Studies on predictability of early graft function after liver transplantation
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chapter 1

Introduction and outline of the thesis
Introduction

For many years liver transplantation has been the preferred treatment for patients with end stage liver diseases, metabolic diseases with their primary defect in the liver and for patients with irresectable tumors of the liver. Although performed for the first time in the early sixties it took till 1983 before liver transplantation was accepted as a non-experimental clinical option during the National Institute Health consensus development conference in Washington USA. At that time perioperative mortality (within one month) was 20-40%. This figure was considered acceptable in view of the imminent death of these patients. Over the years patient and graft survival have improved considerably as can be observed in Figure 1 from the European liver registry (ELTR)

![Survival Graph](image)

**Figure 1** Patient survival according to year of transplantation  
(ELTR results: data analysis 05/1968-2000)

This improvement is likely to be due to a number of factors: surgical experience gained over time, increased knowledge and experience in intensive care medicine and more effective immunosuppression. The majority of patients and grafts are lost in the first months after liver transplantation. After this initial period patient and graft survival curves approximate survival curves of the
age-related general population. As a consequence attempts to improve survival, should be focused on the early phase following liver transplantation. Complications potentially leading to early patient or graft loss can be classified into four main groups (Table 1). In this table a number of complications are listed according to reports in the literature and compared to the incidence in our transplant center in Groningen before the start of the studies presented in this thesis. The first group of patients suffers from graft loss due to vascular problems such as hepatic artery thrombosis, portal vein thrombosis or venous outflow obstruction. Patients having these vascular complications often need either early revision of the anastomosis and thrombectomy or a retransplantation in order to survive.

<table>
<thead>
<tr>
<th>Groups of complications</th>
<th>Percentage of LTx patients (literature)</th>
<th>Percentage of LTx patients (Groningen 1989-1994 n=125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>2-12 (^1)</td>
<td>7</td>
</tr>
<tr>
<td>Portal vein</td>
<td>1-14 (^4)</td>
<td>2</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>1-2 (^6)</td>
<td>-</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>20-70 (^7)</td>
<td>56</td>
</tr>
<tr>
<td>Non liver related</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>50-70 (^8)</td>
<td>60</td>
</tr>
<tr>
<td>Neurological</td>
<td>12-20 (^11)</td>
<td>8</td>
</tr>
<tr>
<td>Cardiac</td>
<td>1-23 (^13)</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>15-75 (^15)</td>
<td>22</td>
</tr>
<tr>
<td>Renal</td>
<td>10-90 (^16)</td>
<td>7</td>
</tr>
<tr>
<td>Multiple organ failure</td>
<td>1-5 (^19)</td>
<td>5</td>
</tr>
<tr>
<td>Primary non function</td>
<td>2-10 (^20)</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1 Complications after liver transplantation as reported in the literature (ranges are given) and the percentage of these complications in our center. (ELTR results: data analysis 05/1968-2000)

A second cause of graft loss is immunological such as early therapy resistant acute rejection, which may lead to destruction of the graft. Ongoing improvement in immunosuppression will reduce the number of grafts lost from acute rejection. A substantial number of patients die with a well
functioning graft. The causes of graft and patient losses in these patients vary from infection with organ failure to bleeding from ruptured splenic aneurysm and cerebral edema with a normal liver graft function. Finally, some patients die or are retransplanted because of primary non function (PNF) or initial poor function (IPF) of the graft. Patients with PNF or IPF are often transplanted with carefully selected grafts of donors thought to be suitable for transplantation. Transplant procedures are often uneventful and nevertheless the grafts fail. IPF and PNF represent the entities of graft failure that are the least well understood. Some of these patients can only be kept alive with a new graft. The need for retransplantation and an observed decreased survival under such circumstances are serious sequelae of non-function and demand better understanding of risk factors possibly involved in its mechanisms.

In the following paragraphs, the current knowledge about primary non-function and initial poor function will be reviewed, with special focus on factors predicting early graft function.

