5.2 | 0.128 | 12.4 ± 3.4 | 20.7 ± 4.5 | **0.011** |
| Number of positive cultures (SEM) | 0.6 ± 0.4 | 2.9 ± 1.1 | **0.015** | 0.5 ± 0.2 | 2.4 ± 0.9 | **0.012** |

NAS, nonanastomotic biliary strictures; PSC, primary sclerosing cholangitis; rPSC, recurrence of PSC.
*P*-values <0.05 are considered statistically significant and are presented in bold.

of them preceded the diagnosis of rPSC. The median (IQR) time between positive blood culture and rPSC diagnosis was 3.055 (0.62–6.02) y. The median time to diagnosis of recurrence PSC was 4.07 (0.87–9.12) y.

Among all tested PSC recipients, intestinal bacteria was identified in 45% of the rPSC group and in only 7% of the nonrecurrence group ($P = 0.004$). Also, the number of positive cultures isolated from PSC recipients that developed recurrence of disease was significantly higher to the number of positive cultures isolated from those that did not develop rPSC ($P = 0.015$; Table 2).

If we look at the relation between intestinal bacteria and the development of rPSC, in the patient group with positive blood cultures for intestinal bacteremia 82% developed recurrence PSC, whereas patients that only had positive blood cultures for nonintestinal bacteremia or no bacteremia at all developed recurrence PSC in 25.0% and 30.3% of cases ($P = 0.039$ and $P = 0.003$, respectively; Figure 3A). When the cumulative incidence over time was monitored, significantly more PSC patients with intestinal bacteremia developed rPSC compared with those with nonintestinal or no bacteremia (Figure 3B; $P = 0.001$).

A similar association was observed in the non-PSC recipient group (n = 179). As shown in Figure 3C, NAS was significantly more common in LT recipients who tested positive for intestinal bacteria compared with recipients who tested negative (36.4% versus 9.6%, $P = 0.004$). The incidence of NAS was not significantly different in patients that had blood cultures from nonintestinal and intestinal origin. Also the number of positive cultures in the non-PSC recipients who developed NAS compared with patients without NAS was significantly higher ($P = 0.012$; Table 2).

Cumulative incidence over time of NAS was also monitored. Significantly more non-PSC patients with bacteremia, specifically intestinal bacteremia, developed NAS early after LT compared with patients with no bacteremia or nonintestinal bacteremia, who had lower incidence or developed NAS later after LT ($P = 0.01$; Figure 3D).

## Relation of *FUT2* Genotype With PSC, IBD, and PSC Recurrence After LT

SNPs in the *FUT2* gene are one of the genetic risk factors associated with PSC and IBD. Using genome-wide association study, one specific *FUT2* SNP (rs601338) has

