Pediatric Liver Transplantation: Improvement in Outcomes

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Statement of Originality

I hereby declare that the contents of this thesis have not been submitted for a degree at this or any other institution. This thesis contains no prior published work except where due acknowledgement has been made.

The work presented here was developed by the candidate with support from the research team including but not limited to, Professor Andrew J. A. Holland and A/Prof. Gordon Thomas. This team is listed as co-authors in publications that have arisen from this work.

The candidate for the degree of Masters of Philosophy was responsible for the development of research questions, reviewing literature, gaining ethics approval through the Sydney Children’s Hospital Network, data collection, statistical analysis of the data, writing of manuscripts and liaising with other members of the research team.

Data collection was aided by the Department of Gastroenterology, The Children’s Hospital at Westmead, who provided patient lists and some demographic data.

In addition to the statements above, in cases where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author.

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To my family and friends who have given me constant support and encouragement I am extremely grateful. In particular, I’d like to thank my partner Hannah Pollard for putting up with late nights, listing to presentation rehearsals and reading over countless drafts and manuscripts.
List of Abbreviations

BA – Biliary Atresia

DPC – Delayed primary closure

E1 – Era 1

E2 – Era 2

E3 – Era 3

FHF – Fulminant hepatic failure

GI – Gastrointestinal

HAT – Hepatic artery thrombosis

LT – Liver transplantation

MTBD – Metabolic disease

PVT – Portal vein thrombosis
Abstract

Introduction and Aims
Although relatively uncommon in children, end-stage liver failure is a potentially devastating condition that occurs secondary to either a congenital anomaly causing cholestasis, metabolic disease (MTBD), neoplasia or fulminant hepatic failure (FHF). When this occurs, patients will likely require liver transplantation (LT). LT remains the gold-standard for children with end-stage liver failure despite high morbidity and mortality associated with the complex pathophysiology of liver failure, the procedure itself and subsequent lifelong immunosuppression.

Surgical complications, often grouped as either general and gastrointestinal (GI), vascular or biliary complications are commonly seen in these patients and can impact on patient morbidity, mortality and graft survival. However, new immunosuppressants, surgical techniques such as in-situ split liver grafts, delayed primary closure (DPC) and microsurgery, as well as better post-operative care involving routine anti-coagulation protocols have improved outcomes significantly. The aim of this work was to assess the impact of new surgical techniques on the incidence of complications following pediatric LT at a single tertiary liver transplant center.

Materials and Methods
To address these aims, this thesis includes two studies. In the first study, we assessed the impact of DPC and the risk of general and GI surgical complications. We retrospectively collected data on children under the age of 18 who underwent LT between April 1986 and May 2014. The primary outcome measured was the incidence of general and GI complications
either in the early (<3 months), intermediate (3 months–1 year) or late (>1 year) post-transplant period.

The second study explored the impact of split grafts, microsurgical anastomosis and routine anti-coagulation on incidence of hepatic artery thrombosis (HAT). We did this by assessing the incidence of HAT in three distinct eras between April 1986 and April 2016, which marked the introduction of microsurgical anastomosis (Era 2) and in addition, a routine anti-coagulation protocol (Era 3). The major outcome measured was incidence and management of HAT in each of the three eras.

Research ethics approval was obtained from the Sydney Children’s Hospital Network Ethics Committee.

Results

In our first study, a positive association was found between biliary atresia (BA) patients and the incidence of bowel perforation post-LT. We confirmed that DPC with or without the use of prosthetic implants was safe and did not increase the rate of general and GI complications.

The second study found that the introduction of a routine anti-coagulation protocol significantly reduced incidence of HAT independent of the introduction of microvascular anastomotic techniques. A secondary finding was that minor bleeding (not requiring operative intervention) was more common Era 3.

In both studies, the use of split liver grafts was not associated with an increase in surgical complications.

Conclusions
Recent advances in pediatric LT are safe and in some cases have been beneficial in reducing the incidence of post-operative complications. The advent of split graft LT has decreased the organ wait time and is a safe alternative to whole or reduced grafts. DPC provides a means of difficult abdominal closure and does not increase the risk of general and GI complications. The introduction of microvascular techniques for vessel anastomosis and a routine anticoagulation protocol have been efficacious, with the later significantly reducing the incidence of HAT.
List of Publications

The following are publications that have arisen from this thesis:

**Refereed Journals:**


**Under Review:**


**Published Abstracts:**


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List of Presentations

International Meetings:

- General and Gastrointestinal Complications in Pediatric Liver Transplant Recipients:
  Annual Scientific Meeting, Pacific Association of Pediatric Surgeons, Jeju Island, South Korea, 20th of May 2015. Presented as a Full Oral Presentation.

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- Abdominal and Enteric Complications Following Paediatric Liver Transplantion:
  Australia and New Zealand Association of Paediatric Surgeons (ANZAPS), NSW/ACT Meeting, 9th of April 2015. Presented as a Full Oral Presentation.

Abstracts Presented on behalf of W. Ziaziaris:

- Improvement in Hepatic Artery Thrombosis in Pediatric Liver Transplantation: Annual Scientific Meeting, Pacific Association of Pediatric Surgeons, Koloa, Hawaii, USA, 27th of May 2016. Presented by A/Prof. Albert Shun. Presented as a Full Oral Presentation.

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CHAPTER I – INTRODUCTION AND BACKGROUND

Aim and Scope of this Thesis

The aim of this thesis is to assess whether new techniques and innovations in the field of pediatric LT are efficacious in reducing post-operative complications. The key techniques examined are DPC, microvascular anastomosis of the hepatic artery and a recently implemented anti-coagulation protocol. A secondary aim is to assess how these complications are diagnosed, their overall incidence and their subsequent management.

The first chapter of this thesis delves into the current state of pediatric LT and recent surgical advances that have been employed in order to improve morbidity and mortality in this group of patients. Later chapters explore how specific surgical techniques and post-operative care have reduced complication rates.
Pediatric Liver Transplantation; technical advancement, surgical complications and their management [REVIEW]

Introduction

Pediatric liver transplantation (LT) is a lifesaving and highly skill intensive procedure, where patients often experience high morbidity due to post-operative complications [1, 2]. Despite this, LT remains the gold standard for children with end-stage liver failure. In recent times, liver transplants in children have experienced excellent results with the implementation of new organ procuring techniques, microsurgery, anti-coagulation protocols and newer immunosuppressive drugs in addition to improved patient selection, nutrition, organ sharing systems, anesthesia and intensive care; reducing both short and long term morbidity and mortality [2].

While previous reviews have looked at either transplantation techniques and operative success or long-term follow up and immunosuppression related complications, this review specifically reflects on transplant specific surgical complications that contribute to patient mortality, short and long-term morbidity.

Indications for Liver Transplantation

The most common indications for LT in the pediatric setting are: (1) cholestatic liver disease, (2) metabolic disease (MTBD), and (3) either neoplasia or fulminant hepatic failure [3].

Cholestatic Liver Disease

Cholestatic liver disease is by far the commonest indication for pediatric LT. It can be subdivided into extra-hepatic cholestasis – primarily biliary atresia (BA) – and intra-hepatic
cholestasis – including Alagille’s syndrome, sclerosing cholangitis, progressive familial intra-hepatic cholestasis and non-syndromic paucity of intra-hepatic bile ducts [4, 5]. In the recent literature, cholestatic disease account for between 55-60% of transplanted patients [5-7]. BA alone accounts for over 40% of all patients and a resounding majority of patients transplanted < 1 year of age [6].

BA patients present soon after birth with worsening conjugated hyperbilirubinaemia, persistent jaundice, clay-coloured stools and hepatomegaly. Kasai portoenterostomy, an attempt to restore bile flow is the initial adjuvant surgical treatment of choice for BA. The clinical basis of this procedure in BA is that intrahepatic bile ducts are patent and a bowel loop can thus be used in lieu of atretic extrahepatic bile ducts. Unfortunately, nearly 66% of patients will develop chronic liver disease requiring LT [8, 9]. Longer term, this proportion of patients can be as high as 80%. After Kasai portoenterostomy, if adequate bile flow cannot be established, patients develop secondary biliary cirrhosis, hepatocyte dysfunction and end-stage liver disease requiring transplantation.

Patients with failed Kasai portoenterostomy who undergo subsequent LT are more likely to have certain complications; particularly bowel perforation [6, 10]. This implies that more stringent selection of surgical options for BA patients may be necessary in the future [10]. Infrequently, patients with BA may be primarily transplanted without prior Kasai. These patients tend to have fewer post-operative complications [6, 10]. Patients with BA also seem to be at greater risk of portal vein thrombosis (PVT) due to hypoplasia of the portal vein [9, 11, 12].

Intra-hepatic cholestatic diseases are by comparison less common and have a strong genetic and familial component. Here, indication for transplantation is to treat severely debilitating symptoms, improve quality of life and prevent the development of future
neoplasia. Alagille syndrome is one example. These patients typically present with jaundice and other congenital anomalies at birth with hepatomegaly and pruritis prior to transplantation [13]. Systemic involvement and signs for all intrahepatic cholestatic disease are common. Unlike untreated BA, intrahepatic cholestatic diseases do not necessarily all require transplantation [14]. Moreover, patients with intrahepatic cholestatic diseases are less likely to require re-look laparotomy for abdominal or bowel complications post-LT [14]. This is probably due to prior Kasai procedures increasing the likelihood of adhesions in BA patients while patients with intra-hepatic cholestasis tend to have virgin abdomens at LT.

Metabolic Disease

Individual metabolic diseases (MTBD) are rare, but combined, form the second most common indication for pediatric LT [15]. These include urea cycle defects, alpha-1 antitrypsin deficiency, cystic fibrosis, Wilson disease, maple syrup urine disease, tyrosinemia, glycogen storage disease, Crigler-Najjar, gestational alloimmune liver disease, primary hyperoxaluria, and inborn errors in bile acid metabolism [15-21]. Of these, urea cycle defects and alpha-1 antitrypsin deficiency are the most common [15, 17, 18].

