Factors Affecting Blood Loss In Liver Surgery For Colorectal Metastases

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The candidate confirms that the work submitted is his/her own and that appropriate credit has been given where reference has been made to the work of others.

Assertion of moral rights:

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• Abstract

ABSTRACT

Introduction

Peri-operative blood loss and blood transfusions are associated with poorer short- and long-term outcomes in patients undergoing hepatectomy. Various techniques are utilised to decrease blood loss though these may also cause an Ischaemia-Reperfusion Injury (IRI). The aim of this thesis was to identify factors which predispose to intra-operative bleeding during liver surgery and to identify methods to decrease blood loss without increasing the likelihood of post-operative liver dysfunction

Methods

In order to address the aim of this thesis, several studies are performed:

1. A systematic review examining non-surgical methods to decrease blood loss. Primary outcome measures included peri-operative blood loss and transfusion requirements. The secondary outcome measure was occurrence of IRI. The review was performed according to the PRISMA guidelines for systematic reviews.

2. A retrospective database analysed the association between blood transfusion and survival. . Uni- and multivariate analysis were performed.

3. A pilot single blinded, randomised control trial (RCT) was undertaken comparing the Pringle manoeuvre (standard) versus Portal Vein clamping.

Results

1. Seventeen studies were included in the systematic review. In 8 studies (n=894) pharmacological methods and in another 9 studies (n=679) anaesthetic methods to decrease blood loss were investigated. In 3 trials potential benefits of anti-fibrinolytics were demonstrated. Six anaesthetic trials demonstrated potential roles for low central venous pressure, acute normovolaemic haemodilution, autologous blood donation techniques and choice of inhalational anaesthetic agent employed.

   Six hundred and ninety patients were included in this study. Median follow-up was 33 months. Sixty-four (9.3%) patients required a peri-operative RBCT. Red cell transfusion was a predictor for decreased OS (median 41 vs 49 months, p=0.04). However, on multivariate regression analyses pre-operative chemotherapy, post-operative complications and Clinical Risk Score (CRS) were independently associated with reduced overall survival, though RBCT was not. There was no association between RBCT and recurrence free survival (median 15 vs 17 months, p=0.28)

2. The main findings of the RCT were that it was technically feasible to perform isolated portal vein clamping in patients and to recruit patients into the trial. However, a larger RCT will be needed to obtain definitive evidence on the role of PVC in hepatic resections in the future

Conclusions

There is potential for use of non-surgical techniques to decrease peri-operative bleeding in liver surgery. RBCT is not independently associated with poorer survival although it may be a surrogate marker for more advanced disease. The RCT confirms that isolated portal vein

clamping is technically feasible and it was possible to recruit into the trial; a multi-centre RCT is required to assess the role of isolated portal vein clamping surgery for colorectal liver metastases.

ABBREVIATIONS TABLE

AAH	Abdullah AlDuwaisan (co-investigator)
AH	Abdul Hakeem (co-investigator)
ALT	Alanine Transaminase
ANH	Acute Normovolaemic Haemodilution
ASA	American Soceity of Anaesthesiologists
BMI	Body Mass Index
BS	Blood Salvage
CD	Clavien-Dindo
CEA	Carcinoembryonic Antigen
CRC	Colorectal Cancer
CRF	Case report Form
CRLM	Colorectal Liver Metastases
CRS	Clinical Risk Score
CTRU	Clinical Trials Unit
CUSA	Cavitron Ultrasonic Surgical Aspirator
CVP	Central Venous Pressure
DM	Danilo Miskovic
FAP	Familial Adenomatous Polyposis
FK	Fadil Khoyratty (co-investigator)
FRV	Functional Residual Volume
HR	Hazard Ratio
INR	International Normalised Ratio
IOUS	Intra-operative Ultra-sound
IPM	Intermittent Pringle Manouevre
IQR	Interquartile Range
IRI	Ischaemia-Reperfusion Injury
IVC	Inferior Vena Cava
KIU	Kallikrein Inactivator Units
KRP	Raj Prasad
NK	Natural Killer
NM	Nafamostat Mesilate

mL	Milliliter
OR	Odds Ratio
OS	Overall Survival
PIL	Patient Information Leaflet
QDS	Quater die sumendus
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QoL	Quality of Life
R+D	Research and Development
RBC	Red Blood Cells
RBCT	Red Blood Cell Transfusion
RCF	Recombinant Factor
RCT	Randomised Control Trial
RFA	Radiofrequency Ablation
RFS	Recurrence Free Survival
SD	Standard Deviation
SHVE	Selective Hepatic Vascular Exclusion
SP	Samir Pathak
SVR	Systemic Vascular Resistance
TA	Tranexamic Acid
THVE	Total Hepatic Vascular Exclusion
WOMEN	World Maternal Antifibrinolytic Trial

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1 Introduction

1.1 Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer in the world after breast and prostate cancer, with nearly 1.4 million new cases annually and the most common gastrointestinal cancer (Haggar and Boushey 2009) (Kingham, Correa-Gallego et al. 2015). In the UK 41,112 new cases of colorectal cancer are diagnosed each year, with a 10 year survival rate of 57% (UK 2009).

