Liver Preservation by Aortic Perfusion Alone Compared With Preservation by Aortic Perfusion and Additional Arterial Ex Situ Back-Table Perfusion With Histidine-Tryptophan-Ketoglutarate Solution: A Prospective, Randomized, Controlled, Multicenter Study

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Background. Arterial ex situ back-table perfusion (BP) reportedly reduces ischemic-type biliary lesion after liver transplantation. We aimed to verify these findings in a prospective investigation. Methods. Our prospective, randomized, controlled, multicenter study involved livers retrieved from patients in 2 German regions, and compared the outcomes of standard aortic perfusion to those of aortic perfusion combined with arterial ex situ BP. The primary endpoint was the incidence of ischemic-type biliary lesions over a follow-up of 2 years after liver transplantation, whereas secondary endpoints included 2-year graft survival, initial graft damage as reflected by transaminase levels, and functional biliary parameters at 6 months after transplantation. Results. A total of 75 livers preserved via standard aortic perfusion and 75 preserved via standard aortic perfusion plus arterial BP were treated using a standardized protocol. The incidence of clinically apparent biliary lesions after liver transplantation (n = 9 for both groups; P = 0.947), the 2-year graft survival rate (standard aortic perfusion, 74%; standard aortic perfusion plus arterial BP, 68%; P = 0.34), and incidence of initial graft injury did not differ between the 2 perfusion modes. Although 33 of the 77 patients with cholangiography workups exhibited injured bile ducts, only 10 had clinical symptoms. Conclusions. Contrary to previous findings, the present study indicated that additional ex situ BP did not prevent ischemic-type biliary lesions or ischemia-reperfusion injury after liver transplantation. Moreover, there was considerable discrepancy between cholangiography findings regarding bile duct changes and clinically apparent cholangiopathy after transplantation, which should be considered when assessing ischemic-type biliary lesions.

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schematic-type biliary lesions (ITBLs) are a major complication occurring in up to 30% of patients who receive orthotopic liver transplantation; typical cholangiography findings include nonanastomotic bile duct strictures, dilatations, mucosal irregularities, and sludge formation, without hepatic artery thrombosis or stenosis. The sequelae, namely, cholestasis and cholangitis, entail interventional or endoscopic treatment, reoperation, and even retransplantation. Several classifications of bile duct changes have been proposed, and lesions affecting extrahepatic and intrahepatic bile ducts are differentiated from those involving the extrahepatic portion alone.

In individual patients, ITBL pathogenesis typically remains obscure. Three interacting pathophysiological concepts are assumed to be involved. The first is ischemia-reperfusion injury, known to be related to donor factors, mode of preservation, ischemia time, and mode of recirculation. Of note, molecular markers of epithelial damage after preservation correlate with the future development of ITBLs. Immunological factors also contribute to the development of ITBLs, and involve ABO incompatibility, rejection, cytomegalovirus infection, genetic polymorphism, and histocompatibility antibodies. Finally, factors, such as cytotoxic hydrophobic bile salts and impaired bile salt/phospholipid ratios, are relevant.

Early-onset ITBLs, obviously related to ischemia-reperfusion injury, are distinguished from late-onset ITBLs, which are attributed to immunological factors. Late-onset biliary strictures in recurrent primary sclerosing cholangitis, as well as other autoimmune diseases, represent a special form of immunological etiology and should be distinguished from ITBLs.

Recently, the question arose if the damage itself or the lack of regenerative capacity is crucial for the development of ITBLs. This question is worth considering, as impaired blood supply to the bile ducts demonstrated as early as after preservation was associated with the development of strictures after transplantation, which is in line with the early hypothesis that damage to the peribiliary vascular plexus may be involved in the formation of biliary strictures. In an attempt to prevent vascular damage, numerous studies focused on the effects of low viscosity solutions, such as histidine-tryptophan-ketoglutarate (HTK) or Marshall solution, and high-pressure perfusion.