MTBD can then be further categorized as either cirrhotic or non-cirrhotic MTBD [18]. Indication for LT is different between these two groups. For patients with non-cirrhotic MTBD, transplantation can not only replace the diseased and poorly functioning liver but may also correct the underlying metabolic defects of the hepatocytes [15, 16, 18]. Here, a normal liver graft replaces a genetically defective native liver providing a unique form of gene therapy [15, 18]. Recurrence of underlying disease is considered rare but some patients may experience extra-hepatic manifestations of their disease [17]. Cirrhotic MTBD patients require transplantation due to progressive liver injury and eventual liver failure [22].
When compared to BA and other cholestatic liver diseases, some studies suggest that patients with MTBD fair better due to their healthier clinical status pre-LT [15, 23]. Others suggest similar outcomes [24]. Generally, patients with MTBD require LT at a later age than those with BA and other non-MTBD [15]. Patients with MTBD are also less likely to be hospitalised, admitted as an intensive care unit inpatient or experience consequences of previous abdominal surgery or portal hypertension prior to LT which supports this hypothesis [15, 17].

Within the subset of MTBD patients, those with cirrhotic liver disease tend to present in a more serious condition and require more urgent transplantation [18]. Subsequent to LT, patients with cirrhotic disease also have poorer survival than those with non-cirrhotic MTBD [18].

In one study, the risk of HAT was significantly higher in patients with MTBD [7]. Another more recent study showed that while HAT rates were higher in patients with MTBD this difference was not statistically significant [15]. In fact, complications such as portal vein thrombosis (PVT), leukopaenia, bowel perforation and bleeding were less likely in this patient subset [15].

Neoplasia

Hepatoblastoma is the most frequently observed liver tumour in the pediatric setting usually diagnosed as an asymptomatic abdominal mass. In the last 3 decades developments in chemotherapeutic and surgical management of hepatoblastoma have improved patient outcomes dramatically. Moreover, unlike adult hepatocellular carcinoma, more advanced stages of pediatric hepatoblastoma seem to be curable with LT [25].
Generally, children with hepatoblastoma are first treated with neo-adjuvant systemic chemotherapy prior to being evaluated for resection or total hepatectomy [25-27]. This allows for tumour shrinkage such that a good surgical margin can be obtained whilst preserving viable native liver [25-27]. Partial hepatectomy is the definitive treatment if the tumour remains confined to 1-3 sectors of the liver [26, 27]. Transplantation is indicated in cases where the tumour is non-resectable. These tumours involve all 4 of the liver sectors and/or are centrally located and thus in close proximity to major veins [28, 29].

An early referral to a pediatric liver transplant surgeon is recommended, with neo-adjuvant chemotherapy, hepatectomy and transplantation the usual management for non-resectable tumours [26, 27]. Neo-adjuvant chemotherapy in such cases is controversial due to the concern of cumulative nephrotoxicity. Hepatoblastoma patients subsequently transplanted seem to have lower tolerance for tacrolimus than patients transplanted for non-malignant conditions, however, this does not seem to significantly impact on rates of acute rejection [26]. Hepatoblastoma staging and the determination of an appropriate management plan is essential, as results of rescue LT after incomplete resection or recurrence post-partial hepatectomy are disappointing [26].

**Fulminant Hepatic Failure**

Fulminant hepatic failure (FHF) is a devastating disease that has a high mortality rate without LT in children [30]. FHF is the rapid development of liver impairment with defective liver synthetic function and hepatic encephalopathy in patients without known prior liver disease. FHF accounts for about 10% of patients in pediatric LT cohorts and is caused by infectious agents (probably viral), drugs, toxins, and metabolic diseases [30, 31]. Occasionally, the cause
of FHF is unknown. Children are more susceptible than adults to drug-induced liver toxicity, particularly with anticonvulsant medication such as valproic acid or diphenylhydantoin [30].

Some causes of FHF are reversible. For example, rates of spontaneous recovery from paracetamol toxicity and hepatitis A infection are high [31]. Other causes are less likely to resolve. In the absence of a treatable or reversible cause for FHF, LT is the only available treatment option.

**Advances in Pediatric Liver Transplantation**

The major issue in transplantation in both adults and children worldwide is donor organ shortage. Though advances in tissue engineering and the idea of transplantation on demand are promising for the future [32-38] this is still an unviable prospect currently. Donor policy in much of the Western world is also limiting. Policy in Australia for example is ‘opt-in’, whereas in Spain and Singapore ‘opt-out’ legislation exists and is successful in increasing donation rates and reducing organ shortages.

**Split Liver Grafts**

In pediatrics, the shortage of size-matched donor livers has led to the development of graft reduction techniques to either split or reduce adult livers and make them suitable for children [39-41]. Reduced sized grafting has successfully allowed for the complementary use of either living donor and cadaveric split grafts [41]. Prior to the advent of these techniques, pediatric recipients had a far greater wait list mortality than adults [42]. Since the 1980’s and 1990’s, these techniques significantly reduced the wait-list mortality for pediatric recipients. In some cases, such as in neonatal LT, hyper-reduced left lateral segments are still too large,
and monosegment grafts are used [40, 43] though not without controversy [44]. For living donor operations monosegmentectomy of the liver is equivalent in terms of safety to standard left lateral segmentectomy [43]. The use of these grafts may be more complex than whole grafts due to the presence of multiple anastomoses or the need for extensive vascular and biliary reconstructions [44]. Moreover, it is likely that traditional left lateral segment liver transplantation managed by an experienced multi-disciplinary team with delayed primary closure is a preferred alternative [44].

Living in-situ split donor grafts were initially conceived to allow transplantation of severely ill children with hepatic failure where cadaveric grafts were otherwise unavailable [45]. Living donor liver transplantation however offers some advantages over the use of cadaveric grafts. In countries such as Japan, where cadaveric donors are not routinely used, organ availability relies almost solely on living-donor grafts. As Mori et al. explain, live donors offer well-functioning grafts that can be pre-operatively histocompatibility and size matched [45]. They also come without the risk of preservation injury [45]. The major detriment of live organ donation is the risk of morbidity and mortality to the donor. At present, the risk of donor mortality is approximately <1% [46-48] however, these results are largely isolated to higher volume centers. Live donor grafts also complicate vessel anastomoses and reconstructions, particularly of the hepatic artery [49]. Here, donor hepatic artery variant typing may be important for planning of reconstruction. One study used pre-operative triphasic computed tomography with three dimensional reconstruction to better plan for intra-operative hepatic artery anastomosis [49].

Living donor, split and/or reduced liver grafts also pose a number of technical issues for the surgeon. Firstly, there are a number of anatomical variations of the right and left liver lobes. Secondly, the quality of the donor liver vessels may be problematic, with vessel calibre
varying between donor and native recipient vessels [50, 51]. This may necessitate more complex vascular and biliary tree reconstruction. Depending on the donor graft used, vessels to be anastomosed may be deeply situated and access partially obstructed by the recipient’s costal margin and the allograft itself [2]. Another challenge arises in patients undergoing additional liver transplants. Here, more imaginative reconstruction of portal vasculature and the biliary tree is necessary due to the delicate and friable native tissues.

*Microsurgery*

To overcome challenges in vessel and bile duct anastomosis some centers have advocated for the use of microsurgical techniques and instruments for pediatric liver transplantation [45, 52-58]. The use of microsurgical techniques for the meticulous reconstruction of delicate pediatric vasculature in liver transplantation was initially recommended by T Starzl in 1976 [58]. Pediatric transplant recipients, particularly biliary atresia patients who tend to be younger at transplantation, have less robust tissues than their adult counterparts [6]. The use of microsurgical instruments is therefore paramount to protecting delicate tissues – namely the vascular endothelium – particularly in cases requiring complex reconstruction and multiple vascular or biliary anastomoses.

For some, microsurgery has helped resolve the high risk of HAT and enabled the reconstruction and anastomosis of vessels of varying calibre [2, 45, 53, 55-57, 59, 60]. In one study it was found that microsurgical anastomosis of the hepatic artery significantly lowered HAT rates from 7.4-26% [51, 61, 62] to 2% compared to anastomosis by either naked eye or loupes [45]. The use of the operating microscope for smaller calibre vessels provides a single, static view of the operating field. This may be problematic during vessel anastomosis where
ventilation moves abdominal organs and thus the vessel being visualised. At this time, it is often difficult to continuously adjust the view of the operative field [57]. Due to this inconvenience, high-powered surgical loupes are often preferred. Ohdan et al. describe a positive experience with an alternative, the Varioscope® AF3 (Life Optics®, Vienna, Austria), a miniature head mounted microscope which allows the surgeon to adapt to any motion in the operative field [57]. A number of centers, including our own, still report good results with surgical loupes [63, 64].

_Anticoagulation_

Both iatrogenic and intrinsic patient factors lead to the development of post-transplant surgical complications. Patients with chronic liver disease have a complex coagulation profile because of decreased pro and anti-coagulant protein synthesis, thrombocytopenia secondary to portal hypertension and platelet dysfunction [65-67]. This is worsened by the stress of LT.

In chronic liver disease, there is a decrease in production of both pro and anticoagulant proteins maintaining the delicate haemostatic balance that ensures there is neither excessive bleeding nor thrombosis [66]. Due to the increase in von Willebrand Factor, factor VIII levels in the plasma remain relatively high despite decreased hepatic synthetic function. It is thought that this, coupled with the decrease in protein C is the underlying cause of hypercoagulability [66]. The haemostatic balance is hence precarious with stresses such as trauma or surgery enough to produce excessive bleeding or thrombosis.
After LT, patient coagulation profiles are equally complex. Synthesis of haemostatic proteins returns at variable rates, with synthesis of procoagulant proteins occurring earlier than anticoagulant proteins. This renders patients hypercoagulable particularly in the first 3-7 days’ post-transplant [68, 69]. It is within this time that the majority of thrombotic complications such as HAT occur. While it was once thought that the major risk for HAT is a direct result of local trauma to the vessels during anastomosis, hypercoagulability certainly plays a significant role. For these reasons, the combination of microsurgical instruments – thought to be less likely to damage the intima of vessels – and early implemented anticoagulation protocols are thought to be beneficial in reducing vascular complications [70].