However, the prognosis of breast and prostate cancer is relatively good with more than 4 out of 5 people surviving. In contrast, CRC has an all cause of mortality in 1 out of 3 patients with the disease (http://www.cancer.org/Cancer/ColonandRectumCancer/index 2011)

Colorectal mucosal tumourigenesis is relatively slow with 90% of new cases being in the over 50 age group, and 94% of deaths also being in this age group. The incidence of CRC has been increasing but the overall mortality has been decreasing over the last 20 years (Jemal, Clegg et al. 2004). The decreased incidence is likely to be due to the early detection of invasive disease via colonoscopy and faecal occult blood tests and improved multi-disciplinary management, as well as removal of adenomatous polyps, preventing adenoma-carcinoma sequence progression (Selby, Friedman et al. 1992, Kronborg, Fenger et al. 1996) (Jemal, Clegg et al. 2004).

Patients with early lesions experience 5-year survival rates of up to 90%, in contrast to those with widely disseminated metastatic disease at presentation who have five year survival rates of < 10% (Nordlinger, Sorbye et al. 2008).

Colorectal cancer is a multifactorial disease process, with aetiology encompassing genetic factors, environmental exposure and also inflammatory conditions of the gastrointestinal tract

(Deen, Silva et al. 2016). These lead to molecular and genetic events leading to transformation from adenomatous polyps to overt malignancy, characterized by Vogelstein and Fearon (Vogelstein, Fearon et al. 1988) . CRC is believed to result from a complex interaction between inherited susceptibility and environmental factors (Cooper, Squires et al. 2010). Observational studies suggest that the development of CRC may be linked to obesity, smoking, high alcohol intake, high calorie intake, high intake of red meats and a low consumption of fruits, vegetables and fibres (NICE 2004). Risk of CRC is also increased in patients with inflammatory bowel disease, with the relative risk increasing dependent on disease duration and severity. (Eaden, Abrams et al. 2001)

From a genetic point of view, a family history of CRC (particularly with a first degree relative diagnosed under the age of 45 years) is associated with an increased risk of CRC development (Hall, Finan et al. 1994). Approximately 5% of cases are associated with either familial adenomatous polyposis (FAP) or hereditary non-polyposis CRC. FAP causes approximately 1% of all CRCs and is caused by a mutation in the adenomatous polyposis coli gene. Patients with FAP may develop hundreds of polyps within the large bowel and by the age of 40, if they have not already had a colectomy, the majority would have developed CRC (Bishop and Hall 1994). Hereditary non-polyposis CRC is caused by a dominantly inherited alteration in one of a number of repair DNA mismatch repair genes; therefore it also conveys a slightly higher risk for the development of other cancers too. (Aaltonen, Salovaara et al. 1998)

Of those patients with colorectal cancer, 20-25% (8 – 10,000 in the UK) have CRLM present when their primary tumour is detected. A further 40-50% of patients develop CRLM within three years of their prior bowel surgery (Weiss, Grundmann et al. 1986, Geoghegan and Scheele 1999, Mueller, Broering et al. 2002).

1.2 Surgery for Colorectal Liver Metastases

Liver resection is the only treatment that offers potential cure in patients with colorectal liver metastases (CRLM). Five year survival rates of 40% in large prospective series and up to 67% in highly selected cases are reported, particularly if surgery is combined with chemotherapy (Hadden, de Reuver et al. 2016).

At presentation approximately only 20% of patients with CRLM have resectable disease(Martinez, Puig et al. 2007). However, the use of chemotherapy and more recently molecularly targeted agents, have been employed to downsize CRLM converting inoperable to potentially operable disease (Cunningham, Humblet et al. 2004, Bipat, van Leeuwen et al. 2005, Grabowski, Mueller-Koch et al. 2005, Gervasini, Garcia-Martin et al. 2007, Martinez, Puig et al. 2007, Zech, Korpraphong et al. 2014).

In contrast median survival without treatment for metastatic colorectal; cancer is typically eight months from presentation(Mueller, Broering et al. 2002) and only 3 – 5% of patients survive to five years (Schnitzbauer, Lang et al. 2012, Ratti, Schadde et al. 2015, Rosok, Bjornsson et al. 2016).