**Definition of early graft function**

If one is to study graft function after liver transplantation, and results should be compared to the literature, this can only be done if endpoints are well defined. Unfortunately thus far, there is no consensus on the definition of primary non function or early graft dysfunction, not only in terms, but also even in the choice of parameters that define the condition. For example, many studies simply presume primary non function to be present in case a patient has to be retransplanted because of liver failure not due to rejection or technical failure within a certain time (range 2-90 days). In case of early graft dysfunction, the criteria broaden even further. Sometimes liver enzymes are used to define this condition, sometimes bile production. In other cases liver function tests or protein synthesis are monitored. In many reports a combination of these test is proposed to qualify graft function. It is easily understood that comparing results is difficult in these situations: choosing different parameters could identify different factors influencing graft failure. Therefore, it is important to realize that the current review includes many
studies not using individual liver graft parameters or graft function but also graft survival in the early postoperative phase as an endpoint.

Donor characteristics

Although donor selection has been a key issue since the start of liver transplantation programs, it is still unclear which criteria should be used for donor selection. Consequently, based on clinical experience acceptance criteria for donors have been widened over the past years. ABO matched, young adult, hemodynamically stable, healthy donors are generally accepted. Matching for blood group remains a prerequisite, despite the fact that ABO mismatch not necessarily leads to graft failure. A positive cross match is also thought to be predictive of early graft loss, however the review by Donaldson and Williams clearly showed that this issue thus far is unresolved with regard to both early graft loss due to (hyper) acute and/or chronic rejection.

Older donors were accepted without apparent effect on outcome. Inotropic support is often necessary in donors and so far no maximum concentration has been identified. Numerous reports have shown that grafts from donors previously infected with hepatitis B or C can be transplanted in selected patients. Also polycystic livers, livers from patients dying from carbon monoxide poisoning, septic donors and even grafts from deceased transplant recipients, have been used with success.

Not surprisingly, donor liver functions have been proposed to predict graft function. For example lidocaine clearance, indocyanide green clearance, clotting factor concentrations and transaminase concentrations have been presented as determinants of early graft function. Grafts from donors that cleared lidocaine at low rates performed less well. However these enthusiastic initial reports on lidocaine clearance as predictor of early graft dysfunction were not supported by later studies.

Another factor that appears to be associated with transplant function is duration of stay of the donor in the intensive care unit (ICU) and/or prolonged ventilation dependency. Grafts from donors that were in the ICU for more than 5 days showed a higher risk for primary non function. Many mechanisms...
might explain this finding. For example, patients in the ICU are more often hemodynamically unstable, hormonal regulation is deranged, fluid and electrolyte balances are disturbed and many inflammatory cascades are upregulated. Especially plasma sodium > 155 mmol/L appears to be an important factor\textsuperscript{57,58}, since grafts from donors with these high levels were associated with decreased graft survival. Fortunately, correction of hypernatremia in the donor can overcome this problem\textsuperscript{59}. Probably closely related to duration of ICU stay is the nutritional status of the donor, which is also thought to influence outcome. Experiments in rats have shown the negative effect of fasting on transplant success\textsuperscript{60-62}. These studies were not confirmed by others\textsuperscript{63}, nor were the results invariable reproducible in pigs\textsuperscript{64,65}. More importantly, it is likely that not feeding itself is the pivotal issue but the depletion of glycogen in the liver as a source for ATP regeneration seems to be essential\textsuperscript{62,65,66,67}. Some reports on initial graft function in humans were able to link increased microscopic fat\textsuperscript{68} up to 30% in graft or increased donor weight with graft dysfunction\textsuperscript{69,70}. However, there are at least as many studies published that do not confirm these initial findings\textsuperscript{71}. Lately donor gender\textsuperscript{72,73} and race\textsuperscript{74,75} have been raised as factors influencing graft survival. Female grafts in male recipients showed decreased graft survival as did race mismatched liver transplant procedures. However, these two factors are given entities and are difficult to balance in a situation of organ shortage. Furthermore the mechanisms of how gender differences in donor recipient combinations might work are difficult to understand.

