Liver transplantation : chimerism, complications and matrix metalloproteinases

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Chapter 1

Introduction

In December 1963 Dr. Thomas Starzl from Denver, Colorado, published his first three attempts of liver transplantation in humans.[1] The first patient he described was a three year old boy with biliary atresia. He bled to death during the procedure. The other two patients had cirrhosis and a malignant liver tumor and were 48 and 67 year old males. Technically the liver transplant procedure was successful. However, both patients died of pulmonary emboli, 22 and 7½ days after the procedure. In the decades that followed, liver transplantation evolved from an extremely hazardous into a standardized procedure with increasing survival rates and in 1983 the NIH declared liver transplantation an accepted therapy for end-stage liver disease.[2]

The first liver transplantation in the Netherlands was performed in 1966 in Leiden University Medical Center, but due to coagulopathy the patient did not survive the procedure. Years later, in 1979, a successful liver transplant was performed in Groningen University Medical Center and in the years that followed liver transplant programs were also started in Rotterdam (1986) en Leiden (1992).

Nowadays, well over 10.000 liver transplant procedures are performed each year worldwide and it is the treatment of choice for acute and chronic liver failure. One year and five year survival rates are around 90% and 85%, respectively.

In the early days surgical techniques and control of hemorrhage were of major concern. The use of cyclosporin A from 1983 on contributed enormously to successful immunosuppression and thus to improved graft and patient survival. Recently, research has shifted towards consequences of long-term survival, such as quality of life issues and recurrent disease within the liver graft. Although the outcome of liver transplantation has improved, the risk of serious complications still remains. Surgical complications, blood loss, rejection, biliary complications and infections all pose serious threats to the graft and its recipient.

The chapters 2, 3 and 7 of this thesis focus on chimerism, that is the coexistence of cells of different genetic origin within one organism. With organ transplantation, cells of two different organisms are brought together. Several questions arise: Do cells of recipient origin replace cells within the graft? Can transplanted blood stem cells develop into mature liver cells? Can cells of the transplanted organ be found elsewhere in the body? What is the clinical relevance of chimerism?

Chapters 4 and 6 focus on a fascinating group of proteolytic enzymes,

matrix metalloproteinases, in relation to complications after liver transplantation. Here questions are: Is the genetic make-up of these enzymes relevant? Is a different genotype of donor and recipient associated with the occurrence of complications like ischemia/ reperfusion injury, rejection and biliary strictures?
More everyday clinical tests are addressed in chapter 5: The value of routinely assessed liver enzymes and the liver ultrasound, for predicting biliary complications after liver transplantation is described using a time-dependent statistical model.
Chapter 7 addresses chimerism in liver tissue biopsies and in peripheral blood after liver transplantation, in those patients with a donor/acceptor mismatch for the studied matrix metalloproteinases.

Chimerism

Chimerism in medicine is defined as the coexistence of cells of donor and recipient origin within a single organism. This phenomenon was first described in autopsies on pregnant women who died from eclampsia, with fetus-derived cells in the maternal circulation.[3,4] Subsequently, chimerism has been described frequently in pregnant women with fetal cells present in maternal blood.[5,6] Similarly, dizygotic twins have shown to be chimeric for each other's blood group.[7,8] Potential sources for chimeric cells, other than pregnancy, are iatrogenic, namely blood transfusion and transplantation.[9]
The possible immunological consequences of chimerism are intriguing. Chimeric cells may be silently present, without interacting with the host's immune system, e.g., resulting from pregnancy. It has also been hypothesized that chimeric cells may induce autoimmune disease by instigating loss of tolerance to self-antigens. This is supported by observations that chimerism is present more often in patients with autoimmune diseases.[10-12] In transplantation medicine chimerism may enhance graft tolerance.
In the early days of solid organ transplantation it has been postulated that cells of the recipient could replace cells in a transplanted organ and that this could lead to graft tolerance. Many studies have addressed this phenomenon with disputing and even conflicting results, and the relevance of chimerism in transplantation is still quite unclear. [13-16]

We studied the existence of chimerism within the transplanted liver, looking at different lineages of non-lymphoid cells. A selection was made of male patients who had received a liver graft from a female

donor. In liver tissue biopsies cells of recipient origin were identified using in-situ hybridization for sex chromosomes. Findings of this study are described in **chapter 2**.
Extensive chimerism within transplanted livers can only be understood if circulating stem cells can develop into liver cells of mesenchymal phenotype. We used a different transplant model to study this. Female recipients were selected that had received allogeneic bone marrow transplantation from a male donor (for hematologic malignancies). Only if liver tissue was available the patients could be included in the study.