Anti-coagulation protocols published in the literature have had success in reducing thrombotic complications, particularly HAT [70]. At our center, the anticoagulation protocol used involves early post-operative replacement of antithrombin using antithrombin concentrate, protein C and S by replacing plasma, vitamin K supplementation and early heparinisation. Synthetic function of the graft is then monitored. Where anti-coagulation protocols have been implemented there has been a dramatic reduction in the incidence of HAT [70].

*Delayed Primary Closure*

Large for size grafts pose the issue of problematic abdominal wall closure. Such mismatching can lead to large-for-size graft syndrome and increased abdominal pressure leading to abdominal compartment syndrome and the compromise of the donor graft [40, 43, 71]. An alternative to hyper-reduced or monosegmental grafts in small patients is delaying primary closure. In one study, only 40% of children’s abdomens were primarily closed post liver transplantation demonstrating the commonplace and importance of delayed primary closure
techniques [72]. Some studies prefer delayed primary closure to the use of monosegmental grafts [44]. Delaying primary closure can be a safe and effective alternative particularly in younger children undergoing liver transplantation allowing for the graft size to reduce and bowel edema to settle, minimising the risk of abdominal compartment syndrome and graft compression [6, 72-75]. In a study published by our center we confirmed that delayed primary closure techniques are safe and efficacious, with a comparable incidence of wound complications [6]. Earlier studies however contradict these findings [72, 76].

A number of implantable prosthetics have also been used to facilitate DPC in children with large-for-size grafts and difficult to close abdomens post-LT [71, 75]. In one study at our center, definitive abdominal wall closure was achieved using a biodegradable membrane derived from porcine intestinal submucosa, Surgisis® (Cook Australia Pty Ltd) [71]. Surgisis® (Cook Australia Pty Ltd) is used to approximate muscle layers and facilitate closure of the abdomen [71]. A follow up study at our center deemed Surgisis® (Cook Australia Pty Ltd) a safe and effective method of abdominal wall closure, with no significant increase in the risk of wound related complications [6]. No patches required removal due to complications [6].

**Surgical Complications Following Pediatric Liver Transplantation**

Surgical complications in liver transplantation are common and contribute to both morbidity and mortality. These complications are subcategorised into vascular, biliary and general surgical complications. Surgical complications following transplantation can be classified as either early, within 3 months, intermediate, between 3 months and 1 year, or late, later than 1 year.

**Vascular Complications**
Vascular complications are those which involve either the hepatic artery, portal vein or the hepatic vein and inferior vena cava confluence. They remain the major cause of graft failure despite recent advancements. Here, vessel specific local factors as well as systemic factors associated with end stage liver disease may increase the risk of vascular complications [2, 68, 69].

**Hepatic Artery Thrombosis**

HAT has the potential to cause graft loss and or long term patient morbidity [77, 78]. In recent times, it has become more treatable, making long-term survival comparable to those without this vascular complication. Rates of HAT in children have been quoted in the literature between 9-20.2% [79] and as high as 30% for children under the age of 1 year [80]. The majority of HAT occurs early, within 3 months of transplantation and are related to technical errors and intrinsic patient factors [42, 49, 81, 82]. HAT is also the commonest indication for re-transplantation [83].

Factors such as hypercoagulability and organ rejection can increase the risk of HAT [49]. Specific surgical risk factors such as anatomical site of arterial reconstruction, size of the donor artery, anastomotic technique and the number of anastomosis revisions may also increase HAT incidence [84, 85].

HAT can potentially lead to graft failure and death in up to 50% of cases [78]. In a single center study, a review of 222 live donor liver transplants found that 5-year patient survival after HAT was significantly lower (58.3% versus. 84.4%) [7]. In the longer term, prior HAT is a risk factor the development of biliary complications such as strictures, bile leaks and, in severe cases biliary sepsis [86, 87].
The treatment for HAT however remains controversial. When thrombosis of the hepatic artery is identified early, patients can either undergo conservative management with intravenous tissue plasminogen activator, laparotomy and surgical revision or hepatic artery reconstruction. In some cases, the HAT is not amenable to surgical revision or reconstruction and requires re-transplantation. In recent years, intervention radiology through angiography and balloon angioplasty has also been promising. Generally, surgical revision and reconstruction of the hepatic artery is commonly employed for early thrombosis while radiological intervention, including thrombolysis, balloon angioplasty and/or stenting, is preferred for HAT that appears later. If graft failure occurs, re-transplantation is the only available treatment.

*Hepatic Artery Stenosis*

Stenosis of the hepatic artery usually occurs at the site of anastomosis and can progress to HAT. Literature on hepatic artery stenosis post-LT in children is scarce. In adults, the majority of stenoses undergo anastomotic revision due to risk of subsequent HAT [88, 89]. Hepatic artery stenosis has an incidence of about 5-11%, with more than a 65% chance of progressing to HAT if left untreated for 6 months [89]. Similar to HAT, stenosis of the hepatic artery also predisposes patients to future biliary complications such as biliary stricture [90]. Revision of the hepatic artery anastomosis thus reduces subsequent biliary stricture rates and gives good long term graft function [88].

Treatment of hepatic artery stenosis is similar to HAT. Patients can either undergo surgical revision via laparotomy or percutaneous interventions through interventional
radiology. Balloon angioplasty and stent placement seem to have similar results and complication rates [91]. Endovascular interventions have been successfully used as an alternative to surgical revisions of the hepatic artery anastomosis with similar survival rates at 5 years and less morbidity and mortality [90, 92-96].

A potential disadvantage of endovascular repair of hepatic artery strictures is the need for surgical revision in a high proportion of patients. The reverse is also true with a proportion of patients potentially avoiding additional abdominal surgery [94]. One study comparing pediatric and adult strictures had comparable results between the two groups though rates of procedural complications such as HAT were higher in the pediatric group and the study was limited by its small sample size of pediatric patients [94].

**Portal Vein Thrombosis and Stenosis**

Like HAT, portal vein thrombosis (PVT) significantly impacts graft and patient survival. Like HAT, the incidence of PVT is greater in the pediatric population, ranging from 0-33% with high morbidity and mortality [9]. In one study, PVT not HAT was linked to increased mortality [97]. The majority of PVTs seem to occur early post-LT [98], however in some studies late-onset PVT was more common [9]. The majority of studies choose not to focus on late PVT due to longer follow up periods. One study quoted a higher incidence of late (7%) versus early (2%) PVT [99].

Risk factors for PVT in the pediatric population undergoing LT are broad. Low weight – some giving the cut off of either 6kg [98] or 8kg [9] - and younger age were associated with post-operative PVT [9, 97]. These findings may be skewed by the fact that patients with BA
tend to be younger and more likely to develop PVT due to native hypoplasia of the portal vein [9, 11, 12]. Other risk factors include small diameter portal veins, poor portal flow, emergency LT, prior PVT and prior porto-systemic shunts [9, 98]. Hepatic vein stenosis, which alters portal flow also seems to be a risk factor for PVT [99]. In a similar way recurrent cholangitis affects intrahepatic vessels and subsequently portal vessel flows [9].

The narrowed portal vein found in BA patients is thought to be secondary to cirrhosis that follows from failure of Kasai portoenterostomy to adequately re-establish bile flow [9]. This complicates portal vein anastomosis in this subset of patients, with PVT uncommon in non-BA patients [9]. In one study by Chen et al. PVT was the most common complication observed in 100 patients undergoing LT for BA [100].

Prompt diagnosis of PVT is essential to prevent subsequent graft loss and long term morbidity [101]. PVT is difficult to diagnose clinically, with symptoms and signs often absent during the early development of a thrombus. However, diagnosis of early-onset PVT and other portal vein anomalies post-LT with routine Doppler ultrasonography is easy and cost effective. On ultrasonography, portal vein flow anomalies are signalled by distal turbulence in both clinically significant and non-significant stenoses caused by thrombus [101]. Despite the high specificity and sensitivity of Doppler ultrasound for detecting narrowing of the portal vein diameter in thrombosis, it is poor at determining the extent of portal vein stenosis [101]. Thus, angiography may also be necessary to determine which patients with PVT or portal vein stenosis require intervention. Late-onset PVTs may be more difficult to detect and subsequently have increased morbidity and mortality [102].

Patients with severe portal vein stenosis (>50%), confirmed with angiography, should undergo either stenting or dilatation of the stenotic segment of portal vein [103].
Conventionally, this has been performed via a percutaneous approach with a high success rate. The most common complications of this procedure are haemoperitoneum and haemothorax [103]. Other approaches include percutaneous trans-splenic, transjugular and intraoperative portal vein access [104, 105]. The transjugular and intraoperative approaches are used when there are contraindications to other approaches [105].

**Biliary Complications**

Anastomosis of the bile duct is commonly referred to as the ‘Achilles Heel’ of the liver transplant procedure. Unlike vascular complications, since initial introduction of LT, incidence of biliary complications has not improved significantly. Anastomoses are generally either fashioned end-to-end or, more commonly, constructed with a Roux-en-Y loop.

Biliary complications following pediatric LT are common with a reported incidence of between 6% and 35% [83, 106-108]. The most common complications are anastomotic bile leaks, biliary fistulas, stricture at the anastomotic site and strictures of the intrahepatic bile ducts [106, 107, 109]. Complications may be linked to biliary anastomotic technique. In one study, Roux-en-Y performed better than end-to-end anastomoses, which had a six-fold increased biliary complication rate [110].

Suspicion of a biliary complication is raised when patients have fever, abdominal pain, jaundice, ileus or bile leaks and/or if there is derangement of liver function tests [107]. Complications are then confirmed by imaging with ultrasound, hepatobiliary scintigraphy or cholangiogram.
Biliary Strictures

Risk for biliary stricture is closely related to development of HAT [109, 111, 112] and anastomotic technique [113]. Risk of anastomotic stricture of the bile duct is higher in patients who received an end-to-end versus those with Roux-en-Y biliary anastomoses [113, 114]. Likewise, HAT increases the risk of biliary stricture. In one study, 25% of patients with biliary stricture had had a prior HAT [109]. This association is well known in both the adult and pediatric liver transplant literature [111, 112, 115, 116].