Recently, brain death with all its pathophysiologic sequelae came to attention as possible cause for poor postoperative graft function\textsuperscript{76}. This interesting issue needs to be pursued further, because it is evident from clinical experience that renal grafts from living donors perform better and have a longer survival compared to grafts from postmortem donors. So far, the importance of this finding however remains under debate since in a canine model, the importance of the relation between brain death and organ function could not be reproduced\textsuperscript{77}. Some studies have explored endotoxin\textsuperscript{78,79} and cytokine concentrations in donors\textsuperscript{80} and have tried to link elevated concentrations of these substances to reduced transplant success, but so far without definite confirmation.
Recipient characteristics

In the search for useful predictors of graft function, not only donor characteristics have been studied. The focus has also been on recipient characteristics. Especially since the recipient was soon recognized as a possible "hostile environment." Although this statement is mainly based on the fact that a recipient will immunologically respond to the new graft, the donor liver is implanted in a recipient with many other problems. These other problems are related to the primary diagnosis and stage of the disease leading to transplantation. Acute hepatic failure in patients needing a liver graft is one of the most important determinants of outcome after liver transplantation. In chronic hepatic failure, the underlying disease might also influence early graft function. There are large differences in 6-month graft survival between groups of patients with different diseases leading to chronic liver failure. So far, this effect is not completely understood. One reason why original disease might lead to differences in IPF is that serum cytokine concentrations differ in some diseases. These differences might lead to differences in early graft function, since cytokines are supposed to play an essential role in ischemia and reperfusion syndromes. Another important issue in chronic liver failure, possible influencing the rate of graft dysfunction is the timing of the transplantation during the course of the disease. For this purpose many scoring systems have been evaluated. For example the Child-Pugh score, the Mayo survival model for patients with primary biliary cirrhosis, the UNOS score and Shaw scoring system are used to identify patients that would benefit from transplantation in the near future. Early data from the UNOS registry reveal that the higher the score in some of these systems the fewer patients survive the procedure. In general most studies showed that high-risk patients (high scores in these systems) tend to do less well. Import to note is that over the last decades the natural history of chronic liver disease has changed in such a way that patients live longer without being transplanted due to the improved non-surgical treatment modalities. Patients might not need a new liver or may need a new liver later in the course of their disease. This affects outcome, since older patients have a decreased patient and graft survival and often also have early graft dysfunction. Timing is even more problematic since increased waiting time results in higher mortality on the waiting list. Renal function is another factor in the recipient determining early
graft function, since patients with decreased kidney function show decreased patient and graft survival. Unfortunately, studies that relate kidney function to graft function or transplantation success have used different parameters to quantify or qualify renal impairment. Such as serum creatinin concentrations\textsuperscript{90}, blood urea nitrogen\textsuperscript{92}, creatinin clearance, hepatorenal syndrome\textsuperscript{93}, or renal insufficiency\textsuperscript{94,95} were used in different studies. Nutritional state has been investigated as a potential predictor of transplant success\textsuperscript{89,95,96}. Malnourished patients represent a group at high risk for infection, graft failure and fatality. However, in patients on the waiting list for liver transplantation it is difficult to assess the nutritional state. Albumin is usually low because of the underlying liver disease and weight is influenced by the presence of ascites or muscle wasting. Other measurements, like deviation of measured from predicted resting energy expenditure or body cell mass, may be difficult to obtain. Moreover, a prospective study, in which improvement of nutritional state is shown to improve outcome, is not available. Recently anthropometrical measurements have been shown to be a possible parameter to use in such studies\textsuperscript{97,98}.

Also as in donors, race\textsuperscript{99,100} mismatch in gender\textsuperscript{75,101} appears to be related to graft function, since graft survival is lower in male recipients from female grafts.