In 2003, we reported on the effect of controlled ex situ arterial back-table perfusion (BP) after in situ aortic perfusion (standard aortic perfusion [SAP] + BP) in terms of preventing ITBLs. Our previous findings were based on a retrospective review of the records of patients who received liver grafts preserved using SAP, which is the routine protocol followed in Germany, or grafts preserved using SAP + BP. ITBLs were diagnosed in 21 (16%) of 131 grafts preserved using SAP, but only in 1 of 59 grafts preserved using SAP + BP. To confirm the role of ex situ BP in preventing ITBLs after transplantation, we initiated a multicenter study involving 13 liver transplant centers in Germany. The rationale of the study involved analyzing the outcomes of controlled flushing of the peribiliary plexus by arterial perfusion after retrieval.

**MATERIALS AND METHODS**

**Study Design**

This randomized, 2-arm, multicenter study included all consecutive full-size liver grafts procured between October 2007 and March 2010 from brain-dead donors aged 15 to 85 years in the Mitte and Nord regions defined by the German Organ Retrieval Foundation. An advisory board was established to control the course of the study and to monitor data safety. Liver grafts were randomly assigned to be preserved using either SAP or SAP + BP. Grafts were procured by 7 organ recovery teams and transplanted in 13 German transplantation centers blinded with respect to the graft preservation mode.

The study was performed per the ethical principles of the Declaration of Helsinki and was approved by the ethical commissions of all participating centers. Primary ethical approval was given by the Ethical Committee of Rhineland-Palatinate under the number 387.364.06 (5462) on December 20, 2006. All patients included in the final analysis provided written informed consent, either before inclusion in the study or before any data acquisition or study-specific investigations. Informed consent before data acquisition or study-specific investigations, instead of consent before inclusion in the study, was stipulated by the Ethical Committee of Rhineland-Palatinate, as both types of perfusion had been routinely used in Germany for several years before initiation of the study, and patients undergoing liver transplantation could receive a graft preserved either by SAP or SAP + BP. Therefore, consent to data acquisition and study-specific workup, but not consent to the type of perfusion, was required for participation in the study.

The study hypothesis postulated superiority of SAP + BP over SAP in preventing ITBLs. A sample size of 75 patients per study group was calculated to be sufficient for detecting a difference of 15% in ITBL incidence at 2 years after transplantation with a statistical power of 80% and a significance level of 5%. The difference in ITBL incidence was anticipated to be 15% based on our previous findings (ie, 16% in the SAP group vs 2% in the SAP + BP group).
The assessment was performed separately for the extrahepatic common bile duct, including the hilar bifurcation (zone A), the right- and left-sided bile ducts between the first- and second-order branches (zone B), the bile ducts between second- and third-order branches (zone C), and bile ducts in the periphery of the liver (zone D). Per these 4 zones, the presence and location of nonanastomotic biliary strictures, dilatations, irregularities, and cast formation were recorded, and the findings were classified as indicative of mild, moderate, or severe ITBLs.

Statistical Analyses
Censored time-to-event data were displayed using Kaplan-Meier estimates and tested with log-rank tests. Proportions were compared using the Fisher test, and the Mann-Whitney test was used for continuous variables. All analyses were performed using IBM SPSS Statistics v20 (IBM Corp., Armonk, NY).

RESULTS
Grafts and Patients
A total of 299 liver grafts were randomized. Fifty-seven grafts had to be excluded from the study due to graft splitting (n = 4), mandatory allocation to nonparticipating transplant centers (n = 33), or refusal of the liver graft by the transplant teams (n = 20). Of the remaining 242 grafts, 92 could not be included in the evaluation because of patient death before consent (n = 44), primary nonfunction of the graft and retransplantation (n = 10), or withdrawal of consent by the recipient (n = 38; Figure 1). The remaining 150 liver grafts were equally distributed between the study groups and form the per-protocol study population used for the evaluations described herein. Demographic and other baseline characteristics of donors, grafts, and recipients were comparable between the SAP and SAP + BP groups (Table 1).

Survival and Incidence of ITBLs
Survival analysis was performed for all patients followed up for at least 2 years. Overall, the patients were followed
up for a median of 31.5 months (0.3-51.1 months) in the SAP group and 25.3 months (0-49.2 months) in the SAP + BP group (P = 0.218). Within the first 24 months after transplantation, 29 of the 150 patients died, of whom 14 in the SAP group and 15 in the SAP + BP group, indicating a 2-year survival rate (Kaplan-Meier) of 79.6% and 76.3%, respectively (P = 0.771).