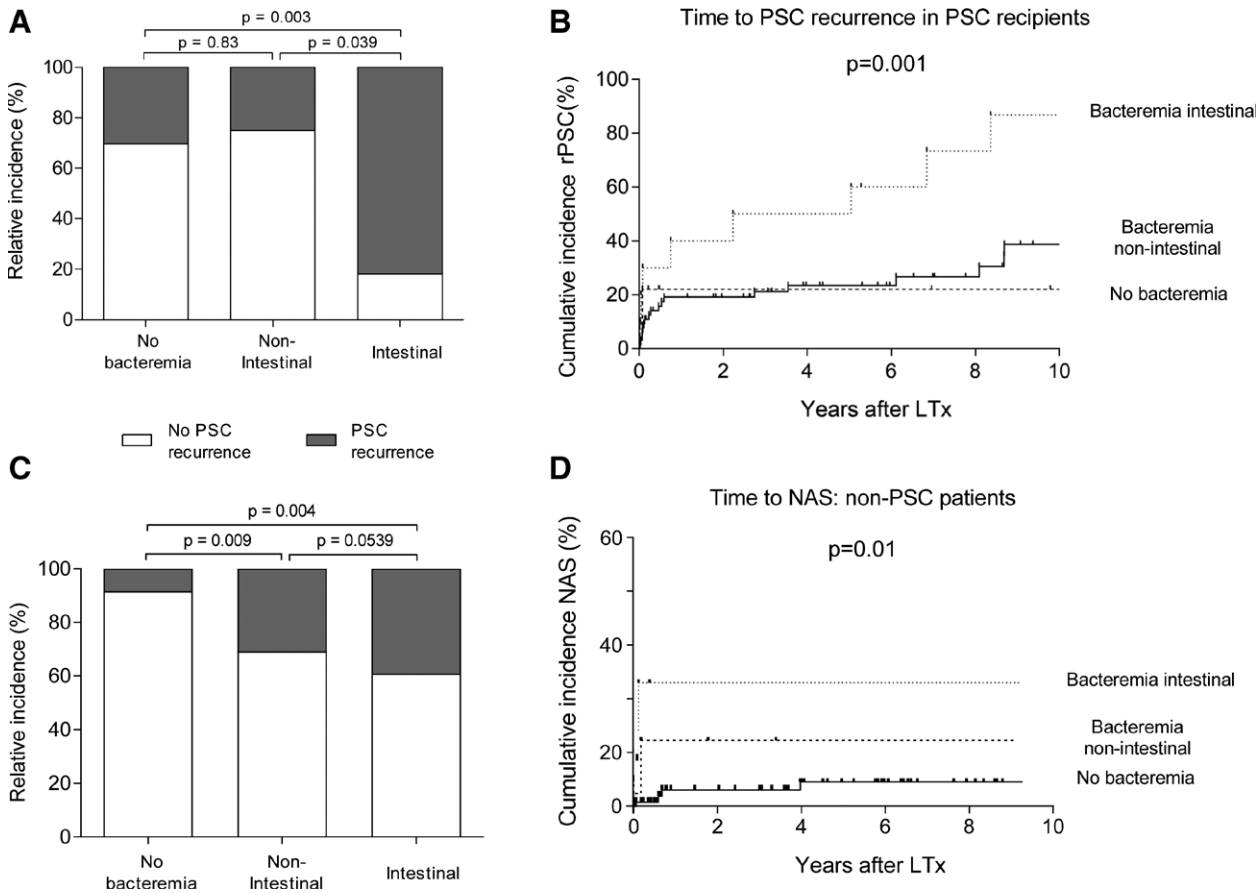


**FIGURE 3.** Relation between bacteremia, recurrence of primary sclerosing cholangitis (PSC) and incidence of nonanastomotic biliary strictures (NAS). Relative (A) and cumulative (B) incidence of recurrence of PSC (rPSC) in relation with detection and type of bacteremia. A, 82% of patients with positive blood cultures for intestinal bacteria developed recurrence of disease; 25.0% of patients with positive blood cultures for nonintestinal bacteremia developed PSC recurrence and 30.3% without positive blood cultures ($P = 0.039$ and $P = 0.003$). B, Cumulative recurrence of disease occurred significantly more often in PSC patients with intestinal bacteremia than in those without bacteremia or with bacteremia with nonintestinal species ($P = 0.001$). C, In non-PSC LT recipients, NAS was significantly more common in recipients who tested positive for intestinal bacteria compared with recipients who tested negative (36.4% vs 9.6%; $P = 0.004$). The incidence of NAS was also significantly different in patients that had blood cultures from nonintestinal origin (28.6% vs 9.6%; $P = 0.009$). D, Cumulative incidence over time of NAS. Significantly more non-PSC recipients with bacteremia, specifically intestinal bacteremia, developed NAS compared with recipients with no bacteremia or nonintestinal bacteremia ($P = 0.01$).

been linked to changes in the intestinal barrier function and changes in the gut microbiome as this mutation results in a truncated, nonfunctional protein.[20,24,25] To confirm this finding in our cohort, the genotype associated with this truncated protein was determined in the donor grafts and in LT recipients with or without PSC and IBD. The homozygous FUT2 nonsecretor status (AA-genotype) was identified in 17.6% of the donors with an allele frequency of 42% (Figure 4A). Recipient genotype distribution in the non-PSC population was not significantly different from that in donors and was therefore also similar to the general population as reported (21.8%, $P = 0.357$). However, analysis of the PSC group showed that the FUT2 nonsecretor status was significantly more frequent in these recipients as compared with donors (33.3% versus 17.6%; $P = 0.006$) or non-PSC recipients (33.3% versus 21.8%, $P = 0.034$; Figure 4A). To determine a possible relationship between FUT2 secretor status and IBD in PSC patients, this cohort was stratified into 3 groups: no IBD, CD, and UC. PSC patients with UC were more likely to have a nonsecretor (AA) genotype compared with patients with CD and non-IBD patients ($P = 0.029$; Figure 4B).