**Graft characteristics**

It appears very attractive to be able to test a graft before implantation. In this way not only all donor variables are included, but also the effects of factors like donor conditioning, organ retrieval, type of preservation fluid, quality of perfusion\textsuperscript{102}, duration of cold and warm ischemia times\textsuperscript{103-105} and machine perfusion\textsuperscript{106,107} can be included. Thus far several approaches have been tried. Grafts biopsies have been tested for normal histology\textsuperscript{24,70,108} viability\textsuperscript{109}, energy content\textsuperscript{110-112} and function\textsuperscript{113,114}. These tests were initiated under the assumption that during cold ischemia changes in different cell types can be identified and therefore correlated to outcome. Nuclear magnetic resonance spectroscopy of the graft\textsuperscript{110} was used to quantify ATP content of the graft. If a graft is preserved, aerobic metabolism is interrupted and therefore ATP synthesis stops and thus ATP levels will drop. Absence of ATP or absence of ATP regeneration capacity
might be associated with postoperative graft function. Our group has shown that it is possible to isolate hepatocytes and that these cells can subsequently be studied for taurocholic acid uptake as a parameter for function. Unfortunately, none of the mentioned approaches proved to be helpful in the clinical situation. An important issue might be that most studies focused on hepatocytes, while nonparenchymal cells are considered increasingly important for adequate function of the liver.

Surgical characteristics

Also surgical factors have influence on graft function. Longer duration of ischemia, whether cold or warm, is a well-known factor that influences postoperative graft function in a negative way. However the physical distance to be bridged between donor and recipient center is a given entity determining for a large part the duration of the cold ischemic time. If for some reason livers have to be reduced or split cold ischemic time will consequently be prolonged. This is reflected in the sometimes-observed rise in transaminases and poor graft function after such extended procedures. Over the years the type of preservation fluid has proven to have influence on early graft function. The introduction of the University of Wisconsin solution made not only possible to preserve grafts for a longer time but also graft function improved after transplantation. The duration of warm ischemia is partly determined by technical difficulties that may be encountered during surgery, such as the need for vascular reconstructions. Also the way the operation is conducted is important. Procedures with careful and meticulous hemostasis with consequently reduced blood loss show a better postoperative graft function.

Some studies have explored the influence of timing of portal and arterial revascularization on early graft function. Simultaneous release of the portal vein and the hepatic artery might be superior to sequential approaches. Some groups do flush the graft with different types of fluids, like Carolina rinse solution or albumin, just before recirculation. The preservation fluid together with possible toxic substances having accumulated in this fluid during storage is removed in this way and organ damage is reported to be less. However, this policy has not been universally accepted in daily practice.
Finally, surgery itself might be an important factor determining postoperative graft function. It has been shown that various acute phase responses are triggered during operations\textsuperscript{126}. These triggered systems range from cytokines to complement system and vascular active substances like nitric oxide. In this respect liver transplantation appears not to be different from other surgical procedures. However the exact role of these substances on early graft function remains to be assessed. The gut is thought to play an important additional role in this cascade\textsuperscript{127}. During the procedure, blood flow through the gut is compromised. This leads to translocation of bacteria or endotoxins from the intestines to the circulation and subsequently to activation of cytokines.

Ischemia reperfusion effects

Ischemia/reperfusion (I/R) is currently the subject that is most studied in the setting of early graft function. Some excellent reviews have been written\textsuperscript{128-132}. Four major effects explain most of the I/R injury, all of which become manifest on reperfusion. These are sinusoidal lining cell injury, white cell adhesion, platelet adhesion and increased coagulation. There is a definite role for free oxygen radicals\textsuperscript{128,133,134} and xanthine oxidase\textsuperscript{135} by inducing cell injury to hepatocytes and other cell types. Cytokines\textsuperscript{136}, like tumor necrosis factor $\alpha$\textsuperscript{137}, interleukin-1 and interleukin-6 also play an important role.\textsuperscript{138,139} Tumor necrosis factor $\alpha$ leads to procoagulant activation, reduction of protein C activity, membrane bound antigen activation and over-expression of adhesion molecules on endothelial cells and leucocytes. It also causes release of cytokines, free oxygen radicals and increased neutrophil aggregation and adherence. TNF-$\alpha$ causes release of vasoactive substances like nitric oxide. Interleukin-1 initiates the secretion of other cytokines and activates endothelial cells. Interleukin-6 leads to induction of acute phase proteins and enhances immune function. In addition to inducing and amplifying a local inflammatory response, this cascade induces both necrosis and apoptosis of hepatocytes, either direct or through induction of nitric oxide expression by the hepatocytes. In animal experiments\textsuperscript{140,141} the role of these cytokines has been extensively explored, but the exact role in human liver transplantation remains controversial and needs to be elucidated\textsuperscript{142-155}. Despite the possible relationship between increased cytokine concentrations
and postoperative organ failure it has been shown that cytokine removal is not helpful\textsuperscript{19}.

