Biliary strictures after liver transplantation : risk factors, diagnosis, treatment and outcome

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Chapter 9

Summarizing discussion

The occurrence of non-anastomotic biliary strictures (NAS) is a troublesome complication after orthotopic liver transplantation (OLT).[1] Strictures located in the biliary tree may lead to cholestasis, which can result in clinical symptoms, e.g., pruritis, fever from bacterial cholangitis and jaundice. In case of inadequate treatment -e.g. due to difficult to reach strictures- persistent cholestasis may lead to graft failure and retransplantation. Re-OLT for NAS is required in up to 18% of cases according to the literature.[2-4] Even though the complete pathophysiological pathway that leads to NAS development is unclear, several risk factors are known. Among those risk factors are ischemia-reperfusion injury, immunological risk factors and pretransplant status of the graft.

Ischemia-reperfusion injury and NAS

It has long been known that ischemia-reperfusion injury (IRI) is related to NAS development. The first reports to describe this association noticed an increased incidence of biliary strictures after the presence of a post-transplant hepatic artery thrombosis.[5;6] Furthermore, it has been described that grafts that are exposed to more severe ischemic injury, e.g., after a procedure with long ischemia times, NAS occurred more frequently.[7-10] Ischemia times are therefore kept as short as possible. However, logistics often dictate the ischemia times. Also, because of shortage of suitable donor organs, grafts donated after circulatory death (DCD) are increasingly used. These grafts are, in contrast to grafts from donation after brain death (DBD), exposed to more severe ischemic injury due to an additional donor warm ischemia time (DWIT) in which metabolic processes shift from aerobic to anaerobic. Numerous reports have confirmed the association between NAS and DCD.[11-14]

The degree of IRI is clearly visible in liver biopsies, but is also reflected by the peak alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the first post-operative week, of which peak ALT is considered the most sensitive. In **chapter 2** we analyzed the relationship between peak ALT within the first 7 days after OLT and the development of NAS in a cohort of 399 DBD livers and 97 DCD livers from two transplantation centers, i.e., the Leiden University Medical Center and University Medical Center Groningen. A log rank test was performed to determine the optimal cutoff-value for peak ALT. Using the calculated cutoff-value, mild IRI was defined as a peak ALT of <1300 IU/L, whereas a high peak ALT of ≥ 1300 IU/L was considered severe IRI. Median peak ALT was significantly higher

after OLT using DCD donors than after DBD-OLT. After DCD-OLT, severe IRI was strongly and independently associated with NAS development, as after a peak ALT of ≥ 1300 IU/L NAS developed in 46% of cases, compared to 10% of cases with mild IRI. Four-year cumulative incidence of NAS development was 49.5% in case of severe IRI, compared to 11.3% when mild IRI occurred. This association was also found when the cohorts of the two transplantation centers were analyzed individually. After DBD-OLT, the incidence of NAS was 13% and no association could be found between peak ALT and NAS development after DBD-OLT. Interestingly, the incidence of NAS after DCD-OLT with mild IRI was similar to the incidence after DBD-OLT. These findings imply that reducing IRI after DCD-OLT to the extent that peak ALT is below 1300 IU/L will significantly reduce the incidence of NAS to the incidence of DBD-OLT. Furthermore, peak ALT can be used to classify patients as high-risk or low-risk for developing NAS, which may be useful to include patients in a more intensive follow-up program. However, the presence of NAS after DBD-OLT and DCD-OLT with only mild IRI, also shows that other factors than IRI are also responsible for NAS development.

Donor specific antibodies and NAS development

Immunological factors are also recognized to be important in NAS development, especially those factors related to hepatic IRI. As a result of ischemia, damage-associated molecular patterns (DAMPs) are released which leads to the activation of Kuppfer cells, dendritic cells, T cells and neutrophils, and subsequently to the release of chemokines, cytokines, matrix metalloproteinases (MMPs) and reactive oxygen species (ROS).[15-17] This sterile immune response ultimately results in a severe tissue damage and an upregulation of human leukocyte antigen (HLA) class molecules on the biliary epithelium.[18-20] In general, the presence of donor-specific alloantibodies (DSA) against HLA molecules is associated with (hyper)acute rejection and shorter graft survival after organ transplantation.[21-24] However, it is unknown whether DSA against the upregulated HLA molecules are also related to increased injury of the biliary epithelium after IRI. In **chapter 3** we evaluated the role of preformed and de novo DSA in the pathogenesis of NAS in patients after ABO-compatible OLT. Therefore, we determined the presence of HLA class I and class II DSA in pre-OLT and 12 months post-OLT serum samples of patients with and without NAS. A time-dependent analysis was performed to assess a possible relation between DSA formation and NAS development. A longitudinal analysis was performed to determine the timeline of DSA formation. Overall, preformed