### PSC Recipients With FUT2 Nonsecretor Status Have an Increased Incidence of rPSC and Impaired Graft Survival Following Transplantation

To determine the role of the difference in FUT2 status in the incidence of recurrence of PSC as well as the impact on graft survival in recipients after transplantation, we compared rPSC and graft survival rate with *FUT2* genotyping results.

Recipient nonsecretor status was significantly associated with the development of rPSC after transplantation ($P = 0.001$; Figure 5A). The cumulative incidence of recurrence was ~50% within the first 5 y following LT in patients in the PSC group that had the FUT2 nonsecretor status, against 10% recipients in the PSC group with an *FUT2* secretor status ($P = 0.001$; Figure 5B). Furthermore, graft survival was significantly decreased in patients with a nonsecretor status in PSC recipients ($P = 0.02$; Figure 5C). A *FUT2* genotype in the non-PSC patients has no significant effect on the cumulative incidence of NAS ($P = 0.383$, data not shown).

Importantly, the FUT2 status of the donor graft did neither have an impact on the incidence of PSC recurrence ($P = 0.379$, data not shown) in the PSC group nor on the incidence of NAS in non-PSC group ($P = 0.696$, data not shown).

### Relation Between Bacteremia and FUT2 Status

To determine the relation between bacteremia and difference in FUT2 status, we analyzed the occurrence of bacteremia in PSC recipients after LT and *FUT2* genotyping results. In the PSC cohort, bacteremia was more often diagnosed in patients with a nonsecretor status ($P = 0.024$; Figure 6A). This effect was even more pronounced in the case when blood cultures were positive for intestinal bacteria ($P = 0.003$; Figure 6B). In addition to this observation, there was a significant difference between the cumulative incidence of positive cultures with higher prevalence in