The primary endpoint of the study (ie, clinically apparent ITBLs at the 24-month follow-up after transplantation) is provided as the Kaplan-Meier estimate (Figure 2). Two years after liver transplantation, the ITBL rate was identical in the 2 groups.

Laboratory Data
Peak AST and ALT levels indicative of parenchymal damage within the first 3 days after transplantation were comparable between the SAP and SAP + BP groups (Table 2). However, the number of patients with marked increase in the levels of transaminases (AST and/or ALT exceeding 2000 U/L) differed between the SAP group (n = 28) and the SAP + BP group (n = 15; P = 0.03). The levels of bilirubin and enzymes reflecting bile duct injury at 6 months after transplantation were comparable between the 2 groups (Table 2).

Cholangiography Findings
Cholangiography data were available for central radiological assessment in 77 patients, of whom 38 in the SAP group loss occurred in 19 cases in the SAP group and 21 cases in the SAP + BP group (25.8% and 30.6% in the Kaplan-Meier estimate, respectively; P = 0.680; Figure 3).

Table 1: Donor and recipient characteristics

|                     | SAP (n = 75) | SAP + BP (n = 75) | P     |
|---------------------|-------------|------------------|-------|
| Donor characteristics|             |                  |       |
| Age, y              | Median (range) | 53.0 (19-86)     | 56.0 (15-85) | 0.824 |
| US-DRI              | Median (range) | 1.8 (1.11-2.73)  | 1.94 (1.13-2.80) | 0.406 |
| ET-DRI              | Median (range) | 2.0 (1.23-3.60)  | 1.96 (1.22-2.94) | 0.775 |
| Body mass index, kg/m² | Median (range) | 25.7 (17.3-39.2) | 26.0 (18-39.8) | 0.806 |
| Cause of death, n   |             |                  |       |
| Cerebrovascular disease | 26          | 22               |       |
| Hypoxia             | 15          | 19               |       |
| Spontaneous vascular event | 24          | 22               |       |
| Traumatic injury    | 10          | 12               |       |
| Ischemia time: median (range), h  | Cold ischemia time | 8.5 (5.12-15.7) | 8.6 (4.0-14.0) | 0.848 |
|                     | Warm ischemia time | 0.67 (0.3-1.3) | 0.67 (0.4-1.3) | 0.978 |
| Recipient characteristics |             |                  |       |
| Sex, n              |             |                  |       |
| Female              | 25          | 21               | 0.596 |
| Male                | 50          | 54               |       |
| Age, y              | Median (range) | 56.1 (17.9-74.1) | 56.0 (17.8-70.0) | 0.913 |
| Primary reason for LT; n |             |                  |       |
| Alcoholic cirrhosis | 18          | 16               | 0.503 |
| HCC                 | 22          | 30               |       |
| Hepatitis B         | 0           | 3                |       |
| Hepatitis C         | 7           | 8                |       |
| PBC/PSC             | 12          | 5                |       |
| Cryptogenic cirrhosis | 7         | 5                |       |
| Fulminant hepatic failure | 5          | 4                |       |
| Others              | 4           | 4                |       |
| MELD score (points) | Median (range) | 22.0 (7-40)      | 22.0 (6-40) | 0.217 |

US-DRI, United States Donor Risk Index; ET-DRI, Eurotransplant Donor Risk Index; LT, liver transplantation; HCC, hepatocellular carcinoma; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; MELD, Model of End-Stage Liver Disease.

![Figure 2](image-url) Incidence of clinically symptomatic ITBLs after transplantation of livers preserved using SAP alone or using SAP + BP. At 2 years after liver transplantation, the ITBL rate was identical in the 2 groups.

![Figure 3](image-url) Graft survival (death or retransplantation) after transplantation of livers preserved using SAP alone or using SAP + BP. At 2 years after liver transplantation, graft survival was comparable between the groups.
and 39 in the SAP + BP group (Figure 4). Bile duct changes on cholangiography were demonstrable in 12 of 38 grafts preserved using SAP and 21 of 39 grafts preserved using SAP + BP (P = 0.07). Of the 33 patients who exhibited bile duct changes on cholangiography, only 10 experienced clinical symptoms of ITBLs (5 of 12 in the SAP group and 5 of 21 in the SAP + BP group; P = 0.43). Notably, a total of 12 patients had clinically apparent ITBLs. Ten symptomatic ITBLs occurred in the 33 patients who showed bile duct changes on cholangiography, compared with 2 clinically apparent ITBLs in 44 patients with apparently normal cholangiography (P < 0.01).

