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Improving efficacy and reducing adverse effects of immunosuppression after liver transplantation

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CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

Chronic liver disease is a global health problem. About two million deaths annually and 4% of all deaths worldwide can be attributed to chronic liver disease[1]. This is because it can lead to liver cirrhosis and its complications, including liver failure and hepatocellular carcinoma. Most cases of liver cirrhosis are caused by viral hepatitis, alcohol consumption and metabolic-dysfunction associated steatotic liver disease (MASLD). Other causes include primary biliary cholangitis, primary sclerosing cholangitis, auto-immune hepatitis and metabolic liver disease from haemochromatosis, Wilson disease or apha-1 antitrypsin deficiency. Liver transplantation can be a lifesaving treatment for patients with end-stage liver disease. The first successful (partial) human liver transplantations were performed by Starzl (1967) in Denver and by Calne (1968) in Cambridge. One decade later, the first successful orthotopic liver transplantations were performed[2]. Over the last decades, surgical- and organ preservation techniques have been improved, which in combination with better immunosuppression, intensive care and medical care in general have led to a current one-year patient survival of 90% and five-year patient survival of 80% in most transplant centers[3]. Despite these acceptable rates, further improvements are required.

Immunosuppression in liver transplantation in the early 60s had been adopted from earlier experiences in kidney transplantation[4]. Initially, the so-called 'double-drug therapy' with prednisolone and azathioprine became the standard regime for years.

Ciclosporin was discovered in the 1970s and the first compound to inhibit lymphocytes specifically and reversibly, representing the prototype of a new generation of immunosuppressive drugs: the calcineurin inhibitors. In 1978-79 the first successful results of the use of ciclosporin in kidney transplantation were reported. Sequentially, in 1980 ciclosporin was introduced in liver transplantation and it significantly improved graft- and patient survival by better preventing acute cellular rejection[5,6]. Tacrolimus (FK-506), another calcineurin inhibitor was introduced in 1989 for patients after liver transplantation, initially it was reserved for those who suffered from repeated acute rejection or chronic ductopenic rejection despite conventional therapy including ciclosporin[7]. The effectiveness and safety profile of tacrolimus was studied in multiple studies since then and it demonstrated to be a very good alternative to ciclosporin. Up till now, calcineurin inhibitors remain the cornerstone of post-liver transplantation immunosuppressive therapy[8]. They can be used as single immunosuppression, but usually are combined with glucocorticoids. Currently they are often also combined with other immunosuppressive drugs like mycophenolate mofetil (MMF) or a mammalian target of rapamycin-inhibitor (mTOR-i), like everolimus, which have been introduced in the field of liver transplantation in the last three decades[9,10].

Calcineurin inhibitors

Over time, the original oral ciclosporin formula has been modified to a microemulsion-based formulation, which led to lower rejection rates and less adverse events compared to the original formulation[11]. Tacrolimus originally was only available as immediate-release formulation, but now is more often prescribed as one of the prolonged-release formulation, which have different pharmacokinetic properties[12]. Therapeutic Drug Monitoring (TDM) has become the gold standard for calcineurin inhibitors. For ciclosporin, most studies and transplant centers still use trough levels, which do not accurately reflect drug exposure. Monitoring at two hours post-dose better correlates with ciclosporin drug exposure and has been associated with reduced rejection and improved renal function compared to dosing based on trough levels[13]. However, for accurate dosing of ciclosporin, dosing on a Bayesian limited sampling model is required[14,15]. For tacrolimus, the area under the concentration-time curve (AUC) is also considered the best monitoring strategy, but dosing based on trough levels better than with ciclosporin reflects the AUC. Since AUC measurements often are considered cumbersome and time consuming most clinics use trough level monitoring for tacrolimus, despite proof that dosing on AUC using a limited sampling model is better[12]. Comparing ciclosporin to tacrolimus has been done in several studies over the last three decades. Earlier meta-analysis[16,17] demonstrated that tacrolimus was superior regarding one-year patient survival and was associated with less hypertension, but it led to more post-transplant diabetes mellitus (PTDM) when compared to ciclosporin. Outcomes regarding graft survival and rejection rates were conflicting. Since then formulations of calcineurin inhibitors have changed, lower blood levels are adhered to and concomitant immunosuppression has become more common. The first meta-analysis included older formulations, the latter is almost 10 years old.