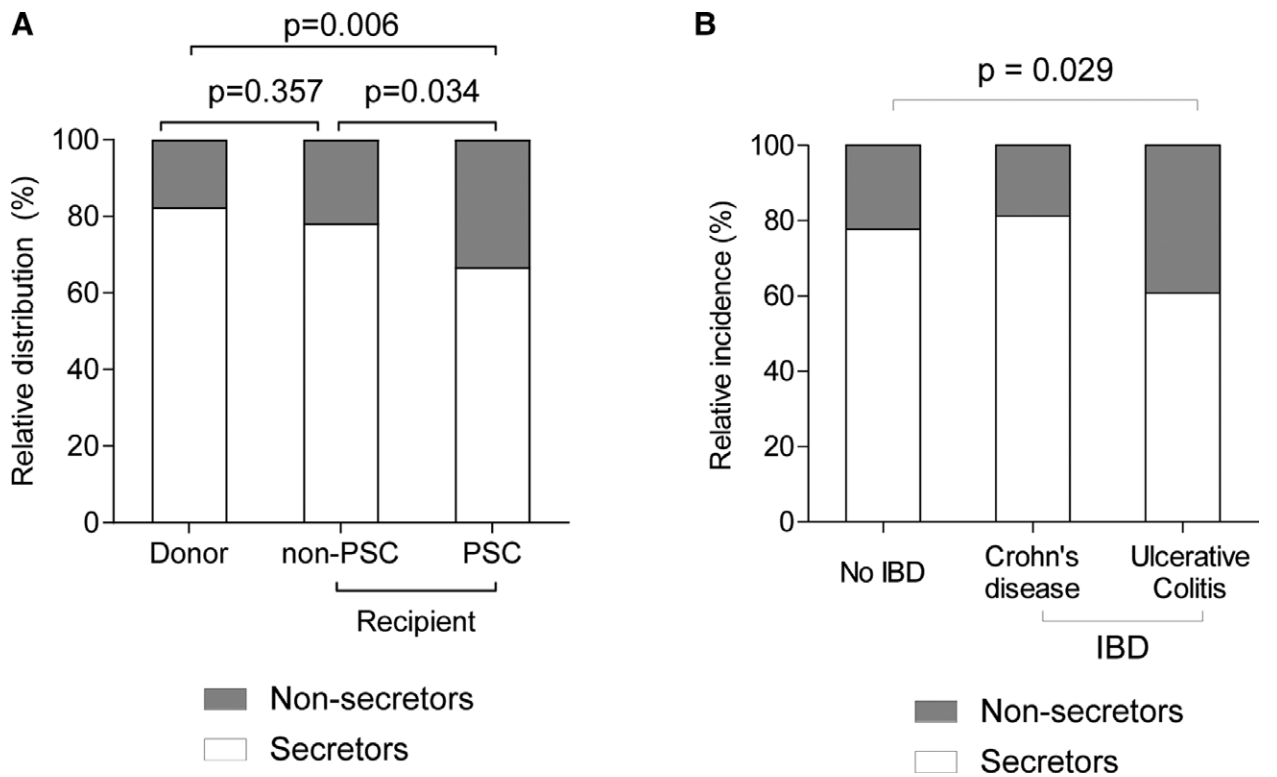


**FIGURE 4.** *FUT2* genotype distribution in donors, nonprimary sclerosing cholangitis (PSC), and PSC recipients. A, In transplant recipients, the percentage of FUT2 nonsecretors (AA-genotype) was significantly higher in PSC patients compared with non-PSC recipients ($P = 0.034$) and with donors ($P = 0.006$). B, FUT2 nonsecretor status in PSC recipients with ulcerative colitis were more likely to have a FUT2 nonsecretor status and is therefore violating the Hardy–Weinberg equilibrium (22.3% vs 18.8% vs 39.2%; $P = 0.029$). FUT2, fucosyltransferase-2.

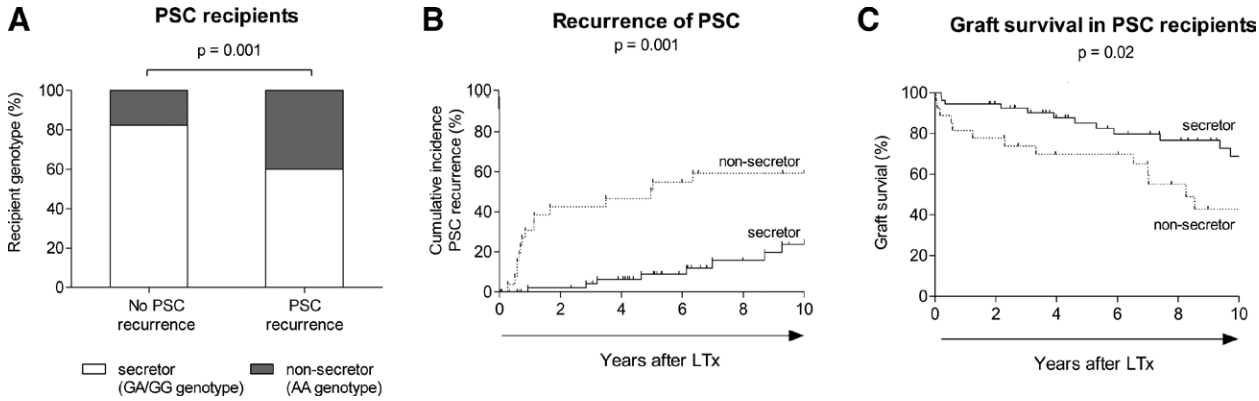


**FIGURE 5.** Cumulative incidence of recurrence of primary sclerosing cholangitis (rPSC) and graft survival percentage in FUT2 secretors vs FUT2 nonsecretors. A, In the PSC cohort, FUT2 secretor status was significantly more frequent in patients with rPSC than in patients that did not have rPSC after transplantation ($P$ = 0.001). B) The incidence of PSC recurrence was 60% in the years following liver transplantation (LT) in FUT2 nonsecretors compared with 10% in FUT2 secretors ($P$ = 0.001). C, PSC recipients with a nonsecretor status (AA) had a decreased graft survival compared with recipients with a secretor status (GA/GG; $P$ = 0.02). FUT2, fucosyltransferase-2.

nonsecretors as compared with recipients with a FUT2 secretor status ($P$ = 0.002; Figure 6C).

## Bacteremia of Intestinal Origin is Associated With *FUT2* Nonsecretor Genotype

Focusing on which type of bacteria were identified in patients that developed the recurrence of PSC and the secretor status, we analyzed intestinal and nonintestinal species isolated from blood.