DSA (mainly class I DSA) were detected in 10% of the patients. Preformed DSA frequently disappeared after OLT, probably due to absorption in the liver. One year after OLT, 21% of patients generated de novo DSA. In the vast majority, the newly developed antibodies were directed against the HLA class II (HLA-DQ and HLA-DR) antigens of the donor. Neither preformed DSA nor DSA generated de novo within the first year after OLT were associated with an increased risk for NAS development. In fact, longitudinal analysis revealed that, in NAS patients, de novo DSA generally developed after the diagnosis of NAS. Similar results were found when class I and class II DSA were analysed individually. However, time-dependent analysis revealed that both NAS as well as de novo class II DSA are independent risk factors for graft loss after OLT. No association was found between de novo class I DSA and graft loss. Graft loss after DSA formation is probably the result of DSA that bind to endothelial cells in the portal triads leading to complement activation. DSA against class II HLA are probably more capable of initiating a clinically relevant immune response than class I DSA. This can possibly be explained because class II antigen presenting cells are abundant in the liver.

Matrix Metalloproteinases and NAS development

As previously mentioned, IRI may lead to a massive release of matrix metalloproteinases (MMPs), especially MMP-9. MMP-2 and MMP-9 (both gelatinases) are capable of degrading different collagen types, of which mainly type IV collagen may be important because this type of collagen is present in basement membranes. Damage to the basement membrane of the bile ducts may produce irreversible injury.[25-28]

The activity of MMPs is regulated by tissue inhibitors of matrix metalloproteinases. Several genetic polymorphisms in the promotor region of MMP-2, MMP-7, MMP-9 and their associated TIMPs are known to alter the mRNA transcription of these MMPs, which may lead to a reduced degradation process of collagen type IV. **Chapter 4** therefore evaluates the risk of NAS development as a results of genetic polymorphisms in the MMP-2, MMP-7 and MMP-9 genes, as well as the TIMP-1 and TIMP-2 genes. Genetic variations in both donors and recipients were determined by extracting DNA from peripheral blood and subsequently perform a restriction fragment length polymorphism (RFLP) polymerase chain reaction (PCR) or tetra primer amplification refractory mutation system (ARMS) PCR. We did not find genetic polymorphisms in either donor and/or recipient in the assessed

MMPs and TIMPs to be associated with a higher incidence of NAS. In addition, NAS patients did not have significantly more mismatches between donor and recipient in any of the evaluated single nucleotide polymorphisms (SNPs). However, the absence of a relation in this study may not completely exclude a role of MMPs and/or TIMPs in NAS development. Possible explanations for our negative findings are a lack of knowledge of the exact (local) function of MMPs, TIMPs and the evaluated SNPs, compensatory mechanisms which prevent alterations in serum MMP and TIMP levels in patients with genetic variations and interactions with other risk factors that alter the susceptibility for NAS. Additional studies to determine the exact functional mechanism should therefore be performed.

Diagnosis and follow-up of NAS

Apart from the challenges to unravel the pathophysiological mechanisms of NAS development, are the difficulties to diagnose and treat NAS in an early stage. Currently, direct cholangiography by endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC) is the golden standard for diagnosis. However, these procedures are associated with major complications.[29;30] Therefore, we evaluated magnetic resonance cholangiopancreatography (MRCP) as a non-invasive tool to monitor the presence or absence of NAS after OLT. **Chapter 5** describes a new scoring model, the Leiden Biliary Stricture Classification (LBSC), which is a modification of a validated scoring model for ERCP/PTC to detect the severity of biliary strictures in patients with primary sclerosing cholangitis. Patients with and without NAS from two liver transplantation centers in whom MRCP and ERCP/PTC (as reference) were performed within less than 6 months were assessed. The MRCP was evaluated by two independent, blinded abdominal radiologists. The presence, localization and severity of biliary strictures was noted and categorized into 4 different hepatobiliary regions. For the non-anastomotic regions a maximum of 15 points could be obtained. The optimal cut-off point, as determined using a ROC curve, was ≥ 3 points. When this cut-off point was applied to the radiologists mean scores, a sensitivity of 98%, specificity of 65%, positive predictive value of 87% and negative predictive value of 92% was achieved. MRCP using the LBSC is thus a reliable tool to detect or exclude NAS after OLT. This is a useful finding, as MRCP can be used to noninvasively diagnose or exclude NAS and plan the optimal treatment prior to direct cholangiography, thereby preventing unnecessary procedures. MRCP performance was better in the evaluation of the intrahepatic than of the extrahepatic bile ducts. The interobserver agreement for

grading biliary strictures severity was poor, indicating that the value of MRCP with current techniques for follow-up of NAS progression is limited. However, for diagnosing or excluding NAS an MRCP with application of the LSBC proved very useful.