1.3 A Brief History of Liver Surgery

Liver surgery for metastatic disease has only become widespread in the last thirty years although the anatomy of the liver was first describe almost 500 years ago by Glisson in 1654 (Foster 1991, Mueller, Broering et al. 2002). The first planned liver resection, removing a left sided liver tumour, was undertaken in 1887 by Carl von Langenbuch (Hardy 1990) and a number of further attempts at liver resection were then reported in both the USA and Europe in the 1880's.

James Hogarth Pringle was an Australian born surgeon who practised in Scotland. In 1908, he published a case series where he proposed that when blood was issued from the fractured liver, it might be possible to temporarily control the bleeding by occluding the portal vein in the free edge of the foramen of Winslow (Pringle 1908). This technique has allowed liver surgery to expand as a specialty, alongside other concurrent advancements in anaesthetics, particularly since the early 1990s (Wong, Hamady et al. 2008)

However, this was followed by a more than 60 year hiatus in this field (Foster 1991). The lack of accurate imaging, effective blood transfusion, modern anaesthesia and lack of critical care all contributed to this gap as without these technological developments, operative risk particularly from bleeding were too high.

Following experiences with hepatic trauma during World War 2 and improvements in anaesthetic expertise, liver surgery was revisited in the 1950's. These developments were also supported by a better understanding of the hepatic anatomy, particularly the patterns of hepatic vascular inflow and outflow as originally described by Glisson (Hardy 1990, Foster 1991). However, the major expansion of liver surgery to what we see today only began in the 1990's (Kopetz, Chang et al. 2009).

1.3.1 **Liver Anatomy**

Although improving technology has helped the evolution of liver surgery, knowledge of the internal anatomy has also significantly improved outcomes. This is largely due to the work of French surgeon and anatomist Claude Couinaud, who published his work in 1957 (Abdel-Misih and Bloomston 2010). He demonstrated that hepatic functional anatomy is based on vascular and biliary relationships rather than external surface anatomy

The liver is located in the right upper quadrant of the abdomen and is the largest organ in the human body typically weighing between 1200g and 1500g. It is suspended from the anterior abdominal wall by the falciform ligament and the round ligament and from the diaphragm by peritoneal reflections known as the triangular and coronary ligaments. Macroscopically the liver is divided in to two lobes and anatomical resections are based on this anatomy (Abdel-Misih and Bloomston 2010, Strasberg and Phillips 2013, Guerra, De Gaetano et al. 2017)

1.3.2 **Liver Surgery**

The majority of liver resections undertaken in the UK are due to colorectal liver metastases (CRLM) (Farid, Prasad et al. 2013). Resections are also performed for other, both benign and primary malignant hepatobiliary tumours such as cholangiocarcinomas, hepatocellular carcinomas, neuroendocrine tumours and live-related or -unrelated donation for transplantation and for trauma (Tsim, Frampton et al. 2010, Farid, Prasad et al. 2013).

Liver surgery previously carried significant risk however developments in surgical and anaesthetic techniques have resulted in a typical mortality risk for all comers of 1-3%. These developments have also resulted in some improvements in short term morbidity and mortality (Melendez, Arslan et al. 1998, Jarnagin, Gonen et al. 2008, Agrawal and Belghiti 2011). However, reported morbidity in spite of these advances is still high ranging from 19.7% to 52.5% in elderly patients (Nagano, Nojiri et al. 2005, Mazzoni, Tocchi et al. 2007, de Liguori Carino, van Leeuwen et al. 2008, Mann, Neal et al. 2008, Adam, Frilling et al. 2010, Reddy, Barbas et al. 2011). Complications include respiratory events such as pneumonia (5.5 – 17.9%), cardiac events (arrhythmias and infarctions; 7.6%), abdominal (2.3 – 7.8%) and wound collections (3.6 - 7.4%). Less common complications included thromboembolic

events (pulmonary embolus and deep venous thrombosis; (1.0 – 3.5%), bile leaks (5.3-5.5%) and rarely liver failure (1.4-3.0%) (Cannon, Martin et al. 2011, Reddy, Barbas et al. 2011).

The lack of consensus as to how to grade surgical complications led to widespread variability in reporting outcomes and hampered surgical evolution. The Clavien-Dindo classification was developed for use in all fields of surgery to be simple and reproducible. It is how the majority of hepatobiliary studies grade their complications (Clavien, Barkun et al. 2009).