When we look at the type of bacteria that were isolated from all positive blood cultures and compare the FUT2 nonsecretors group with the secretors group, the frequency of *E. faecium* ($P$ = 0.019), *E. faecalis* ($P$ = 0.023), and *E. coli* were isolated significantly more often from the blood of nonsecretors ($P$ = 0.043), whereas in secretors, blood cultures were significantly more often positive for *Corynebacterium* ($P$ = 0.047), *Klebsiella* ($P$ = 0.040), and *S. epidermidis* ($P$ = 0.032; Figure 7).

## Intestinal Bacteremia and FUT2 Status are Both Risk Factors for PSC Recurrence

To identify all risk factors for rPSC recipients, we performed a multivariate analysis (Table 3). Variables with a $P$-value ≤0.1 were selected for univariate Cox regression analysis (**Table S1, SDC**, http://links.lww.com/TP/C716). These included bacteremia with intestinal bacteria, FUT2 nonsecretor status, recipient age and gender, and cytomegalovirus mismatch between donor and recipient.

Gender mismatch between donor and recipient at the time of transplantation was 42% and 50% in recipients with PSC and without PSC, respectively. Recurrence of PSC was observed in 30 out of 81 PSC patients. In 6 cases, a gender mismatch was observed in the rPSC group, one female-to-male (F/M) and 5 male-to-female (M/F) transplantations. In the nonrecurrence group arm of the PSC group, we identified 26 cases of gender mismatch (15 M/F and 11 F/M). This showed that the gender mismatch observed in the rPSC versus non-rPSC was highly significant ($P$ = 0.006, $\chi^2$ test). When looking at F/M or M/F separately, only a significant difference for F/M mismatch in the rPSC versus nonrecurrence PSC groups was observed and not for the M/F mismatch ($P$ = 0.03 and $P$ = 0.20, respectively). This suggests a protective effect of recurrence in F/M mismatched pairs.

In a multivariate analysis, however, donor–recipient gender mismatch remained an independent risk factor for the development of PSC recurrence (HR, 0.57; $P$ = 0.002).

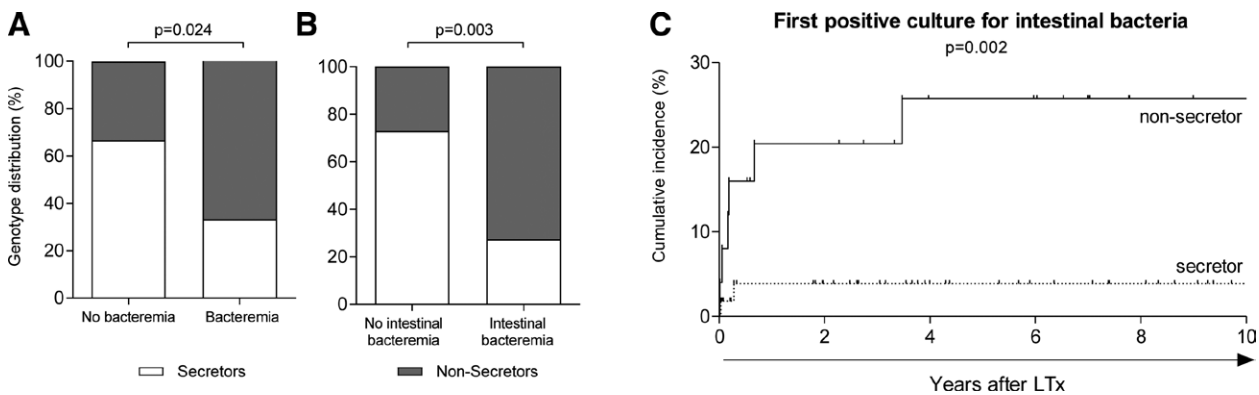


**FIGURE 6.** Prevalence of (origin of) bacteremia in patients with FUT2 secretor vs FUT2 nonsecretor status. A, Primary sclerosing cholangitis (PSC) patients with a FUT2 nonsecretor status were more often diagnosed with bacteremia ($P$ = 0.024). B, In a subanalysis for bacteremia with intestinal bacteria, recipients with a nonsecretor status were more at risk ($P$ = 0.003). C, Recipients with a nonsecretor status were more likely to have a first positive blood culture with intestinal bacteria ($P$ = 0.002). FUT2, fucosyltransferase-2.