Transient Elastography (TE) is an upcoming non-invasive tool in the follow-up of patients after OLT. TE can reliably measure liver fibrosis, by assessing liver stiffness.[31] Even though normal TE values have not been established, its use has especially proven valuable to monitor the recurrence of hepatitis C virus (HCV). Because it is yet unknown whether NAS is associated with fibrosis of the transplanted liver, in **chapter 6** we assessed TE values in a cohort of patients with and without NAS. Interestingly, liver stiffness was significantly increased in patients with NAS with persistent cholestasis after treatment. Patients with NAS in whom cholestasis had resolved with dilatation and/or stenting had TE values similar to those without NAS or cholestasis. The relation persisted when patients with HCV after OLT were excluded from the analysis. No association between increased TE values and the presence of anastomotic biliary strictures (AS), recurrence of HCV, type of donation and previous episodes of rejection was found. However, there was a relationship of current TE values with peak-ALT in the first week after OLT as a marker of ischemia-reperfusion injury. Unfortunately, we could not find an optimal liver stiffness cut-off value for the prediction of NAS. In controls, median TE values were relatively stable over time. These data indicate that repeated TE may be helpful during follow-up of patients with and without NAS after OLT.

Treatment and outcome of biliary strictures

In **chapter 7** we focused on (longterm) outcomes of the cholangiographic treatment of biliary strictures after OLT. Biliary strictures located at the anastomotic site or in the choledochal or hepatic ducts can usually be treated with an endoscopic procedure in which a balloon dilatation and/or the placement of a stent is performed. In case of more intrahepatic strictures, PTC with drainage is usually the treatment option of choice.

We found that, in the majority of patients with AS, cholangiographic abnormalities were already present in the first weeks after OLT. Successful treatment of AS was achieved in 45.5% of the patients that were treated with ERCP only and 3% of patients that were treated with PTC only. Cholangiographic success rate was higher in patients who received early treatment, whereas the simultaneous

presence of other biliary complications, i.e., NAS or anastomotic leakages was associated with a worse outcome. Patients with endoscopic success required a median of 2.5 procedures.

NAS was found to develop slightly later than AS. Treatment of NAS in the majority of cases consisted of combined balloon dilatation and stent placement. However, even though an aggressive approach with multiple stenting was performed in most patients, cholangiographic success rate in NAS was lower than in AS, i.e., 31.5%. A higher success rate was found for extrahepatic than for intrahepatic biliary strictures. Furthermore, a trend was found towards a lower success percentage in patients with early presentation, probably due to a more severe character of the biliary strictures. Not only was success rate lower in patients with NAS as compared to AS, patients also required more cholangiographic procedures. In general, complications after cholangiographic procedures were rare. This study therefore indicates that timely cholangiographic treatment is important in all cases to prevent cholangitis and progression to graft failure.

From the literature it is known that perceived quality of life in general improves after OLT for end-stage liver disease. Even though biliary strictures may partly be asymptomatic and complications of their treatment are rare in our center, it is not unthinkable that their presence and related interventions may have an effect on patients' perception of general well-being and quality of life (QOL). Therefore, **chapter 8** focussed on differences in QOL in patients with and without biliary strictures. In addition, patients after OLT were compared to matched healthy controls to evaluate the impact of a liver transplantation. In a cross-sectional case-control study, a general background questionnaire and four validated QOL questionnaires were evaluated.

Overall, we found that QOL was significantly worse in post-OLT patients, as compared to matched healthy controls. Patients after OLT with and without biliary strictures reported similar QOL. In addition, no difference was found when AS and NAS were evaluated separately. Because biliary strictures usually occur within the first year after OLT, a subgroup analysis of patients with a follow-up of ≤ 4 years was performed. In this subgroup, patients with biliary strictures had lower QOL scores in all domains, although this was, probably due to a small sample size, not statistically significant in any of the subscales.