Clinically, biliary strictures can be asymptomatic or cause abdominal pain, fever and/or jaundice. This varied clinical presentation makes detection problematic. There is also contention in the literature as to what clinical investigations should prompt suspicion of biliary stricture, with some using liver biopsy showing mechanical cholestasis after raised serum GGT as an indication for aggressive percutaneous intervention [117]. Conversely, the Brussels group uses ultrasound evidence of proximal bile duct dilatation as indication for intervention [107].

Ultrasound is often the first imaging modality employed when stricture of the bile duct is suspected clinically. It is quick, cheap and allows for good visualisation of the biliary tree. Subsequently, percutaneous transhepatic cholangiography plus dilatation and/or stenting is useful diagnostically and for the management of biliary stricture in pediatric transplant recipients [113, 118-122]. In the recent literature, most stricture related complications of the biliary tree are managed without the need for re-operation [109]. Rarely, patients may require re-transplantation. In older studies, operative intervention was more commonly used [111, 116]. Complications from percutaneous cholangiographic interventions such as haemobilia, fever and sepsis, are uncommon and successful bile drainage is achieved in 76-
89% of cases [109]. Thus the more modern approach to stricture treatment is through interventional radiology.

**General Surgical Complications**

General surgical complications are those involving the post-operative wound itself or complications secondary to iatrogenic injury to peritoneal structures. These include wound infection and dehiscence, incisional and diaphragmatic hernia, bowel obstruction and bowel perforation. They are frequently observed but are generally not life-threatening [6]. As such, literature regarding these complications in pediatric LT is scarce.

**Wound Infection**

Wound infection is a common minor complication that can occur after surgical procedures. Pediatric liver transplant recipients are predisposed to infection due to a comparatively high net state of immunosuppression [29, 123, 124]. This is a result of the complex interaction between immunosuppressive therapy, presence of infection with immunomodulating viruses and surgical complications relating to the transplant procedure such as indwelling foreign bodies, injured tissues and fluid collections or ascites [29]. Moreover, transplant patients are in a parlous state pre-transplantation due to malnourishment and bleeding tendencies. Despite this risk, our center and others report relatively low rates of wound infection comparable to routine abdominal procedures [6, 28, 125]. This may be in part due to underreporting of minor complications not managed directly by specialist transplant teams.

Infections post-LT vary depending on timeline. Wound infections tend to appear within a month and do not usually involve opportunistic organisms as patients will not yet
have had prolonged exposure to immunosuppressants [124]. Ideally, most wound and other infections will be prevented with appropriate vaccinations for age, surgical antibiotic prophylaxis and universal prophylaxis for at risk populations.

_Bowel Perforation_

Perforation of gut is commonly due to iatrogenic injury during LT and has a reported incidence of up to 20% [7, 13, 21, 126, 127]. Unlike other general surgical complications, which tend to be minor, bowel perforation has a reported mortality rate of 30-50% [13, 126-129]. In a study at our center we published an incidence of 2.5% and a mortality rate of 0% more closely reflecting the recent literature [6].

At the time of LT, patients with BA with prior Kasai portoenterostomy often have dense adhesions complicating dissection [128, 129]. Thus, diathermy induced iatrogenic injury to the bowel and or diaphragm can occur while dissecting adhesions during the hepatectomy phase of LT. Moreover, BA patients tend to be younger and their tissues less robust at transplant making iatrogenic injury more likely in this population. One study suggested a link between adhesion severity in BA patients and the delay in transplantation [130]. At our center, patients undergoing primary transplantation without Kasai portoenterostomy were less likely to have gut perforation [6]. Management of bowel perforation usually necessitates laparotomy.

_Diaphragmatic Hernia_

Diaphragmatic hernia post-LT is a rare complication. Unlike congenital diaphragmatic hernias, after transplantation these complications are more likely to occur on the right side. It is usually associated with younger patients who tend to be lighter and/or malnourished leading
to a thinner diaphragm more easily damaged intra-operatively [131]. Oversized grafts increasing intra-abdominal pressure and positioning of split grafts more medially may also increase the propensity for herniation of abdominal contents through the diaphragm [131]. We also postulate that dense adhesions at the time of surgery – particularly in BA patients - complicating native liver dissection during hepatectomy may play a role.

The hepatectomy phase of LT may cause trauma to the right hemi-diaphragm particularly in the region overlying the bare area of the liver where there can be greater intra-operative bleeding [131]. Due to the severity of the bleeding, more extensive hemostatic efforts are often required that may injure the diaphragm. Trauma is not the only risk factor, however, with other patient characteristics clearly playing a role in the development of this complication.

Generally diaphragmatic hernia presents with respiratory symptoms and is readily diagnosed on chest radiography and/or ultrasound. Diaphragmatic defects in reported cases are predominantly in the right postero-lateral hemi-diaphragm [131]. Hernias are managed operatively by reducing the bowel contents and directly closing the diaphragmatic defect.

**Diaphragmatic Palsy**

Diaphragmatic palsy secondary is an uncommon complication caused by injury of the phrenic nerve in close anatomical proximity to the inferior vena cava [132, 133]. Early studies cite rates of partial right phrenic nerve injury and hemi-diaphragm paralysis post-LT as high as 80%, and complete paralysis as high as 30-40% [132, 133]. Recovery of normal function depends on the location and severity of the initial injury, but in severe cases can take up to a year [134, 135]. Conversely, there is some suggestion that iatrogenic injury to the phrenic nerve by crush injury – most likely caused by vascular clamps – rarely resolves [136, 137].
Though respiratory failure is unlikely following ipsilateral phrenic nerve injury, hemidiaphragmatic palsy can further predispose pediatric liver transplant patients to already common post-operative respiratory complications [133].

Management of paralysis to the right hemi-diaphragm is either with mechanical ventilation or with diaphragmatic plication [138]. As mechanical ventilation may be long-term and prone to complications, diaphragmatic plication is often preferred. It is a safe and effective procedure that allows for rapid weaning off mechanical ventilation with good long-term outcomes [138-140].

Conclusion

Pediatric LT outcomes are improving. The advent of new liver graft procuring techniques has somewhat alleviated stress on donor availability, microsurgery assisted anastomosis and anticoagulation have bettered vascular complication rates and delayed primary closure has been safe and allows for expected graft to recipient size mismatch in children. Incidence of complications is now more rare, however, if they do occur either interventional radiology and or surgery can be used to adequately treat patients with good long-term morbidity and mortality outcomes.

Remarks on Chapter I

Chapter I provides a brief overview of pediatric LT; its indications, recent advances and the most common complications observed. This review of the literature represents perspectives from transplant units around the world and thus these units and their patient demographic may differ significantly from our own. For example, in much of Asia and in particular Japan, the use of cadaveric grafts is rare with living related donor liver grafts far more prevalent.
Moreover, the use of particular surgical techniques is not universal and is instead highly variable between centers.

The key underlying theme here is that sick children with significant disturbance of their physiology undergo a major operative procedure and can subsequently develop complications. However, these are now less common and better managed than in the past thanks in part to the development of new surgical techniques and a greater understanding of the complex pathophysiology of end-stage liver failure. Moreover, multi-disciplinary specialized post-operative care is fundamental in the prevention and/or management of these complications should they arise.

Surgical advances in pediatric LT have been beneficial, with split liver grafts easing the pressure of donor organ shortage, and a combination of microsurgery and routine anti-coagulation reducing the incidence of hepatic artery complications. If vascular or biliary complications do occur, interventional radiology can now be used in their management. What used to require re-look laparotomy can now be successfully managed with less invasive percutaneous interventions.
References


65. Valla DC, Rautou PE. The coagulation system in patients with end-stage liver disease. Liver Int 2015;35:139-144.


CHAPTER II

Introduction

While Chapter I provided an introduction to pediatric LT and its common complications, Chapter II and III now delve into specific developments introduced at the Children’s Hospital at Westmead and their subsequent effect on complication rates. This chapter specifically explores the effect of DPC on general and GI complications. As DPC involves keeping the abdomen ‘open’ and requires a subsequent operation to achieve closure, some hypothesise that it may increase the risk of wound related and gastrointestinal complications. Thus, this study aimed to determine the safety of this surgical technique in our patient cohort.

Primary closure of wounds following surgery is defined as closure at the time of operation. By contrast, DPC involves partial or incomplete closure of the child’s abdomen at the time of LT and a subsequent closure usually 2-5 days’ post-transplantation. After implantation, donor liver grafts may become oedematous and swell, increasing intra-abdominal pressure and subsequently predisposing patients to abdominal compartment syndrome. Here, high intra-abdominal pressures can impede blood supply to the abdomen and cause organ hypoperfusion and ischaemia.

DPC was an important development in pediatric LT. Until the advent of split liver grafts, transplant surgeons more commonly used whole grafts which could be too large for pediatric recipients. The large-for-size grafts made primary closure difficult and predisposed patients to abdominal compartment syndrome. In more recent times, split grafts and even hyper-reduced or monosegmental liver grafts have now been well described and are widely implemented. However, as surgeons aim to avoid closure under tension, DPC techniques are still useful even with the use of smaller or more appropriately size-matched donor grafts.
An adjunct to DPC is the use of prosthetic implants to reinforce the anterior abdominal muscle wall. In this chapter we also investigated the safety of Surgisis®, a bovine derived bioprosthetic membrane, that can be used to better approximate abdominal muscle layers and allow for safe, delayed closure of abdomens post-LT.

A secondary aim of this study was to assess the general and GI complications that occur following LT in children and whether there was any notable patient, graft or operative predisposing factors.
Abstract

Purpose: To analyze the general surgical complications in pediatric liver transplant recipients and the safety of delayed primary closure at a single tertiary center.

Methods: A retrospective review of all liver transplant recipients between April 1986 and May 2014. All general and gastrointestinal complications, were recorded and analyzed. The incidence and risk of these complications were compared between children who had a primary versus those who had a delayed closure, with or without the use of Surgisis®, of their abdomen.