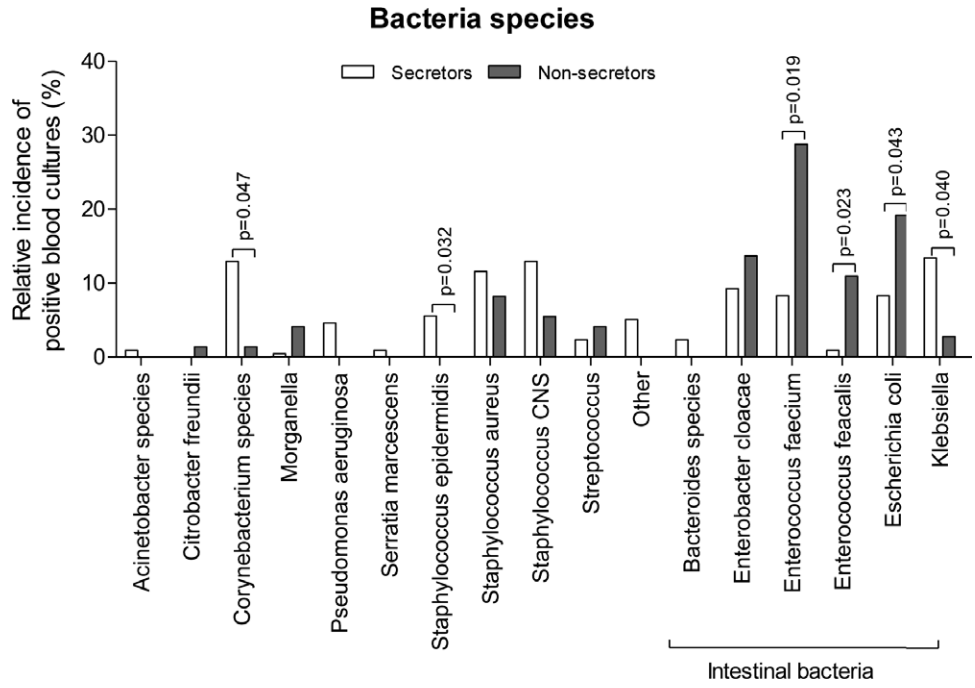


**FIGURE 7.** Bacteremia with species of intestinal origin is associated with *FUT2* nonsecretor genotype. *E. faecium* ($P = 0.019$), *E. coli* ($P = 0.043$), and *E. faecalis* ($P = 0.023$) were relatively most prevalent in nonsecretors. *Corynebacterium* ($P = 0.047$) and *Klebsiella* ($P = 0.040$) and *S. epidermidis* ($P = 0.032$) were more prevalent in secretors. FUT2, fucosyltransferase-2.

Because of the high correlation between *FUT2* genotype and the incidence of bacteremia as shown previously, these variables were combined in a single variable with 4 categories. FUT2 secretor status combined with no intestinal bacteremia was not significant ($P = 0.903$, data not shown). FUT2 secretor status (genotype GA or GG) combined with bacteremia was a significant predictor of PSC recurrence (HR, 2.54; $P = 0.014$). Similarly, FUT2 nonsecretor status without intestinal bacteremia was an independent predictor of PSC recurrence but less significant (HR, 1.96; $P = 0.023$). When both FUT2 nonsecretor status and bacteremia of intestinal origin were present, this was a highly predictive factor for PSC recurrence (HR, 15.36; $P < 0.001$; Table 3). This suggests that both recipient FUT2 status and bacteremia independently are highly associated with PSC recurrence after LT, with a strong additive effect of the 2 when both are present.

## DISCUSSION

In this article, we show that PSC patients with bacteremia from intestinal origin have an increased risk of rPSC after transplantation and that this increased risk is associated with the FUT2 nonsecretor status (genotype AA of the sr601338 SNP). This study is the first to analyze the association of bacteremia after LT and FUT2 status in PSC patients within one cohort. Although our study does not contain an independent validation cohort and the number of patients in the PSC cohort was relatively small with only 81 recipients, the data presented here do confirm the findings of previous large genome-wide association studies in the context of PSC, a common indication for LT.[24] Identifying bacterial species based on rRNA16s is an advanced method for bacteremia research and has the power to identify species that are otherwise undetected using classic culturing conditions. As rRNA16s sequencing, metagenomics, and transcriptomics are not