1.3.3 **Indications and Contraindications to Liver Surgery**

Liver resection for CRLM is safe and feasible in many patients and with normal liver parenchyma a functional residual liver volume (FRV) of as little as 20% can be safely tolerated although complications, particularly hepatic impairment are a significant risk (Kanas, Taylor et al. 2012). A more conservative approach is taken if patients have received chemotherapy or significant hepatic steatosis is present when a post-operative functional liver remnant of 30–60% is desirable. In patients with cirrhosis only relatively small volumes of liver can be resected and a functional liver remnant of 50 - 70% or more is optimal to minimise the risk of liver insufficiency (Charnsangavej, Clary et al. 2006). Those patients who demonstrate an adequate FRV would typically proceed to liver surgery without or without chemotherapy depending on the clinical presentation.

Anatomical resection over the years has been the standard modality of resection for hepatocellular carcinoma to achieve adequate tumour free margin (Spelt, Andersson et al. 2012) . For CRLM, a parenchymal sparing approach is being increasingly adopted keeping in mind chemotherapy induced hepatotoxicity and the need for repeat liver resection as an

option in multimodal treatment of recurrent colorectal liver cancer (Gold, Are et al. 2008). This paradigm shift in management of CRLM from 'what comes out' to 'what stays in' has led to non-anatomical resections/parenchyma preserving surgery being increasingly utilised. Several studies, comparing anatomical and non-anatomical liver sparing surgery have published conflicting results with some showing better survival with anatomical resections (DeMatteo, Palese et al. 2000), whilst others showed no difference (Kokudo, Tada et al. 2001, Lalmahomed, Ayez et al. 2011) although, a recent meta-analysis comparing the 2 techniques has shown that margins and oncologic outcomes were comparable (Sui, Cao et al. 2012)

1.3.4 **Neoadjuvant Chemotherapy**

The past decade has seen the criteria for resectability in CRLM being extended with more patients being eligible for hepatectomy with curative intent. However, a small future liver remnant volume (FLR) is associated with significant morbidity and mortality (Azoulay, Castaing et al. 2000, May, Talenfeld et al. 2013). It is therefore critical to leave a sufficient volume of liver coupled to the functional capacity of the underlying liver (Shindoh, Tzeng et al. 2013). Several strategies have been implemented to increase the FLR including portal vein embolization, portal vein ligation and two-stage hepatectomies (Abulkhir, Limongelli et al. 2008, Popescu and Alexandrescu 2012, Lam, Laurence et al. 2013, van Lienden, van den Esschert et al. 2013).

Pre-operative chemotherapy has also been used to increase the pool of patients amenable to resection. Current treatment algorithms classify patients with liver only CRLM as being resectable, borderline resectable or unresectable. The borderline resectable category facilitates chemo-responsiveness to be established, as well as identifying patients with

aggressive tumour biology (Adam, Delvart et al. 2004). Furthermore micro-metastatic disease can also be treated.

Patients previously considered unresectable are now being considered for curative therapy with a recent phase III trial demonstrating that a 60% response rate in such patients with 15% going on to have R0 resections (Falcone, Ricci et al. 2007). Biological therapies and molecular targeting have also played key roles in advancing management for patients with Stage IV CRC, with response rates for unresectable liver disease approaching 70% (Folprecht, Gruenberger et al. 2010). An aggressive line of management in patients with unresectable metastatic disease is therefore now justified with studies showing five year survival rates for patients who are "chemo-converted" to resectability being the same as patients who were initially considered resectable (Adam, Avisar et al. 2001, Adam, Delvart et al. 2004, Lam, Spiro et al. 2012, Jones, Hamann et al. 2014). Furthermore, the addition of monoclonal antibodies against the epidermal growth factor receptor (EGFR), such cetuximab, have facilitated response rates of up to 80% in the treatment of liver metastases (Hurwitz, Fehrenbacher et al. 2004).

Regimen-specific liver injury may be caused by neoadjuvant chemotherapy, which adds further complexity to the timing of such therapies. The use of 5-FU, leucovorin and irinotecan may predispose to development of steatosis (and hence, bleeding) and oxaliplatin use may cause sinusoidal obstruction syndrome (Wagman 2013). Such injury may increase surgical risk and therefore by default, overall prognosis. Hence utilisation and timing of chemotherapy needs careful evaluation, particularly in this cohort of patients.