In 36 patients, the quality of cholangiography allowed for an assessment of all 4 bile duct regions (zones A-D), and the results of this detailed analysis are listed in Table 3.

**Safety Analysis**

The safety analysis showed no significant differences between the treatment groups. All adverse events were related to problems and injuries caused by liver transplantation, side effects of immunosuppressive drugs, procedural complications, or infection, and could not be attributed to the perfusion mode.

**DISCUSSION**

The prevention of ITBLs after liver transplantation remains challenging. The use of grafts from marginal donors and elderly donors has steadily increased during recent years,9,13 leading to higher risk of developing ITBLs. Donor age is particularly high in Germany (Table 1). Because it is currently not possible to control immunological pathomechanisms or the detrimental influence of bile salts and other molecular mechanisms, prevention of ITBLs has been focused on clinical issues such as stabilization of the potential donor, improvement of graft retrieval, usage of improved preservation solutions, mode of preservation, reduction of ischemia time, and bile duct reconstruction.7,13,16

When SAP + BP was adopted in our procurement region in the year 2000, we followed the rationale of hydrodynamics in the cardiovascular system, as described by R. M. Anderson.38 Specifically, the principal requirement of normal circulation is a closed circuit filled with liquid at a positive mean pressure. During aortic preservation, the system is no longer a closed circuit, as it has usually been opened at the intracardiac portion of the caval vein to allow free outflow of perfusate. Positive intravascular basal pressure can, therefore, never develop in this system, not even if high pressure is applied to the perfusate bag during aortic perfusion. It is the lack of this positive basal intravascular pressure which theoretically prevents adequate perfusion of arteries deriving from the aorta. Hemodynamic measurements corroborated this explanation, as the amount of perfusate reaching the hepatic artery during aortic perfusion proved to be negligible.39 This shortcoming of SAP may lead to inadequate protection of the bile duct vasculature during cold storage. In view of this theory, our former results36 demonstrating a reduction in the incidence of ITBLs from 16% (SAP) to 2% (SAP + BP) appeared reasonable. Nevertheless, further confirmation was required before changing the general recommendation for preservation mode in our country; hence, the present randomized, multicenter study was initiated.

In this study, the 2-year incidence of clinically apparent ITBLs was comparable between the SAP and SAP + BP groups, as was graft survival rate. Based on these results, the superiority of back-table pressure perfusion in preventing ITBLs can be excluded. Similarly, no difference was noted between the groups in terms of graft damage, as assessed by

| Grading of ITBL | Lesion A (n) | Lesion B right (n) | Lesion B left (n) | Lesion C right (n) | Lesion C left (n) | Lesion D right (n) | Lesion D left (n) | ITBL total (n) |
|-----------------|-------------|-------------------|------------------|-------------------|------------------|-------------------|-----------------|---------------|
| None            | 22          | 22                | 27               | 26                | 30               | 36                | 36              | 11            |
| Mild            | 2           | 4                 | 2                | 6                 | 2                | 2                 | 2               | 1             |
| Moderate        | 7           | 6                 | 3                | 2                 | 4                | 2                 | 3               | 4             |
| Severe          | 5           | 4                 | 4                | 2                 | 3                | 1                 | 1               | 0             |

The most severe damage identified in 1 of the 4 zones (A-D) was used to classify the total radiological severity of the biliary damage (last columns; significance of differences between the last 2 columns: P = 0.03).

**FIGURE 4.** Cholangiography and clinical findings of ischemic-type biliary lesions after transplantation of livers preserved using SAP alone or using SAP + BP.
peak levels of transaminases within 3 days after transplantation. The only difference between the groups was in terms of the number of patients with marked increase in transaminase levels (28 in SAP vs 15 in SAP + BP; P = 0.03), but this observation may related to the arbitrary choice of the cutoff value of 2000 U/L.