**TABLE 3.**

**Cox regression analysis for potential risk factors for PSC recurrence**

| | Multivariate | |
|---|---|---|
| | **Hazard ratio** | ***P* (95% CI)** |
| Variables in PSC recipients | | |
| Gender mismatch | 0.57 | 0.002 (0.27-0.90) |
| FUT2 + intestinal bacteremia | | |
| Secretor status (GA/GG) – no bacteremia | NA. | |
| Secretor status (GA/GG) – bacteremia | 2.54 | 0.014 (1.98-4.11) |
| Nonsecretor status (AA) – no bacteremia | 1.96 | 0.023 (1.43-2.47) |
| Nonsecretor status (AA) – bacteremia | 15.36 | <0.001 (8.45-19.75) |

Cox regression analysis of potential risk-factors for the development of recurrence of disease in PSC recipients including the combined factors of intestinal bacteremia and FUT2 status in 4 different categories. Factors from univariate analysis with a *P*-value <0.05 were included for multivariate analysis.
CI, confidence interval; FUT2, fucosyltransferase-2; PSC, primary sclerosing cholangitis.

yet implemented in clinical practice, this information is, unfortunately, unavailable because all samples were taken for clinical purposes and analyzed in a retrospective manner, not for research.

Confounding factors related to bacteremia, such as the type of antibiotics used by the individual patients, were not examined in this study. Although antibiotics as a treatment for bacteremia in PSC patients might select multidrug-resistant species, further research should take the use of antibiotics both pre- or posttransplantation, into account.

We demonstrated that bacterial translocation is highly associated with recurrence of PSC after LT. Also, a FUT2 nonsecretor status is an independent risk factor for the development of recurrence of disease in patients with PSC following LT. We also showed that the FUT2 nonsecretor status is also associated with bacterial translocation as measured by positive blood cultures for intestinal bacteria. Therefore, patients with a FUT2 nonsecretor status have an increased risk of bacteremia and subsequently, of recurrence of PSC after transplantation.

The characterization of the gut microbiome and determination of differences in IBD, PSC, PSC-IBD, and healthy controls has been of major interest in the last years.[33,34] Despite the fact that recent literature identified IBD, and in particular UC, as an independent risk factor for rPSC,[35] this was not confirmed by our study. There is, however, also evidence contradicting this association and provided evidence that neither high IBD activity nor the presence of IBD flares before or after LT were associated with rPSC.[36]

Analysis of patients with or without active IBD was not possible as all patients receiving a liver transplant were without active signs of IBD. Moreover, preoperative concomitant active IBD was a relative contraindication to LT due to an increased risk of thrombosis, escalation of the IBD, possible generalization of infection, and septic status in the postoperative period. Cheng et al[27] showed that *FUT2* loss-of-function mutations are highly prevalent and are associated with IBD. Our results also confirmed the hypothesis that *FUT2* loss-of-function mutation participates in the IBD pathogenesis by decreasing binding sites for adherent bacteria, thus altering the gut microbiome. Decreased abundances of adherent bacteria may allow the overgrowth of bacteria that induce inflammatory T cells, leading to intestinal inflammation.

PSC patients showed reduced microbial gene richness and a difference in abundance of several species in feces.[13,17,19,32] In addition, these PSC patients also showed dysbiosis in fecal and salivary samples, independent of the co-existence of IBD.[30] On the species level, PSC patients showed a decrease in the relative abundance of commensal bacteria and an increase of potentially pathogenic species as compared with healthy controls.[37–39] Although the observations made in these studies are sometimes heterogeneous, several similarities are described for the fecal microbiome: PSC patients present lower alpha diversity as well as dysbiosis compared with healthy controls, although this alpha diversity does not seem to be correlated with FUT2 status. Although detailed analysis of taxonomic levels was not always consistent, a few common patterns can be observed: elevation of *Veillonella* and *Clostridium* species and of the genera *Streptococcus*, *Lactobacillus*, and *Enterococcus*, and, with a lower abundance, of *Faecalibacterium* and *Coprococcus*.[40]

The prevalence of the *FUT2* genotype AA is increased in recipients with PSC and has a pronounced effect on the incidence of recurrence of disease. We furthermore show that FUT2 nonsecretors have an increased incidence of bacteremia, especially with bacteria from intestinal origin. Bacteremia was also one of the predictors of recurrence of disease in our cohort. The increased risk on rPSC after LT in nonsecretors is a novel finding in PSC patients. Our data are consistent and supported by recent literature that showed that nonsecretor status is strongly associated with adverse outcomes in patients with PSC and UC, including overall mortality and death from sepsis supports our data.[22–24] Not only do the results of our study confirm the pathophysiological role of bacteremia and altered glycosylation in PSC as identified by previous studies; it is also the first study that shows that the *FUT2* genotype is an independent risk factor for transplant outcome following LT.