Metabolic complications after liver transplantation

Improvements in long-term survival have lagged behind those in one-year survival. The metabolic syndrome, including PTDM, dyslipidemia, hypertension and obesity, is a common complication after liver transplantation and can effect long-term outcomes.

The development of PTDM is associated with an increased risk of infection, graft failure and mortality. Incidence rates vary due to diagnostic criteria, but are reported to be as high as 50%. Risk factors are male gender, the use of tacrolimus, recipient age and body mass index (BMI). Maintenance immunosuppressive therapy with mTOR-inhibitors (especially sirolimus) is also associated with an increased risk of developing PTDM[18].

Dyslipidemia is very common after liver transplantation, with a prevalence as high as 70%[19]. Risk factors are preexisting metabolic factors (MASLD, diabetes

mellitus) and the choice of immunosuppression. It is associated with an increased cardiovascular risk and requires individualized management.

Immunosuppressive therapy is the main cause leading to post-transplant hypertension. The chronic use of glucocorticoids and/or calcineurin inhibitors causes increased vascular resistance, renal arterial vasoconstriction and sodium and water reabsorption[20].

Screening for the metabolic syndrome is of great importance in post-transplant care, in close collaboration with primary health care physicians. Individualized management should consist of adapting immunosuppression, lifestyle management and specific pharmaceutical therapies.

Renal impairment after liver transplantation

The development of renal impairment post-transplantation is multifactorial, involving both pre-existing conditions and post-transplant factors. Incident rates of renal impairment after liver transplantation vary, partially because of different definitions, but 5-8% of the patients require renal replacement therapy within ten years after transplantation[21]. Additionally, liver transplant recipients appear to have a higher incidence of renal impairment compared to recipients of other types of organ transplants[22].

Pre-existing renal impairment is a strong predictor of post-transplant renal dysfunction and may sometimes be associated with underlying liver disease and degree of liver failure. For example, hepatitis C infected patients can develop immune-complex glomerulonephritis and patients with advanced liver cirrhosis are susceptible for the development of hepato-renal syndrome.

Immediately after liver transplantation, acute kidney injury is frequently observed, especially because of hypovolemia and hemodynamic instability peri-operatively.

In post-transplant patients, calcineurin inhibitors are a major cause of nephrotoxicity by reducing renal blood flow due to vasoconstriction of the glomerular arterioles. Adjusting immunosuppressive therapy to minimize calcineurin inhibitor exposure, can help to preserve renal function in the short term, but the long-term benefit of such dose modifications are questionable[23].

Regular monitoring of renal function and long-term follow-up are critical for preventing the progression of renal impairment and thereby probably enhancing patient survival[24].

Infectious complications after liver transplantation

Early postoperative infection rates, specifically surgical site infections, are generally estimated to be between 1% and 3%. However, these rates can vary depending on the type of surgery, patient factors, and hospital practices. Infections after liver transplantation are challenging, because of the necessity of immunosuppressive therapy following a large abdominal operation. Particularly in the early postoperative period infections can lead to graft dysfunction and increased morbidity and mortality.

Bacterial infections account for the majority of post-transplant infections, comprising approximately 70% of cases. Most of these infections are intra-abdominal, especially in the early post-liver transplantation period in which they are often related to surgical complications. The use of immunosuppression can result in a less symptomatic course, potentially leading to a diagnostic delay and a more serious outcome[25,26].

Viral infections are another significant concern post-liver transplantation. Apart from recurrent hepatitis viruses, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are the most clinically relevant viral infections post-liver transplantation. CMV infection, occurring in up to 30% of recipients, can occur as reactivation or 'de-novo' infection. CMV infection can lead to CMV disease, which is characterized by systemic inflammation and (multiple) organ involvement, potentially leading to significant morbidity and graft failure[27]. However, monitoring, prophylaxis and (pre-emptive) treatment are now available. EBV infection is a major risk factor for developing post-transplant lymphoproliferative disease (PTLD), particularly in pediatric recipients who are EBV-seronegative at the time of transplantation[28]. PTLD can also occur in adult recipients, leading to morbidity and mortality, but risk factors and ways of prevention are less well known.