Results: 242 patients underwent 281 liver transplants. The median age of the children was 31 months. Whole (77), reduced size (91), split (96), and living related grafts (17) were used. General surgical complications were observed in 33 cases (11.7%). 135 cases underwent delayed primary closure (DPC) of their abdomen, 35 with Surgisis®. Patients with biliary atresia had a higher rate (4.6%) of bowel perforation (p = 0.013). The majority of complications occurred within 3 months of transplantation.
Conclusion: General surgical complications post-pediatric liver transplantation were common but usually not life threatening. Delayed primary closure was safe, had no significant long-term issues and was not associated with higher incidence of wound related complications.

Introduction

Development of surgical techniques, improved post-operative management and the introduction of refined immunosuppressive regimens have continued to improve the outlook for pediatric liver transplant (LT) recipients [1]. Pediatric transplant recipients often suffer high morbidity and remain more prone to post-operative complications [2]. Pre-operative patient factors, operative management and the post-operative regimens of immunosuppressive drugs [3] impact upon the development of surgical complications following transplantation which contribute to patient morbidity. General and gastrointestinal (GI) complications – including wound infection, wound dehiscence, incisional hernia, bowel obstruction and bowel perforation – continue to be important issues in the post-operative management of pediatric LT recipients.

Previous abdominal surgery, such as the Kasai procedure for patients with biliary atresia (BA), may also increase the likelihood of complications versus those with no prior abdominal surgery. Commonly, pediatric LT recipients receive size-mismatched grafts over which abdominal incisions cannot always be primarily closed. This has led to the employment of delayed primary closure (DPC) techniques, where the abdominal incision is closed at a later date allowing time for the liver graft and bowel edema/distension to settle, with or without the use of a prosthetic Surgisis® (Cook Australia Pty. Ltd., Brisbane, Queensland, Australia) graft [4-7]. It is possible that delaying the closure of abdominal wounds also contributes to the incidence of post-transplant complications.
We studied the incidence of general and gastrointestinal surgical complications in this cohort of patients and assessed potential factors that contributed to increased patient risk.

**Materials and Methods**

Children under 18 years of age who underwent LT between April 1986 and May 2014 were identified via The Children’s Hospital at Westmead (CHW) Gastroenterology Department and the Department of Pediatric Surgery databases. CHW, part of the Sydney Children’s Hospital Network (SCHN), represents the largest pediatric teaching hospital in Sydney and the sole center for pediatric LT in the state of New South Wales (NSW) and the Australian Capital Territory (ACT), serving a combined population of approximately 7.3 million.

Data collected included age, gender, ethnicity, pre-transplant diagnosis, previous transplant/surgical history, operative techniques, graft type, additional surgeries in the immediate post-transplant period (including DPC), general and GI complications. Unfortunately, data for native and transplanted liver graft weights, as well as operative time including warm/cold ischemic time were not available for all patients, particularly those in the late 1980s and early 1990s. These risk factors and outcomes were analyzed separately, with SPSS version 22 (IBM, St. Leonards, NSW, Australia) used for all statistical analysis. Chi-square tests were used for the comparison of grouped patient risk factors and odds ratios generated where possible to determine levels of risk. The primary outcome measured was the incidence of general and GI complications either in the early (<3 months), intermediate (3 months – 1 year) or late (> 1 year) post-transplant period.

Research ethics approval was obtained through SCHN Ethics Committee.
Results

281 liver transplants in 242 patients were completed in the study period: 128 (52.9%) were female. Overall, 25 received a second and 7 a third graft. The median age of the study population was 31 months (2 months – 18 years), with the majority of patient less than 50 months at time transplant. Whole (77), reduced size (91), split (96), and living related grafts (17) were used, with split graft LT being introduced in the year 2000. The most common indication for transplant was BA, accounting for 56.2% of the study cohort. 16 underwent primary LT and 129 had a Kasai portoenterostomy prior to transplantation. Patients were routinely immunosuppressed with adequate levels of tacrolimus, high dose steroid that was rapidly tapered, and azathioprine. Mycophenolate was rarely used. Demographic and outcome data are summarized in Table 1.

Wound infection and superficial wound dehiscence occurred in 7 (2.5%) and 6 (2.1%) cases respectively. Incisional hernia and diaphragmatic hernia were complications in 0.7% and 1.1% respectively, while bowel obstruction and perforation were seen in 3.2% and 2.5% of cases. 3 patients with general and GI complications died during follow up due to unrelated hepatic artery thrombosis, overwhelming sepsis associated with biliary stricture, and acute rejection respectively.

There was no correlation between patient age, gender, weight and cadaveric grafts with the incidence of general and gastrointestinal complications. Children who received whole grafts tended to have higher rates of bowel perforation (5.4% versus 1.4% for all other grafts) though this was not statistically significant (p = 0.061).

When grouping by transplant indication, patients with BA had a higher rate (4.6% versus 0%) of bowel perforation (p = 0.013). Bowel perforation only occurred in BA patients who underwent a Kasai portoenterostomy (5.1%). When compared with primarily
transplanted BA patients (n = 16) the relationship between prior Kasai and bowel perforation failed to reach significance (p = 0.353). BA patients also tended to be younger than patients undergoing LT for other indications (mean age 31.5m versus 90.3m p < 0.001). Patients with acute hepatitis were at an increased risk of developing post-operative adhesive bowel obstruction (p < 0.01). There was an increased trend for wound complications in the DPC group, excepting for incisional hernias, although this was not significant. Patients requiring Surgisis® (Cook Australia Pty. Ltd.) for primary closure had moderately higher rates of wound infection (5.7% versus 2%, p = 0.191) and dehiscence (5.7% versus 1.6%, p = 0.117) and lower rates of incisional hernia (0% versus 0.8%, p = 0.592). None of the patients required removal of Surgisis® patch due to infective complications. Patients undergoing re-transplantation were not found to be at increased risk of developing general or GI complications when compared to those receiving their first transplant (p > 0.5), the only exception being a non-significant increase in wound dehiscence (p = 0.164). Risk factor analysis is summarized in Table 2.

Discussion

Pediatric LT remains a skill intensive, lifesaving operative procedure with high rates of post-operative surgical complications [8, 9]. Despite this, LT remains the gold standard for the treatment of end stage liver disease. In children, LT has been challenged by allograft size discrepancy and the now more common use of marginal and split grafts, which may potentially increase the risk of surgical complications. Conversely, the use of split and other marginal liver grafts, either from a cadaveric or living related donor, allows for timely transplantation, reducing wait list morbidity and mortality [10]. Post-operative morbidity in children arises due to a number of contributing factors including pre-transplant morbidity,
such as malnutrition, graft type and quality, previous abdominal procedures, surgical and anesthetic expertise and post-operative management [9].

Bowel perforation has a reported incidence between 2.5-20% and a related high mortality rate of 30-50% [11-15], making early diagnosis and appropriate post-operative management essential. Our incidence of 2.5% for bowel perforation with 0% related mortality in 281 transplants compares favorably with results from other centers. Much of this success may be attributable to the implementation of a specialized pediatric team in the care of these children from the inception of the transplant program. Pediatric surgeons perhaps are more familiar with the handling of delicate tissues and detect subtle deteriorations in children post-operatively.

Interestingly, bowel perforation occurred in only one subset of patients in our series, leading to a statistically significant association between BA who previously had a Kasai portoenterostomy and bowel perforation compared to all other indications for LT. As a consequence of recurring cholangitis and previous portoenterostomy, patients with BA almost invariably have severe intra-abdominal adhesions by the time they are transplanted [11, 12]. At the time of surgery, therefore, these patients commonly have dense bowel to bowel, bowel to liver, and liver to diaphragm adhesions. Often there is no obvious plane of dissection, and the separation of these adhesions becomes both time consuming and technically difficult. A consequence of dense adhesions remains diathermy-induced injury. This may present as bowel perforation post-transplant a few days later. Iatrogenic injury to the diaphragm can also occur while separating adhesions consequently potentiating diaphragmatic hernia development. Sanada et al. postulated a link between the severity of adhesions and the length of time the native liver remains in situ in BA patients [11]. Furthermore, BA patients tend to be younger and their tissues less robust at the time of
transplant, increasing the risk of bowel perforation during the hepatectomy phase of the transplant procedure. In our cohort, the average age of patients with BA undergoing transplantation was 32 months compared to an overall average age of 59 months, though age was not found to be an independent risk factor for the development of complications.

Patients receiving whole donor liver grafts had moderately increased rates of bowel perforation (OR = 3.67) but this did not reach significance. This link was questionable, having been influenced by the relatively higher proportion of BA patients receiving whole grafts (37.2%) earlier in the series. We do not have an explanation of the increased incidence of adhesive bowel obstruction for patients transplanted for acute hepatitis and this may represent a chance occurrence.

The majority of other general and gastrointestinal complications occurred within 3 months of transplantation, with the exception of incisional hernias which appeared later. This stresses the importance of post-transplant clinical vigilance in order to appropriately manage these complications in a timely fashion. Moreover, education of families is vital in ensuring the success of longer-term surveillance of patients in detecting complications of more delayed-onset. Our rates of wound infection (2.5%), wound dehiscence (2.1%) and incisional hernias (0.7%) were low and compared favorably with literature [3, 9]. This low instance of wound infection may have been underreported, as this is usually a comparatively minor complication and hence less accurately recorded. However, wound dehiscence and incisional hernias were proactively managed and documented by the transplant surgeons at our center, and was therefore much less subject to a reporting bias.

Particularly in younger infants, the size of donor liver graft is an important concern. To overcome this discrepancy and organ shortage, left lateral segment grafts either from cadaveric or live donors are commonly used. In these children some centers have proposed
the use of monosegmental grafts in order to prevent complications associated with large-for-size graft syndrome, increased abdominal pressure [16, 17], and avoid DPC with or without the use of synthetic mesh. A recent publication has suggested that the use of monosegmental grafts may be unnecessarily complicated, however, favoring instead DPC techniques [18]. The use of Surgisis® (Cook Australia Pty Ltd) allows for approximation of muscle layers facilitating safe closure of the abdominal wall in transplanted patients [19]. Our data suggests that Surgisis® (Cook Australia Pty Ltd) is a good alternative to achieve closure in cases with thick liver grafts and those with increased abdominal pressure threatening to complicate the liver grafts’ vascular integrity.