(Hurwitz, Fehrenbacher et al. 2004)

1.4 Significance of bleeding in CRLM surgery

Despite recent advancements, intra- and postoperative hepatic bleeding remains an important operative risk with negative impact on short-term outcomes resulting from organ hypoperfusion (Kooby, Stockman et al. 2003, Ibrahim, Chen et al. 2006, Shiba, Ishida et al. 2013, Sui, Onyeji et al. 2016). In addition, there is increasing some evidence that the need for blood transfusions also results in poorer long-term survival in surgical oncology (Parrott, Lennard et al. 1986, Little, Wu et al. 1990, Tartter 1992, Panagopoulos, Karakantza et al. 2008, Wang, Jiang et al. 2015, Cata, Lasala et al. 2016). Attempts to explain this observation include host immunosuppression due to the transfusion resulting in decreased tumour surveillance and earlier recurrence (Kooby, Stockman et al. 2003), a condition termed transfusion-related immune modulation (TRIM) (Cata, Wang et al. 2013, Youssef and Spitalnik 2017). Mechanisms for TRIM include suppression of cytotoxic cell and monocyte activity, release of immunosuppressive prostaglandins, inhibition of interleukin-2 (IL-2) production, and increase in suppressor T-cell activity (Vamvakas 2002{van Twuyver, 1991 #103, Sui, Onyeji et al. 2016)}. The immunosuppressive effects of allogeneic blood transfusion were even used therapeutically to reduce renal allograft rejection before effective immunosuppressant drugs became available (van Twuyver, Mooijaart et al. 1991)

It is also worth considering the circumstances in which patients are given blood transfusions peri-operatively which may also influence survival. These include preoperative nutrition and functional status, the presence of preoperative anaemia, tumour type and stage, degree of resectability, duration and type of anaesthesia, amount of blood loss, perioperative stress response, and the presence of postoperative complications.

Furthermore, tumour manipulation during surgical resection increases the load of circulating malignant cells (Bosch, Guller et al. 2003). Secondly, volatile anaesthetics and opioids depress the function of host cellular defenses, especially NK cells and cytotoxic lymphocytes. Thirdly, perioperative factors such as inflammatory response to injury, physiological stress response to surgery, hyperglycaemia, and hypothermia may result in a pro-tumour environment (Cata, Wang et al. 2013)

Hence, there are also several confounding factors to consider when examining retrospective studies looking at blood loss in cancer surgery. Blood transfusions are administered to approximately one third of all patients undergoing liver resections, although this number is decreasing (Nagino, Kamiya et al. 2005, Pulitano, Arru et al. 2007, Verma and Schwarz 2007). Worse outcome in patients requiring blood transfusions or blood products have also previously been shown in some studies within the context of liver surgery (Gruttadauria, Saint Georges Chaumet et al. 2011, Shiba, Ishida et al. 2013). However, the data is limited regarding the impact of red blood cell transfusion (RBCT) on long-term impact in liver surgery for CRLM with single centre studies demonstrating conflicting evidence (Younes, Rogatko et al. 1991, Gruttadauria, Saint Georges Chaumet et al. 2011, Jiang, Fang et al. 2013, Hallet, Tsang et al. 2015, Schiergens, Rentsch et al. 2015). The largest of these studies was by Kooby et al (Kooby, Stockman et al. 2003) where over 1000 patients underwent liver resections between 1986 and 2001, with 55% requiring a blood transfusion. They found transfusion to be linked to poorer short term outcomes but on multivariate analysis for long term survival, transfusion was not significant whereas a positive resection margin, primary tumour histology, the number and size of the liver lesions and the disease free interval were significant. Conversely, a more recent study by Hallet et al on regression modelling found transfusion, Clinical Risk Score and age to be predictive of poorer long term outcomes.

Bennett at al (Bennett, Baker et al. 2017) performed a systematic review looking at the effect of RBCT in patients undergoing liver resection for all causes. They found that transfusion requirements were decreasing with time and that the majority of papers showed worse survival on univariate analyses. However, after multi-variate analyses only 10 out of 18 studies demonstrated a poorer outcome of RBCT with long-term outcome with only 4 of the studies being specific to CRLM.

The majority of the studies regarding CRLM and transfusion are historical and perhaps not relevant today where modern chemotherapy along with biological agents and advancements in surgical and anaesthetic techniques have changed the way liver resections are approached. It is therefore vital to ascertain the impact of blood transfusions in a UK based population undergoing CRLM surgery and to identify strategies to further reduce transfusion requirements during liver surgery. .

1.5 Balancing Bleeding and Ischaemia Reperfusion Injury (IRI) in Liver Surgery

Although intraoperative bleeding in liver surgery can be prevented by clamping blood vessels leading to the liver, this increases the risk of Ischaemia-reperfusion injury (IRI). IRI is defined as the tissue damage caused when the vascular supply to the organ is returned after a period of ischaemia (Nadarajah, Yaqoob et al. 2017) . The absence of oxygen and nutrients to the tissue during the ischaemic period creates a condition in which restoration of the blood flow results in inflammatory and oxidative damage via the induction of oxidative stress (Fondevila, Busuttil et al. 2003, Teoh and Farrell 2003). IRI can be a serious complication of major liver surgery if the procedure involves some form of vascular exclusion (Abu-Amara, Gurusamy et al. 2010). It may cause a local and systemic inflammatory response and its

clinical manifestations may vary between transient arrhythmias to multi-organ dysfunction (Eltzschig and Collard 2004).