How can the present negative result be explained in context of the superiority of SAP + BP observed in our previous, retrospective study? Several aspects may have contributed to this discrepancy. For example, when the former study was performed, University of Wisconsin solution was the standard preservation solution used in Germany. However, it appears highly improbable that the different viscosity of the preservation solutions (University of Wisconsin solution versus HTK) could have accounted for the negative results observed in the present study, because animal experiments indicated that arterial flow rates are not significantly affected by the viscosity of the solution used for SAP and BP. The volume of perfusate used for BP may also play a role. Nevertheless, in the former study as well as in the present study, BP was performed with 300 mL of the respective solution. Because the same volume of preservation solution was used in both studies, this factor does not explain the discrepancy in the results. Moreover, this volume of solution is expected to be sufficient for rinsing the hepatic arteries and arterioles several times, because experiments using vascular corrosion casting have indicated that the volume of these vessels hardly exceeds 50 mL. Therefore, we believe that the negative results obtained in the present study provide a realistic view of the comparative outcomes expected for SAP and SAP + BP perfusion.

Despite the overall negative outcome as to the primary and secondary endpoints, some findings noted in the present study deserve consideration. The great discrepancy between visible bile duct changes on cholangiography and clinical manifestation of ITBLs represents an interesting point. In 77 imaging studies, the rate of radiologically apparent cholangiopathy compared with clinically symptomatic ITBLs differed by a factor of 3 (33 radiological vs 10 symptomatic ITBLs). This remarkable discrepancy may explain the wide range of ITBL rates reported in the literature. Different attitudes toward posttransplant workup of bile ducts and lack of unequivocal definitions result in variations of reported ITBLs. Accordingly, the results of our former analysis, which prompted the present multicenter study, may be explained as follows: Due to the principles of organ allocation in Germany, grafts are usually transplanted in centers different from the retrieving center. In the authors’ center, routine radiography is performed in all patients through a biliary drainage placed during transplantation, which resulted in a high rate of “visible” ITBLs. In other transplantation centers that used grafts treated with BP and retrieved by the authors, reports mostly cited clinical ITBL rates. Therefore, it was not BP, but the approach to diagnostic workup and definition which led to the observation of the supposed “superiority” of BP in our previous study.

Another point was the time to clinical manifestation of cholangiopathy. Sixteen of 18 patients experienced symptomatic ITBLs within the first year after transplantation, while just two (1 in each group) were diagnosed later than that. Although the late occurrence of ITBLs (ie, after the observation period had ended) cannot be excluded, the pathomechanisms affecting biliary integrity before or shortly after transplantation are responsible for most ITBLs and therefore deserve attention. Accordingly, major studies focused on primary damage. Numerous alterations in the epithelial layer and the wall of the bile ducts are detectable as early as at the end of cold storage, and this type of injury becomes more pronounced after reperfusion. Injury to the deep peribiliary glands and microvasculature was found to be associated with the development of ITBLs. Hansen et al reported that arteriolar necrosis was the most prominent predictor of ITBL development, whereas epithelial damage was visible after reperfusion in almost all patients (99%). So far, it remains obscure if insufficient regeneration due to impaired blood supply or due to loss of biliary stem cells located in the epithelial crypts is pivotal for the development of ITBLs.

In this context, the implementation of machine preservation, which provides a more physiological method of perfusion, should be noted. Particularly, regarding donation after cardiac death (which is not performed in Germany) and extended criteria donation, encouraging results have been reported after machine preservation in comparison to those noted for cold storage. So far, it remains obscure whether continuous perfusion, the avoidance of hypoxia or hypothermia, rewarming after storage, or all the abovementioned procedures are crucial for the effectiveness of machine preservation. Both experimental models and clinical studies have used perfusate oxygenated under hypothermic or normothermic conditions and it was found that using perfusate containing red blood cells led to attenuation, but not complete prevention, of biliary complications. In view of these conflicting findings, further clinical studies are warranted to clarify the pathomechanism of early cholangiopathy after liver transplant.

Our randomized, controlled multicenter study did not confirm the previously suggested superiority of liver preservation using arterial ex situ BP in addition to SAP over preservation using SAP alone in terms of preventing ITBLs after liver transplantation. Likewise, ischemia-reperfusion injury, graft loss, and patient survival rates did not differ with perfusion mode. The considerable discrepancy between cholangiography findings of bile duct changes and clinically apparent cholangiopathy should be considered when assessing ITBLs after liver transplantation.

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