DPC allows time for the graft to reduce in size and bowel edema/distension to settle, minimizing the risk of graft compression and abdominal compartment syndrome [4, 6, 20]. As only 40% of infants undergoing LT are able to have their abdomen closed primarily, DPC has become common practice even in this era of reduced-sized liver grafts [4]. Some studies have indicated that DPC increases the risk of wound complications including wound infection, dehiscence, and incisional hernias [4, 21]. Our study refutes these findings. In our experience, DPC was safe with no significantly increased risk of general and gastrointestinal complications. A recent meta-analysis has suggested that DPC may reduce the incidence of wound infections in patients with dirty or contaminated abdominal wounds, though further well-designed randomized clinical trials with larger sample sizes would be warranted [22].

Re-transplantation post graft failure can be accompanied by a number of issues such as extensive blood loss and the need for more creative vascular anastomotic techniques [23]. In our study having multiple transplants was not a risk factor for the development of general and GI complications.
In conclusion, general and GI complications appear commonly but are usually not life threatening. In our study, 3 patients with these issues died due to other concurrent LT complications. We re-affirmed the association between BA and bowel perforation, and that DPC is safe with no long-term issues.
### Table 1: Demographic and Outcome Data

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Table 2: Risk Factor Analysis

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<td>0.569</td>
<td>*</td>
</tr>
<tr>
<td>Surgisis</td>
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<td>2.9</td>
<td>0.191</td>
<td>0.545-15.669</td>
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<td>Wound Dehiscence</td>
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<td>3.7</td>
<td>0.117</td>
<td>0.646-20.805</td>
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<tr>
<td></td>
<td>Incisional Hernia</td>
<td>0.0</td>
<td>*</td>
<td>0.592</td>
<td>*</td>
</tr>
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</table>

* Odds ratio could not be computed as either the rate or the comparison rate is equal to 0.
Remarks on Chapter II

Chapter II primarily demonstrates the general and GI complications seen in children post-LT. Specifically, we also assessed if there was a link between DPC and the incidence of wound related complications such as wound infection and dehiscence.

Our findings suggest that DPC with or without the use of prosthetic implants such as Surgisis® (Cook Australia Pty Ltd) is a safe and effective technique for closure of abdomens post LT. DPC allows time for the donor liver graft swelling and other post-operative oedema to subside, reducing the risk of abdominal compartment syndrome and graft compression. In addition, our study showed no increase in the risk of wound related complications observed in this cohort compared to those who underwent primary closure.

The secondary aim in this study was to deduce whether general and GI complications were linked to patient or operative factors. Of note, BA patients who had undergone prior Kasai portoenterostomy had a higher incidence of bowel perforation. We postulated that this may be due to dense adhesions seen at the time of LT secondary to prior abdominal surgery. Adhesions make surgical dissection of the native liver difficult during explantation, predisposing patients to iatrogenic diathermy induced injury to local structures.
References


CHAPTER III

Introduction

While Chapter II looked at general and GI complications, this chapter is assigned to HAT and recent advances in both surgical technique and post-operative management which have reduced the incidence of this complication. As described in Chapter I, HAT is a potentially devastating complication which is more common in pediatric versus adult liver transplant recipients. Its incidence, as will be explored, is thought to be linked to surgical technique as well as the complex hypercoagulable state observed pre and post LT.

In light of this, microsurgical techniques were introduced to more delicately handle and avoid damage to the intimal layer of vessels during anastomosis. Similarly, in response to reports demonstrating hypercoagulability following LT, a new routine anticoagulation protocol was introduced.

In this study we examined the effect of these on the incidence of HAT. To do so, our cohort was divided into three eras. In the first, normalisation of coagulation physiology was allowed to return passively with only a single standardised dose of heparin given. In the second era, microvascular techniques were introduced and in the third era, in addition, a routine anticoagulation protocol was implemented which involved replacement of antithrombin 3, protein C and S, and appropriate heparinisation. Subsequently, we analysed the incidence of HAT in all three eras to determine the efficacy of these new surgical and post-operative management techniques. In addition, we also reported how HAT was managed in each era.
**Paper 2 - Reduction in hepatic artery thrombosis in pediatric liver transplantation with the introduction of microvascular techniques and a customized anticoagulation protocol – Presented as it was submitted to Pediatric Transplantation.**

**Abstract**

Aim: To assess the incidence of hepatic artery thrombosis (HAT) over 3 eras following implementation of microvascular techniques and a customized anticoagulation protocol in a predominantly cadaveric in situ split liver transplant program.

Methods: Retrospective review of pediatric liver transplants performed at our centre between April 1986 and April 2016. Incidence of HAT over 3 eras was analysed. In the first era, 1986-2008, each patient received a standard dose of 5U/Kg/hour of heparin and coagulation profiles were allowed to normalize passively. In the second era, 2008-2012, microvascular techniques were introduced. In the third era, 2012-2016, in addition, a customized anticoagulation protocol was introduced which included replacement of Antithrombin 3, Protein C and S and early heparinization.

Results: 317 liver transplants were completed during the study period, with a median age of 31.7 months. In the first era, 22% of grafts were cadaveric in situ split grafts while the second and third eras used split grafts in 59.0% and 64.9% of cases respectively. HAT occurred in 9.5% in the first era, 11.5% (p = 0.661) in the second and dropped to 1.8% in the third era (p = 0.043).
Conclusions: A routine anti-coagulation protocol has significantly reduced the incidence of HAT post liver transplantation in children in a predominantly cadaveric in-situ split liver transplant program.

Introduction

Pediatric liver transplantation (LT) remains a lifesaving and skill intensive procedure, where patients often experience high morbidity due to peri-operative complications (1, 2). Despite this, LT remains the gold standard for children with end-stage liver failure. In recent times, liver transplants in children have enjoyed excellent results with the implementation of new organ procuring techniques, microsurgery, anti-coagulation protocols and newer immunosuppressive drugs; reducing both short and long term morbidity and mortality (2).

Hepatic artery thrombosis (HAT) represents an uncommon complication of LT with the potential to cause graft loss and/or long term patient morbidity (3, 4). Paediatric liver transplant recipients have historically had a higher incidence of HAT than their adult counterparts (5).

The incidence of HAT is related to both technical and graft related factors. Prolonged cold ischemic preservation, ABO incompatibility, rejection, anatomical site of arterial reconstruction, size of the donor artery and the number of anastomoses have all been found to be associated with an increased incidence of HAT (5-7).

Post-operatively, HAT frequently causes graft failure and death in up to 50% of cases (4). In the longer term, HAT correlates with the development of biliary complications such as strictures, bile leaks and severe biliary sepsis (6, 8). Patients also have significantly lower 5-year survival rates after HAT (9).
The advent of split liver grafts has eased the pressure of donor organ shortages but has contributed to increased technical difficulties and further increased the risk of HAT (10-12). Attempts to reduce the incidence of HAT using microsurgical techniques either with or without the use of an operating microscope have shown to be of some but not consistent benefit (13-15).

Patients with chronic liver disease have decreased levels of both pro-coagulant factors as well as naturally occurring anticoagulants such as Anti-thrombin 3, Protein C and S, as almost all factors responsible for haemostasis are produced in the liver (16-18). This then makes patients susceptible to bleeding but also paradoxically to vascular thrombosis as well. In the immediate post-transplant period, this risk is amplified due to a lag in the production of Antithrombin 3, Protein C and S by the transplanted liver, placing the patient at greater risk of vascular thrombosis (19).

An understanding of this phenomenon in the more recent past, has prompted centres to correct this with the introduction of thrombin inhibitor replacement protocols which include early replacement of Antithrombin 3, Protein C and S coupled with heparinization. Such protocols have demonstrated clear improvements in the rates of HAT (10, 12).

Our program in Sydney is a predominantly cadaveric split donor program. In order to reduce our incidence of HAT, we introduced the use of microsurgical techniques for hepatic arterial reconstruction in 2008. In 2012, in addition, we introduced a thrombin Inhibitor replacement protocol along with early appropriate heparinization.

We hypothesize that the incidence of HAT in our cohort has decreased throughout 3 distinct eras after the introduction of microsurgical techniques and with the implementation of a customized anticoagulation protocol.
Materials and Methods

Pediatric liver transplants completed at a single tertiary referral centre in Sydney, Australia between April 1986 and April 2016 were retrospectively identified. Pre-transplant patient demographic data including age and weight at transplant, gender, indication for LT, and relevant medical and surgical history was obtained. Transplant specific details such as liver graft type and weight, number and type of HA anastomosis, HA flow and the use of microsurgery and or a routine anticoagulation protocol were also recorded. The primary outcomes measured were the incidence and subsequent management of HAT.

Patients were then divided into 3 eras depending on the date of implementation of microvascular techniques and an anticoagulation protocol. In the first era (E1) between 1986 and 2008, the normalization of coagulation by the liver graft was permitted. Use of platelets and Fresh Frozen Plasma (FFP) were avoided unless there was problematic bleeding. A standardized 5 U/Kg/hr of post-operative heparinization was initiated when the International Normalized Ratio (INR) dropped to normal (1.2). In the second era (E2), microvascular techniques were introduced between 2008 and 2012. Here, the heparinisation protocol remained the same. In the third era (E3) from 2012 to the present, a customized anticoagulation protocol was introduced in addition to the use of microvascular techniques. In all eras, we aimed to keep the hematocrit to around 30%.

Risk factors and outcomes underwent statistical analysis using SPSS version 22 (IBM, St. Leonards, NSW, Australia). The primary outcomes measured were the incidence in each of the three eras and the subsequent management of HAT. Early HAT, defined as evidence of thrombosis within the first 30 days of LT (5) is diagnosed by occlusion of the HA on angiography or at exploratory laparotomy at our centre.
Standard and Microvascular Techniques for Arterial Reconstruction

In the first era, standard vascular instruments and bulldog clamps for vascular control were used. Interrupted or continuous 7/0 or 8/0 polypropylene sutures were used for arterial anastomoses.