Hence, managing the balance of haemostasis and prevention of IRI is a crucial concept in liver surgery in order to optimise both short and long-term outcomes.

Chapter 2

Aims and Objectives

2 Aims and Objectives

2.1 Aim

Based on the review of the literature, I identified three main areas with minimal or incomplete evidence in the current literature: (1) non-invasive/ non-surgical methods to reduce intraoperative blood loss, (2) comprehensive analysis of risk factors for bleeding and the impact of blood transfusion on survival in patients undergoing hepatectomy for CRLM, and (3) randomised controlled trials on isolated vein clamping.

Based on these findings, the aim and objectives of the research was defined. The aim of the thesis was to study risk factors for bleeding and to perform a feasibility study looking at the role of isolated portal vein clamping during CRLM surgery.

2.2 Objectives

In order to achieve this aim, the objectives were defined as follows:

a) To review surgical techniques utilised in liver surgery (**chapter 3**)
b) To perform a systematic review looking at pharmacological and anaesthetic methods which could complement surgical techniques to reduce blood loss (**chapter 4**)

c) To set up a retrospective database of patients undergoing liver surgery from 2005-2012 and determine the effects of blood transfusion on long term outcomes of patients undergoing CRLM resection. (**chapter 5**)

d) To conduct a pilot, randomised control trial looking at the Pringle Manoeuvre versus Isolated Portal Vein Clamping (**chapter 6**)

Chapter 3

Review of Peri- and intra-operative Techniques used to minimise blood loss in liver surgery

3 Review of Peri-operative Techniques

3.1 Introduction

Minimal intra- and postoperative blood loss is crucial to achieve good results in liver surgery and a number of surgical and non-surgical techniques have been developed to reduce bleeding. This chapter will describe peri- and intra-operative techniques which are routinely used to decrease blood loss during liver surgery.

3.2 Peri-operative techniques

Risk factors to predict the likelihood of blood loss can be summarised into patient factors (body mass index, recent chemotherapy exposure, underlying liver disorders, co-morbidities and cardiovascular fitness, medication history) and tumour factors (number and size, distribution and relationship with major vascular structures and tumour vascularity) (Gayowski, Iwatsuki et al. 1994, Nordlinger, Guiguet et al. 1996, Robertson, Stukel et al. 2009, Mathur, Ghaferi et al. 2010). Hence, preoperative considerations include patient selection and patient optimisation. Chronic liver failure predisposes to intra- and postoperative bleeding due to its associated coagulopathy, volume and electrolyte disturbances, hepato-renal and hepato-pulmonary syndromes and low cardiovascular reserve capacity (Dagher and Moore 2001, Mazzeo, Lucanto et al. 2004, Tympa, Theodoraki et al.

2012). Assessment of severity of liver dysfunction before surgery is important and the risk benefit of the procedure needs to be carefully assessed via modified Child-Pugh or MELD scores. Liver resections in patients with underlying hepatic cirrhosis and portal hypertension still represent a medical challenge with regard to perioperative morbidity, surgical management and postoperative outcome although peri-operative advancements have meant resections in such patients are still feasible, though the risk-benefit margins need careful analysis (Hackl, Schlitt et al. 2016).

Surgery should be avoided if possible in the setting of acute and alcoholic hepatitis, in a patient of cirrhosis who is child class C or has a MELD score more than 15. In this subset of patients, the patients should be managed without an operation (Rai, Nagral et al. 2012) . Pre-operative optimization necessitates correction of electrolyte imbalance and improving renal dysfunction, cardiorespiratory assessment, correcting any sepsis and correction of coagulation. Intra-operatively, safe anesthetic agents like isoflurane and propofol with avoidance of hypotension are advised. In general, nonsteroidal anti-inflammatory drug and benzodiazepines should not be used (Rai, Nagral et al. 2012, Hackl, Schlitt et al. 2016).

General risk factors for bleeding such as anti-thrombotic drugs, a family history of bleeding disorders, known clotting abnormalities and evidence of previous excessive post traumatic or post-surgical bleeding should also be considered (Chee, Crawford et al. 2008).