Again, sex chromosome identification was used to identify the origin of cells in liver specimens, as reported in **chapter 3**.
If chimerism is a persisting feature after liver transplantation, one would expect donor-derived cells even in peripheral blood samples late after transplantation. The study reported in **Chapter 7** not only focuses on chimerism in liver biopsies after transplantation, but also on chimerism in peripheral blood samples.

Matrix metalloproteinases and biliary complications

Matrix metalloproteinases
The matrix metalloproteinases (MMPs) are a group of proteolytic enzymes that are important in many physiologic processes requiring matrix turnover. Basement membrane and matrix components like collagen, elastin, gelatin and casein are major components cleaved and degraded by these MMPs. The breakdown of these components is essential for many physiological processes such as embryonic development, growth, reproduction, tissue resorption and remodelling.[17-19] MMPs are also implicated in a variety of pathological processes such as arthritis, inflammatory bowel disease, cancer, and ischemic cardiovascular and neurological diseases.[20-22]
Among the different MMPs, the gelatinases MMP-2 and MMP-9 are of particular interest in liver pathophysiology. The main cellular source of MMP-2 is the hepatic stellate cell, whereas the principal sources of MMP-9 are the leukocytes and Kupffer cells. Expression of MMP-2 is increased in patients with chronic liver disease.[23,24] Different MMP genes have been shown to contain polymorphisms in their promoter region. These promoter polymorphisms have specific effects on the regulation of both MMP gene transcription and expression.

The donor liver graft is exposed to warm and cold ischemia with severe hypoxia before and during the transplant procedure. While ischemia

primes the cells for damage, the actual injury usually becomes manifest after the restoration of blood flow, i.e. the reperfusion. Many factors contribute to the extent of this ischemia-reperfusion (I/R) injury. **Chapters 4** and **6** address the genetic MMP make-up of both donor and recipient, in relation to clinical complications as I/R injury, rejection and the development of biliary complications.

Biliary strictures

Biliary complications are a significant cause of morbidity and even graft loss after liver transplantation. The most common biliary complications are biliary leakage and biliary tract strictures.[25-27] Anastomotic leakage occurs early after the transplantation procedure, whereas strictures occur later. Strictures can be divided into anastomotic and non-anastomotic. Anastomotic strictures occur at the anastomosis of the donor common bile duct and the recipient common bile duct (duct to duct anastomosis) or of the donor common bile duct with a recipient jejunal Roux-en-Y limb (hepaticojejunostomy). Strictures occurring at the anastomosis are usually due to surgical difficulties and/or local ischemia. Non-anastomotic strictures are thought to result from ischemia of the biliary epithelium by compromised arterial blood flow, hepatic artery thrombosis and/or ischemia/reperfusion injury. More complex immunologic factors and cytotoxic injury by bile salts may also contribute to non-anastomotic biliary strictures.[28,29]

We studied the relationship between MMP-2 and MMP-9 gene promoter polymorphisms in the donor and recipient DNA and the development of non-anastomotic biliary strictures after liver transplantation, the findings of which are described in **chapter 6**.

Biliary stricture formation is often insidious and typically first detected when biliary obstruction results in serum liver enzyme abnormalities, intrahepatic bile duct dilatation and/or infection. Imaging of the biliary tree is mandatory to make a definitive diagnosis. A cholangiography can be obtained endoscopically (ERCP), percutaneously (PTC) or by using magnetic resonance imaging (MRCP). Although ERCP and PTC are considered the golden standard to diagnose and treat strictures, they are invasive procedures. The predictive value of serum liver enzymes and abdominal ultrasonography for the development of non-anastomotic biliary strictures has been investigated before, but the results were not conclusive.[30,31] We performed a time-dependent statistical analysis to assess the predictive value of serum liver enzymes and abdominal

ultrasound as a first step in the diagnosis of biliary strictures after liver transplantation **(chapter 5**).

A summarizing discussion of the results obtained in the different studies as described in the separate chapters of this thesis is given in **chapter 8**. Finally, **chapter 9** provides a general discussion of the findings of this thesis in the Dutch language.

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