Other factors implicated in I/R injury\textsuperscript{128-130} range from Matrix Metallo proteases, calcium, phospholipase A\textsubscript{2}, eicosanoids to platelet activating factor, endothelin and adhesion molecules. All these substances initiate an inflammatory response with upregulation of adhesion molecules, neutrophil sequestration and priming of neutrophils and macrophages for increased cytotoxicity.

Finally endotoxin should be mentioned, since it may also contribute to I/R injury. The presence of endotoxin has been shown during liver transplantation and has been linked to adverse outcome\textsuperscript{157-159}. Since the liver plays a key role in clearance of endotoxin and is probably deficient before and definitely absent during the anhepatic phase of the transplantation, endotoxin could enhance ischemia reperfusion effects. Endotoxin can damage hepatocytes, render macrophages cytotoxic and induce release of biologically active mediators and free oxygen radicals.

**Conclusion**

This review shows that many factors can influence early graft function. Unfortunately most of them are still only partly understood and definitive proof of their influence and mechanisms of action are not always present. Several handicaps exist for adequate comparison of studies reported in the literature. Studies are often done in different species or in populations with a different composition (non homogeneous populations). The lack of consensus on the definition of early graft dysfunction is still a major issue.

Among the donor factors accepted as playing an important determining role in early graft function after liver transplantation are; duration of ICU stay, an increased serum sodium (above 150 mmol/L), and possibly brain death has in itself detrimental effects on graft function.

In liver transplantation clinicians are aware that also recipient factors play an important a role in the development of postoperative graft function. Factors proven to correlate significantly with early graft function are pretransplant kidney function and diagnosis and stage of disease.

Surgical factors like length of cold ischemia time, blood loss and probably rinsing...
are important as well. Finally, it is obvious that preservation-reperfusion injury also plays a key role in postoperative graft function. Despite increasing knowledge of the mechanisms at the cellular and molecular levels, more studies are needed before effective interventions can be implemented in human liver transplantation.

Outline of the thesis

The investigations, described in this thesis, approach the problem of initial liver graft function from various angles. Chapter 2 investigates the incidence of early graft dysfunction in a homogeneous group of patients in our center. Since there is a lack of consensus on the criteria for early graft dysfunction we also studied whether two commonly used sets of criteria showed concordance. Finally, we analyzed whether any particular donor, recipient or transplantation related parameters could predict early graft function in a well-defined homogeneous patient cohort. Chapter 3 and 4 focus on the analysis of a number of tests performed in materials obtained from the graft before implantation (biopsies, cells, slices) and their ability to predict graft function after transplantation. In chapter 3 the impact of the monoethylglycinexylide (MEGX) test in the donor is assessed. In addition, the results of a MEGX test performed in vitro in isolated liver slices are presented. In chapter 4 tests for hepatic transport function and metabolic tests are studied in isolated hepatocytes and liver slices of donor livers to determine whether they can predict early graft function. In chapter 5 through 7 peroperative recipient variables are assessed in order to clarify their role in the clinical setting. These parameters (gastric mucosal pH, cytokines and endotoxin) were chosen because they are closely related to one another and either animal or clinical experiments suggest they might play a role in early graft failure. In chapter 5 the gastric mucosal pH as a measure of splanchnic perfusion of the recipient is investigated to determine whether it correlates with early graft function and clinical outcome. In chapter 6 the role of endotoxin and cytokines like TNF-alpha and IL-1 and 6 is studied in relation to early graft function.
Finally, Chapter 7 investigates the effect on early graft function of selective decontamination of the digestive tract on endotoxemia, cytokine concentrations and early graft function. The thesis is concluded by a summary and conclusions in chapter 8.
Chapter 1

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