The positive blood cultures for intestinal types of bacteria were also significant different in the group of non-PSC patients with nonanastomotic strictures ($P = 0.003$). This finding confirmed the thesis that bacteria can cause NAS, as mentioned in previous publications.[41–43] Although the study focuses on risk factors for PSC recurrence, we find it relevant and important to include the non-PSC transplant recipients in our analyses. The fact that we observe that intestinal bacteremia is also a risk factor for NAS in the general (non-PSC) LT population further supports our conclusions. Both NAS and PSC recurrence are assumed to share some pathophysiological mechanisms. The fact that intestinal bacteremia is both associated with NAS and PSC recurrence provides cross-validation of our findings. For this reason, we want to remain the non-PSC recipients in our study. In our PSC cohort, we showed not only prevalence of some of the different types of bacteria associated with PSC but also the dysbiosis in relation to FUT2 status (secretors versus nonsecretors) where bacteremia was diagnosed more often in patients with a nonsecretor status. This effect was even more pronounced when only intestinal bacteria were taken into consideration. Over time, we observed a significant difference between the cumulative incidence of positive cultures with higher prevalence in nonsecretors. Out of all positive blood cultures obtained from nonsecretors and compared with secretors, *E. faecium*, *E. faecalis*, and *E. coli* were identified with significantly higher frequency. In blood cultures from secretors, *Corynebacterium*, *Klebsiella*, and *S. epidermidis* were more prevalent.

Moreover, FUT2 nonsecretors have a different, less diverse, bacterial composition of their intestine.[44] These factors could lead to increased bacterial translocation to extraintestinal sites and might explain the involvement of FUT2 dysfunction in other diseases like chronic pancreatitis and autoimmune disorders, for example, psoriasis and Bechet's disease.[45–48] Remarkably, the correlation between FUT2 dysfunction and UC is less clear, with contradictory results in various studies.[8–26]

In the current study, we observed a higher frequency of FUT2 nonsecretors in LT recipients also suffering from UC, compared with recipients with CD or without IBD. This suggests that rPSC is a consequence of intestinal barrier defects that also affects the intestine and the intestinal microbiome and is not an intrinsic liver-originating phenotype.

Beside the intestine, Folseraas et al[20] demonstrated that a FUT2 nonsecretor status also changes the bacterial composition of bile. This finding supports the hypothesis that in patients with PSC, adhesion of specific bacteria to the cholangiocyte epithelium could provoke recurrent episodes of cholangitis and subsequent recurrence of disease. Because the intestinal epithelium is affected by the *FUT2* mutation, bacteria or bacterial endotoxins from the intestinal site can translocate to the liver via the portal circulation and cause inflammation in the liver.[18] Due to the immunosuppressive therapy all LT recipients receive, the immune response of the recipient might be decreased, and bacteremia occurs more frequently. The detection of *Enterococcus* (in particular *Enterococcus faecalis*), *Escherichia coli*, and *Candida* in bile of LT recipients with posttransplant biliary complications has been associated with shortened retransplantation-free survival.[49,50]

This study demonstrates that bacterial translocation is highly associated with rPSC after LT. FUT2 nonsecretor status is an independent risk factor for the development of recurrence of disease in patients with PSC following LT and for bacterial translocation as measured by positive blood cultures for intestinal bacteria. Therefore, patients with a FUT2 nonsecretor status have an increased risk of bacteremia and subsequently, of rPSC, after transplantation. Although an important determinant in the development of rPSC, it is evident that the FUT2 nonsecretor phenotype is not the only explanation for recurrence of the disease. A large number of patients in the rPSC have at least 1 functional *FUT2* allele. Other risk factors for rPSC, for example, age, tacrolimus treatment will also play an important role.[36]

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