Exercise or pharmacologic stress testing is useful for the preoperative assessment of cardiovascular status providing information regarding myocardial contractility and the mechanics of blood flow (Redai, Emond et al. 2004). More recently, several centres have advocated the use of Cardiopulmonary Exercise testing to evaluate peri-operative risk (Junejo, Mason et al. 2012). Recent improvements in radiology have also led to better

evaluation of the tumour relation to the major vessels, including three dimensional imaging of the liver (Saini 1997, Israel, Mor et al. 2002, Numminen, Sipila et al. 2005).

3.3 Intra-operative Anaesthetic Techniques

3.3.1 **Anaesthetic agents**

Considerate selection of anaesthetic agents is important for hepatic vessel clamping techniques. In animal models both isoflurane and sevoflurane decrease portal vein resistance (Hoetzel, Geiger et al. 2002), whereas in humans, sevoflurane also increases hepatic arterial blood flow (Kanaya, Nakayama et al. 1995). Theoretically, this may be relevant for isolated portal vein clamping as evidence suggests that ischaemic pre- and post-conditioning with sevoflurane prior to inflow occlusion may reduce post-operative liver dysfunction (Beck-Schimmer, Breitenstein et al. 2008, Beck-Schimmer, Breitenstein et al. 2012)

3.3.2 **Hypothermia**

Inadvertent perioperative hypothermia is common and preventable. In the first hour of anaesthesia a patient's core temperature may drop to below 35^0C due to loss of the behavioural response to cold, impairment of thermo-regulatory mechanisms and anaesthesia induced peripheral vasodilation and heat loss. This increases blood loss secondary to associated coagulopathy whilst increasing the likelihood of significant cardiac events, wound

complications and altering drug metabolism (NICE 2008). During the pre-operative phase, clinical staff should ensure patients stay warm by use of additional clothing or blankets and forced air warming. A risk assessment should also be performed encompassing American Soceity of Anaesthesiologists (ASA) grade, type of surgery being undertaken, pre-operative temperature and comorbidities (NICE 2008, Knaepel 2012). Intra-operatively the temperature should be measured and documented every 30 minutes. Intravenous fluids and blood products used should be warmed to 37^0C using a warming device prior to use. Internal and external warming techniques such as forced air warming should also be used peri-operatively to prevent hypothermia-related coagulopathy (Redai, Emond et al. 2004).

3.3.3 **Central Venous Pressure**

Blood loss reduction by using low central venous pressure anaesthesia (CVP between 2 and 5 mmHg) is also employed however it use must be weighed against its risks of injury due to reduced end organ perfusion (Rees, Plant et al. 1996, Johnson, Mannar et al. 1998, Jones, Moulton et al. 1998, Melendez, Arslan et al. 1998). The use of low CVP anaesthesia to reduce intra-operative blood loss during liver surgery is now well established however measurement of urine output, pulse, blood pressure and arterial pulse pressure waveforms to assess fluid status and minimise end organ harm are recommended (Smyrniotis, Kostopanagiotou et al. 2004) .

3.4 Intra-operative Surgical Techniques

3.4.1 **Intra-operative Ultrasound (IOUS)**

Localisation of the hepatic lesions that are to be resected and also identification of their relation to major vascular structure can also assist in the reduction of blood loss. Intra-operative ultrasound (IOUS) has a valuable role as allows identification of lesions for resection including additional lesions not found on pre-operative imaging in a significant number of cases (Cervone, Sardi et al. 2000, Zacherl, Scheuba et al. 2002). In addition IOUS also accurately assesses proximity and/or invasion to major vascular structures. This allows an operative approach to be devised that minimises blood loss. (Cervone, Sardi et al. 2000, Ellsmere, Kane et al. 2007, D'Hondt, Vandenbroucke-Menu et al. 2011)

3.4.2 **Intra-operative Dissection Techniques**

A number of strategies to transect the liver parenchyma can be employed and appropriate selection of these can also minimise blood loss.

3.4.2.1 Crushing Technique

Traditionally the liver tissue is fractured between fingers or surgical clamps ("crush-clamp" technique) under intermittent inflow occlusion (Pringle manoeuvre) and vessels and hepatic ducts are ligated or clamped (Aragon and Solomon 2012) with the crush clamp technique offering superior control to finger fracture when transecting the parenchyma (Poon 2007, Kim and Lee 2008, Aragon and Solomon 2012). Diathermy or argon beam coagulation can be applied to the remnant parenchyma during reperfusion, to achieve haemostasis (Postema,

Plaisier et al. 1993). This is a simple, quick, efficient and cost-effective technique however a number of newer techniques have developed and it is against this crushing technique that newer techniques detailed below have been measured (Pamecha, Gurusamy et al. 2009, Rahbari, Koch et al. 2009).