Fungal infections, although less common, are associated with high mortality rates. Invasive candidiasis and aspergillosis are the predominant fungal infections in liver transplantation recipients, often occurring in patients with prolonged intensive-care unit stays and the use of pre-transplant immunosuppression[29].

Aims of the thesis

The studies presented in this thesis aimed to better tailor and individualize immunosuppression after liver transplantation in order to improve the balance between optimal graft survival and adverse outcomes, like opportunistic infections, metabolic complications and malignancies.

In **chapter 2**, we present the results of the DELTA study, which was a randomized controlled trial, designed and conducted by the three liver transplantation centers in the Netherlands, comparing ciclosporin and tacrolimus de novo after liver transplantation. In contrast to most previous trials, dosing of ciclosporin was based on blood levels two hours after dosing.

A partial superiority of tacrolimus over ciclosporin was demonstrated by two earlier meta-analysis[16,17], although certain outcomes were conflicting. In the first meta-analysis older formulations of the drugs were included. Since the publication of the last meta-analysis from 2016[16], dosing aiming at lower blood levels and more use of other concomitant immunosuppression were introduced, and more data became available. We therefore conducted a new systematic review and meta-analysis of randomized controlled trials conducted between 2000 and 2024 comparing ciclosporin and tacrolimus de novo in the first year after liver transplantation. The results are presented in **chapter 3**.

When choosing tacrolimus as calcineurin-inhibitor, most centers use trough levels for therapeutic drug monitoring. Although this method seems practical in the first place, patient education and outpatient clinic scheduling can be challenging. An earlier developed model with a derived Bayesian limited sampling method demonstrated a better correlation between AUC and a single point measurement of the blood tacrolimus concentration four hours after dosing[30]. We therefore conducted a randomized controlled trial in which we compared trough monitoring to four-hour monitoring of tacrolimus after liver transplantation. This so called FK-04 study is presented in **chapter 4** and focused on renal function as primary outcome, with rejection and metabolic parameters as secondary outcomes.

Besides therapeutic drug monitoring, other strategies for individualizing immunosuppression can be used. While initially the focus was on preventing rejection, the focus has shifted to better avoiding over-immunosuppression and adverse effects. Reactivation of CMV and EBV can be a sign of potential over-immunosuppression. EBV has been demonstrated to be a potential risk factor for PTLD in liver transplantation recipients[31-33]. In children, who are susceptible for primo EBV infection, a viral load monitoring strategy -with reduction of immunosuppression in case of primo EBV infection- resulted in a lower incidence of PTLD[34]. In adult liver transplantation recipients, EBV primo infection is less

common, but reactivation frequently occurs. Since 2003, we routinely monitor post liver transplantation patients for EBV reactivation, combined with adjustments in case of reactivation. In **chapter 5**, we investigated the value of this EBV viral load monitoring strategy and the risk of PTLD in adult liver transplantation recipients.

While over two-thirds of PTLD cases are associated with EBV[35], other risk factors for PTLD in adult liver transplantation recipients—unlike in pediatric cases—are not well established. Consequently, **chapter 6** describes the investigation of potential independent risk factors for PTLD in adults who have undergone liver transplantation.

Chronic immunosuppression carries an increased risk of infections, especially during the first year after liver transplantation, in which immunosuppression is usually higher dosed. Bacteria are responsible for up to 70% of these infections, with 40% of cases occurring within the first month after liver transplantation[36,37]. A strategy in trying to prevent these infections is the use of prophylactic selective digestive decontamination (SDD) directly after liver transplantation. In **chapter 7**, we investigated infection type and bacterial species within the first year of liver transplantation when routinely using standardized SDD.

In **chapter 8**, the results of our studies are summarized, and the implications for the current practice and future perspectives are discussed.

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