In E2 and E3, microsurgical techniques included the use of microsurgical instruments to minimize trauma to the vessels. Vessels were handled with jewellers forceps and micro-scissors were used to divide the vessels and de-sleeve the adventitia. Either single or double atraumatic micro-clamps (Acland or Kleinert type) were used to avoid clamp induced endothelial trauma. Interrupted 8/0 or 9/0 sutures were used for the anastomosis. The operating microscope was used only on a few occasions with the vast majority of surgeries done using 3.5 or 4.0-loupe magnification.

Post-Operative Anticoagulation Protocol (Figure 1).

In E1 and E2, heparin therapy was initiated usually by the 3rd or 4th post-operative day as a standard 5U/Kg/Hr continuous infusion for 4 or 5 days or until oral aspirin (40mg daily) was tolerated. Coagulation parameters from the new liver graft were allowed to normalize passively. Aspirin was commenced post-operatively when the child was able to take orals and continued for 6 months.

In E3, the first dose of Antithrombin 3 concentrate, Thrombotrol®-VF, CSL, Australia, was commenced intra-operatively after satisfactory haemostasis was achieved. A further 2 doses were given 24-hours apart with the expectation that the donor liver would by day-4, produce its own Antithrombin 3. Antithrombin 3 levels were maintained between 70-100% of normal.
Initially, at least 20 mL/Kg of FFP was given daily for replacement of protein C and S. In cases with significant ascitic losses, additional FFP was given as needed. This volume has since 2015 been reduced to 10mL/Kg plus replacement for ascitic losses due to concerns with over-hydration.

Intravenous unfractionated heparin was commenced as soon as the INR fell to less than 2 at an initial dose of 10 U/Kg. The maximum dose of heparin given was 20 U/Kg. Although anti-Xa levels were measured daily, no attempt was made to achieve a therapeutic anti-Xa level. Our intention was to achieve a very modest degree of heparinzation in order to reduce the risk of post-operative bleeding. The dose of heparin was titrated on a twice-daily basis using the Thromboelastography as a rough guide (Figure 2). A modest prolongation of the R time on the Thromboelastogram between 1.5 to 3 times greater than the length on the Citrated Kaolin Heparinase curve was considered evidence of a gentle or modest degree of heparinization. Heparin was continued for up to 2 weeks until oral aspirin was commenced.

Daily Protein C and S levels as well as Antithrombin 3 levels were measured for a week to ensure levels were close to or within the normal range.

**Results**

Between April 1986 and January 2016, 273 children underwent 317 LTs at our centre. The median age of our cohort was 31.7 months (IQR = 91.46) at LT with a slight preponderance of females (51.6%). The number of liver transplant cases within each era was 199 for E1, 61 for E2 and 57 for E3. Biliary atresia (BA) was the most common indication for LT representing 53.0% of the cohort. In the first era, whole or reduced graft LT was preferred, accounting for 75.4% of patients. Split grafts were only used in 22.1% of cases. In the subsequent eras, split grafts predominated being used in 59.0% of cases in E2 and 64.9% in E3. Living related donor
grafts were used in only 19 (6.0%) cases. Graft type utilization in each era is summarized in Figure 3.

HA anastomoses were either standard end-to-end (81.1%), comprised of multiple anastomoses (4.4%), required arterial reconstruction (5.4%) or involved a jump graft (4.7%). Demographic data is summarized in Table 1.

During the study period there were 27 instances of HAT giving an overall incidence of 8.5% in 317 liver transplants. All HATs manifested early, occurring within two weeks of transplantation in all but one case and were confirmed with either exploratory laparotomy or angiography. Rates of HAT differed between the 3 eras, with 19 (9.5%) seen in E1, 7 (11.5%) in E2 (p = 0.661) and 1 (1.8%) in E3 (p = 0.043). Rates of HAT were not influenced by graft type. Rates of HAT were highest for patients with intrahepatic cholestatic disease, 20.0% (6), than for patients with other indications for LT, 7.3% (21) (p = 0.018). The rate of HAT in patients receiving split grafts (9.4%) was similar to HAT rates in all other graft types (8.0%) across all eras (p = 0.666) and in each individual era respectively (pE1 = 0.296, pE2 = 0.478 and pE3 = 0.170).

Anastomotic technique did not impact on the incidence of HAT. Standard end-to-end anastomoses and multiple HA anastomoses had the lowest rates of HAT (8.1%) while jump grafts had the highest (13.3%) though these differences did not reach significance (p = 0.478). Minor bleeding not requiring surgical intervention was more common in patients in E3, occurring in 24.6% (14) of cases versus 11.5% (30) in E1 and E2 (OR = 2.496, p = 0.01).

Management of HAT varied between cases and eras particularly with the advent of interventional radiology. Re-transplantation was necessary in 11 (40.7%) cases. The number of re-transplants for each era was 9 (47.4%) in E1, 1 (14.3%) in E2 (p = 0.124) and 1 (100%) in E3, however these did not differ significantly. For the remaining 16 (59.3%) cases, laparotomy
and revision of HA, portal vein and or biliary anastomoses was necessary. In one case in E2, the patient rapidly deteriorated and care was withdrawn. For a further two patients in E2, good patency of the HA was achieved after balloon angioplasty and with angiography plus local thrombolysis respectively. Despite remaining patent for some months, both these cases subsequently required laparotomy and revision of the HA anastomosis due to poor flows.

Management of HAT is summarized in Table 2.

Our overall Kaplan-Meier patient survival over the entire study period is 70% and out unit’s current 1-year patient survival is 94%.

Discussion
This represents the largest single centre study on HAT incidence in a predominantly cadaveric split graft pediatric liver transplant program. Only three other studies in the literature have larger pediatric cohorts and mostly represent living donor or whole graft liver transplant programs. In this cohort of pediatric patients from a single tertiary liver transplant centre in Sydney, we have shown that an appropriate anticoagulation protocol significantly decreases the rate of HAT.

The conditions immediately post LT creates “a perfect storm” for vascular thrombosis to occur. This includes a combination of large fluid shifts, multiple vascular anastomosis with “intimal damage”, sluggish flows secondary to recovering but stiff grafts and a prothrombotic state brought on by critical reduction in the levels of Antithrombin 3, Protein C and S.

After LT, synthesis of procoagulant proteins by the transplanted liver occurs almost immediately but there is a 3 to 7 day lag in the production of anticoagulant proteins, rendering patients prothrombotic in the first 3-7 days post-transplant (12, 19). It is within this time that the majority of thrombotic complications such as HAT can occur.
HAT post-pediatric LT has a reported incidence between 1.0-20.2% in the recent literature (5). In one study, incidence of HAT was reported as high as 30% in recipients under 1-year of age (20). In a systematic review, Bekker *et al.* report the mean incidence of pediatric HAT post-LT as 8.3% with a mortality rate of 25% across 43 studies (5). This mortality rate varied significantly depending on the era and the transplant centre (11). Our rate of HAT across all three eras (8.5%) and particularly in E3 (1.8%) compares favourably with results from other institutions. Some studies suggest that in-situ split graft LT in children increases the risk of HAT [11-13]. Our findings however, do not support this.

We believe our improvement is probably due to multiple factors. In part, it is due to the learning curve of the multi-disciplinary team caring for these very complex patients and improved operative techniques. As the donor selection process, methods of retrieval, continual use of mostly cadaveric grafts, peri-operative IV fluid management, immunosuppression regime and the surgical personnel have been relatively constant over the latter 2 eras, the major contributing factor seems most likely to be the implementation of the customised thrombin inhibition anticoagulation protocol. This came with an increased risk of minor bleeding, which, in this study was always managed conservatively without surgery.

Our success echoes that of Sugawara *et al.*, who have had a similar outcomes in reducing thrombotic complications, particularly HAT (12). In our study, although this appears to be independent of the adoption of microvascular techniques, the results could reflect a learning curve with adoption of a large number of in-situ split grafts during that period.

The use of microsurgical techniques for the meticulous reconstruction of delicate pediatric vasculature in liver transplantation was initially recommended by Starzl in 1976 and is critical when small vessels, complex reconstructions and multiple vascular anastomoses are involved (14, 21). The use of an operating microscope for HA anastomosis has not been
universally adopted by many transplant centres as it can be cumbersome and difficult to use in a narrow and moving field.

While it is thought that the major risk for HAT is a direct result of local trauma to the vessels during anastomosis, our results suggest that understanding the pro-thrombotic state in the early days post LT and correcting it appropriately appears to be the intervention that has made the most significant difference in preventing HAT. Thus, both the use of microsurgical techniques and the appropriate management of coagulopathy are essential in minimizing risk of HAT (2, 10, 15, 22-28).

The major strength of this study is that it may offer a potential solution to reduce the rate of HAT in cadaveric split graft LT. There are several limitations that should be considered when evaluating our results. This was a single-centre retrospective study that relied upon data spanning over 30-years. Thus, it is likely that there are subtle changes in techniques, management improvements and other risk factors over this long time period that could not be controlled for. Future studies may wish to focus on the long-term complications and outcomes of patients with successfully managed early HAT.