However, patients with biliary strictures reported significantly more anxiety and depression on both the EQ-5D and Liver Disease Symptom Index questionnaires. Surprisingly, worries about biliary strictures and invasive treatment procedures did not negatively affect QOL scores, as the frequencies of reported disease- and treatment-related worries that contributed to depression did not differ between patients with and without biliary strictures. The results indicate that reducing anxiety and depression should be an important target in patient-care. This could possibly be achieved by adequate patient education and counselling during consultations.

General conclusion and future perspectives

Orthotopic liver transplantation is considered the only definite treatment option for end-stage liver failure. In the past decades, the many improvements regarding surgical technique, immunosuppression and post-transplantation care have made OLT a treatment with very acceptable patient and graft survival.[11;12;32] Even though post-transplant complications are to be taken into account, in patients with advanced chronic liver disease the benefits of OLT often far outweigh the risk of complications.

This thesis describes several aspects and the clinical importance of one of the most frequent complications after OLT, i.e., the development of biliary strictures. Biliary complications are known to have a significant clinical and financial impact on post-OLT care.[33] We attempted to facilitate diagnosing their presence by proposing the use of non-invasive screening tools, i.e., MRCP and transient elastography. We also demonstrated that most biliary strictures can be well-treated, although NAS seems to be more difficult to treat and more therapy-resistant than AS. Much is known about the development of anastomotic biliary strictures and therefore is seems that currently the greatest challenge is unravelling the pathways that lead to NAS formation. This knowledge is essential to change donation procedures in order to prevent NAS development after future OLTs. Their multifactorial etiology makes it difficult to have a complete insight in this pathway, but with the current knowledge risk factors that seem to have the greatest contribution in this process are immunological factors resulting from ischemia and reperfusion.[15-17;34-36]

This thesis confirmed that, even though strict protocols for donation after cardiac death are applied in the Netherlands, the incidence of NAS is still significantly

higher after this type of donation, than after donation after brain death.[12] This is probably due to the additional warm ischemia time leading to more severe IRI, but other factors may also contribute to this process. We have shown that severe IRI, as reflected by a peak ALT above 1300 IU/L, is associated with NAS development after DCD-OLT, but not after DBD-OLT. The main focus in the future should therefore be on improving the quality of DCD grafts. Following the current results a peak ALAT below 1300 IU/l may serve as a surrogate endpoint in studies aiming at prevention of NAS in DCD OLT. This can possibly be achieved by keeping ischemia times as short as possible and by a careful selection of grafts, but the greatest improvement in graft quality can be expected from machine perfusion.[37;38] This technique can likely not only improve the quality of donated grafts, but may also make grafts suitable for donation that would otherwise be discarded, thereby increasing the donor pool and decreasing waitlist mortality. The optimal conditions for machine preservation need yet to be established and currently several studies are ongoing on this subject. One of the most important questions that needs to be answered is whether machine perfusion should be normothermic, hypothermic or a combination thereof. However, excellent results have been described so far in pilot studies, and the expectation is that machine preservation will prove useful for prevention of NAS in further studies.[37;38]

Another possibility for decreasing the incidence of biliary complications theoretically might be improvement of the allocation process. However, currently, no screening tool is available to predict the risk of biliary strictures post-OLT, but recently the Eurotransplant Donor Risk Index was introduced as a valuable tool to predict the outcome of liver transplantations in general.[39;40] Since immunological factors are also important in the development of NAS, it would be interesting to see whether this could be incorporated in the allocation process. Unfortunately, so far we could not find an association between immunological factors of donor and recipient (i.e., donor specific antibodies and mismatches in SNPs of matrix metalloproteinases) and NAS formation. However, it is not unthinkable that matching donor and recipient for other (yet unknown) factors may reduce the incidence of NAS.

Until dramatic decreases in the incidence of biliary strictures can be achieved, high risk patients should be monitored and screened on a regular basis. We demonstrated that magnetic resonance pancreatocholangiography may have an

important role in non-invasively detect or exclude the presence of biliary strictures. We have also shown that early treatment can dissolve biliary strictures in many cases and this may possibly prevent repeated episodes of cholangitis, hospital readmissions and progression to graft failure. Furthermore, patients with biliary strictures report increased symptoms of anxiety and depression, which can hopefully be prevented by timely and adequate treatment.

In conclusion, this thesis demonstrated that the development, diagnosis and treatment of biliary strictures remains a complex issue and should be taken into account in future studies related to OLT.

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