In conclusion, HAT has decreased in incidence in our centre where predominantly cadaveric in-situ split grafts are used. We believe this is due primarily to the implementation of the thrombin inhibition and anticoagulation protocol. Microvascular techniques for HA reconstruction have a smaller role to play.
### Tables and Figures

#### Table 1. Demographic data and incidence of HAT.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
<th>HAT (N%)</th>
<th>p-value</th>
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<td><strong>Gender</strong></td>
<td></td>
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<tr>
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<td>141</td>
<td>51.6%</td>
<td>8.8%</td>
<td>0.881</td>
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<td>Male</td>
<td>132</td>
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<td>8.3%</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;10kg</td>
<td>128</td>
<td>40.4%</td>
<td>7.8%</td>
<td>0.711</td>
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<tr>
<td>≥10kg</td>
<td>189</td>
<td>59.6%</td>
<td>9.0%</td>
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</tr>
<tr>
<td><strong>Era</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Era 1</td>
<td>199</td>
<td>62.8%</td>
<td>9.5%</td>
<td>-</td>
</tr>
<tr>
<td>Era 2</td>
<td>61</td>
<td>19.2%</td>
<td>11.5%</td>
<td>0.661</td>
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<tr>
<td>Era 3</td>
<td>57</td>
<td>18.0%</td>
<td>1.8%</td>
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<td><strong>Transplant Indication</strong></td>
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<td>Biliary Atresia</td>
<td>150</td>
<td>54.9%</td>
<td>8.9%</td>
<td>0.781</td>
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<td>19.0%</td>
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<td>Acute Hepatitis</td>
<td>33</td>
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<td>7.5%</td>
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<td>Intrahepatic Cholestasis</td>
<td>23</td>
<td>8.4%</td>
<td>20.0%</td>
<td>0.018*</td>
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<td>Miscellaneous</td>
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<td>2.9%</td>
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<td>Neoplasia</td>
<td>6</td>
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<td>1</td>
<td>0.4%</td>
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<td>0.595</td>
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<tr>
<td><strong>Graft Type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Split</td>
<td>116</td>
<td>36.6%</td>
<td>9.5%</td>
<td>0.666</td>
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<tr>
<td>Cutdown</td>
<td>94</td>
<td>29.7%</td>
<td>10.6%</td>
<td>0.380</td>
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<tr>
<td>Whole</td>
<td>88</td>
<td>27.8%</td>
<td>5.7%</td>
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<td>19</td>
<td>6.0%</td>
<td>5.3%</td>
<td>0.600</td>
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<td><strong>Hepatic Artery Anastomosis</strong></td>
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<td>Standard End-to-End</td>
<td>257</td>
<td>81.1%</td>
<td>8.2%</td>
<td>0.648</td>
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<td>Arterial Reconstruction</td>
<td>17</td>
<td>5.4%</td>
<td>11.8%</td>
<td>0.851</td>
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<td>Jump Graft</td>
<td>15</td>
<td>4.7%</td>
<td>13.3%</td>
<td>0.622</td>
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<tr>
<td>Unknown</td>
<td>14</td>
<td>4.4%</td>
<td>7.1%</td>
<td>-</td>
</tr>
<tr>
<td>Multiple Anastomoses</td>
<td>14</td>
<td>4.4%</td>
<td>7.1%</td>
<td>0.494</td>
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<tr>
<td><strong>Number of Grafts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>First graft</td>
<td>273</td>
<td>86.1%</td>
<td>8.1%</td>
<td>0.466</td>
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<tr>
<td>Multiple grafts</td>
<td>44</td>
<td>13.9%</td>
<td>11.4%</td>
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Table 2. Incidence and Management of Hepatic Artery Thromboses

<table>
<thead>
<tr>
<th></th>
<th>Era 1</th>
<th>Era 2</th>
<th>Era 3</th>
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<tr>
<td>Re-Transplantation</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Laparotomy</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Thrombectomy</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trial of Balloon angioplasty/Local Thrombolysis</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Withdrawal of Care</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
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</table>
Figure 1. Schematic diagram of anticoagulation protocol used from 2012 for the prevention of HAT.

### Patient Monitoring

**12-Hourly Monitoring**
Commenced upon reaching ICU

- FBC
- PT
- APTT
- INR
- Fibrinogen**

**Daily Monitoring**

- Vitals
- Fluid Status

Blood Tests:
- Fibrinogen**
- Anti-Xa (upon commencement of heparin)
- Antithrombin 3 (first checked 18-24 hours after first dose)
- Protein C and S levels
- Thromboelastography

Additional Monitoring:
- Doppler ultrasound

### Anticoagulant Replacement

**Intra-Operative After Haemostasis**

Antithrombin 3 at:
- 1,000 units for 0-30Kg BW*
- 2,000 units for 30-60Kg BW
- 3,000 units for 60+Kg BW

Vitamin K 1mg

**Daily from Day 1-3**

Antithrombin 3 regardless of serum levels given 24hr apart at above doses

Commence unfractionated heparin when INR falls to less than 2.

- Initial dose of 10U/Kg/h
- Maximum dose of 20U/Kg/h

**FFP**
- 10-20mL/Kg/day
- Additional FFP if ascitic losses significant

**After Day 3**

Continue heparin for up to 2-weeks or until aspirin is commenced

*BW – Body weight
** – fibrinogen is monitored daily unless there is evidence of hemorrhage
Figure 2. Sample thromboelastogram (TEG) showing modest prolongation of R-time on the heparinize sample.
Figure 3. Percentage of patients receiving whole, split, reduced and living related donor liver grafts per transplant era.
Remarks on Chapter III

Chapter III looked at HAT incidence across three eras after the introduction of microsurgical anastomosis of vessels and a new routine anticoagulation protocol. These have both been shown to be beneficial in other centers, but at our transplant unit, incidence of HAT was only lowered when the anticoagulation protocol was introduced. This was at the expense of increasing the rate of post-operative bleeds, however, these were minor and did not require operative intervention.

Unlike general and GI complications, HAT certainly has the propensity to cause graft failure, biliary complications in the long term and/or death. It is also more common in the pediatric liver transplant population. Therefore, the prevention of this complication is an important determinant of patient short and long term morbidity and mortality.

The major benefit of this study is the assessment of risk factors potentially predisposing to HAT and the offering of a solution to help reduce HAT incidence. The consistent finding in the literature of a reduction in HAT with aggressive and appropriate anticoagulation clearly confirms that a hypercoagulable state at the time of and following transplantation is important in the pathogenesis of this complication.
References

CHAPTER IV – CONCLUDING REMARKS

Summary of Findings

Despite the frequency of complications, LT remains the gold-standard treatment for children with end-stage liver disease. The purpose of the work presented in this thesis was to evaluate the safety and efficacy of new surgical techniques and post-operative management in reducing post-operative complications introduced at a single tertiary referral center in Sydney, Australia. Introduced to common practice at our center were DPC and microsurgical techniques as well as a post-operative anticoagulation protocol. We therefore assessed the incidence of general and GI complications to determine the safety of DPC and then examined the incidence of HAT after the introduction of microsurgical techniques and then again after the routine use of an aggressive anticoagulation protocol.

In our first study, we found that DPC was safe and allowed for appropriate closure after LT. In addition, the use of prosthetic implants such as Surgisis® (Cook Australia Pty Ltd) to help approximate muscle layers was also safe and did not increase the risk of wound related complications. We also reaffirmed the association of BA with the incidence of bowel perforation. Here, we believe that this may be due to iatrogenic injury at the time of explantation secondary to complicated dissection in the presence of dense intra-abdominal adhesions.

Next, we assessed the efficacy of microsurgery and a routine anticoagulation protocol on the incidence of HAT. While at other centers, microsurgical techniques have been shown to reduce the incidence of vascular complications such as HAT, at our center this was not demonstrated. Conversely, the introduction of a routine anticoagulation protocol significantly
reduced the incidence of HAT but also increased the rate of minor bleeding. This was mostly self-limiting and never required operative management.

It is hoped that the studies presented in this work will contribute to the existing body of knowledge on pediatric liver transplant complications and their patient specific or operative risk factors. With the advent of new surgical techniques and post-transplant management protocols, the prevention, detection and management of complications is certainly improving.
Site Authorisation Letter

Dear Mr Ziaziaris,

HREC reference number: LNR/14/SCHN/416
SSA reference number: LNRSSA/14/SCHN/493
Project title: The predictability of general surgical complications after liver transplantation in children based on clinical and patient factors
Site: The Children’s Hospital at Westmead

Thank you for submitting an application for authorisation of this project. I am pleased to inform you that authorisation has been granted for this study to take place at the above site.

The following conditions apply to this research project. These are additional to those conditions imposed by the Human Research Ethics Committee that granted ethical approval:

1. Please advise us of the date when the project starts at this site.
2. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, and which are submitted to the lead HREC for review, are copied to the research governance officer.
3. Proposed amendments to the research protocol or conduct of the research which may affect the ongoing site acceptability of the project are to be submitted to the research governance officer.

Yours sincerely,

Eman Nafea, PhD
Research Governance Officer
Declaration by the Head of Department/School/Research organisation

Project title: The predictability of general surgical complications after liver transplantation in children based on clinical and patient factors.

Where an investigator for the study is also the head of department, certification must be sought from the person to whom the head of department is responsible. Investigators must not approve their own research on behalf of the department.

1. I certify that I have read the project application named above.

2. I certify that I have discussed this project and the resource implications for this department with the Co-ordinating/Principal Investigator.

3. I certify that the Co-ordinating/Principal Investigator and other investigators involved in the project have the necessary skills, training and experience to undertake their role, and where necessary, appropriate training and supervision has been arranged.

My signature indicates that I support this project being carried out using the required resources, based on the information provided by the Co-ordinating/Principal Investigator

Name of department: Department of Paediatric Surgery

Name of head of department: A/Prof. Ralph Cohen

Signature: [signature]

Date: 26/9/14

Comments:
Author Contributions

William A Ziaziaris – Obtained ethics approval. Conceived the idea and design of both studies under the guidance of GT and AS and conducted the literature review. Acquired, analyzed and interpreted data. Computed statistics. Produced figures and tables. Drafted and revised both papers.

Alexandre Darani – Drafted and revised both papers.

Andrew JA Holland – Helped conceive the idea and design of the study. Aided with statistical analysis. Drafted and critically revised both papers.

Angus Alexander – Drafted and revised manuscript.

Jonathan Karpelowsky – Aided with statistical analysis. Drafted and critically revised both papers.

Pasquale Barbaro – Drafted and critically revised Paper 2.

Michael Stormon – Aided acquisition of data. Drafted and revised Paper 2.

Edward O’Loughlin – Aided acquisition of data. Drafted and revised Paper 2.

Albert Shun – Helped conceive the idea and design of the studies. Drafted and critically revised both papers.

Gordon Thomas – Guided and helped WZ conceive the idea and design of the studies. Drafted and critically revised both papers. Revised all figures and tables. Approved final version of both papers.