3.4.2.2 Ultrasonic Dissection

The Cavitron Ultrasonic Surgical Aspirator (CUSA) combines ultrasonic energy with aspiration to divide the liver parenchyma and skeletonise blood vessels and biliary structures greater than 2 mm in width (Andrus and Kaminski 1986) which are subsequently ligated. CUSA is able to dissect parenchyma but does not directly contribute towards haemostasis however accurate identification of blood vessels with minimal damage facilitates vascular control and can reduce blood loss. CUSA use is associated with low blood loss and low risk of bile leak, though the transaction time is longer than with the crushing technique (Aragon and Solomon 2012). A recent RCT showed ultrasonic dissection and the crushing technique to be comparable in terms of blood loss and other outcome measures though the crushing technique was faster. (Doklestic, Karamarkovic et al. 2012)

The harmonic scalpel again uses ultrasonic technology like the CUSA, however the high frequency waves generate heat between the jaws of the instrument and achieve a heat based sealing of structures and can contribute towards haemostasis (Gertsch, Pelloni et al. 2000, Abbasoglu and Sayek 2003, Arru, Pulitano et al. 2007). It is associated with decreased operative time and decreased blood loss and transection times. However, a retrospective study showed there was a significant increase in the incidence of post-operative bile leak as smaller ducts were likely to remain patent [p=0.01] (Kim, Ahmad et al. 2003).

3.4.2.3 Bipolar Sealing Devices

Bipolar vessel-sealing devices such as the Ligasure vessels sealing system are hypothesized to seal blood vessels up to 7mm in size, thereby decreasing operative time. Although a small single centre study supported the hypothesis of less blood loss and faster operative time (Saiura, Yamamoto et al. 2006), a larger RCT comparing this technology with the crush-clamp technique failed to show differences in both blood loss and operative time (Ikeda, Hasegawa et al. 2009). The discrepancy between the two studies may be explained by the fact that different techniques may have been utilised between the studies; the number of structures ligated per transection was much higher in the study by Ikeda et al, which may be because they ligated all structures greater than 2mm in size. Furthermore, different hand pieces were used in the two studies.

3.4.2.4 Radiofrequency Assisted Liver Resection

Radiofrequency Ablation (RFA) has been one of the favoured thermal local ablative techniques and has demonstrated to achieve good local control of the tumour in cases where resection was not feasible (Abitabile, Hartl et al. 2007). RFA probes can also be used to treat the parenchyma inducing coagulative necrosis prior to division using a scalpel. However, it takes more time and is associated with higher complication rates such as biliary fistula, biliary stenosis and abscess formation (Li, Zhang et al. 2012).It is likely that this is due to the "heat sink" effect of nearby biliary and vascular structures (Pathak, Jones et al. 2011).

Therefore, RFA-assisted liver resection should only be performed for peripherally located tumours.

3.4.2.5 Water Jet Dissection

As the liver consists of a three dimensional structure of afferent and efferent duct and vessel systems, which are higher in collagen and elastin and therefore differ from the surrounding liver parenchyma it is possible to mechanically separate the ducts and vessels from the underlying liver parenchyma (Rau, Duessel et al. 2008). A high pressure water jet is utilised to dissect the parenchyma and isolate small vascular and biliary structures, which can then be ligated and divided (Rau, Duessel et al. 2008). The residual parenchyma is spared from the effects of coagulation or charring. However, the technique is time consuming and no advantage has been demonstrated in pooled data analyses (Pamecha, Gurusamy et al. 2009, Rahbari, Koch et al. 2009).

3.4.2.6 Vascular Staplers

Stapling devices have been introduced for safety and to reduce the overall operative time in several types of surgery (Schemmer, Friess et al. 2007). Within liver surgery staplers are routinely used to control inflow and outflow vessels. It can also be used to facilitate parenchymal transection where a large clamp is used to fracture the liver parenchyma, followed by serial firings of the surgical stapler with a vascular load. Reddy *et al* showed in a retrospective series of 112 patients that use of a vascular stapler when compared to crush

clamp technique was associated with less operative time, blood loss and transfusion requirements (Reddy, Barbas et al. 2008). The CRUNSH trial was a prospective RCT comparing crush-clamp to vascular stapler use in elective liver resections. The trial included 65 patients in each arm, who were comparable in terms of baseline demographics. There was no difference in the amount of intra-operative blood loss between the two groups. However, when blood loss was normalised to transection area there was an apparent advantage in using staplers. These findings do not justify the routine use of staplers but they do suggest further studies in high risk groups to ascertain an potential benefits of staplers (eg cirrhotics requiring a resection) (Rahbari, Elbers et al. 2014). A further trial demonstrated staplers to be safe and faster than CUSA, with a diminished inflammatory response (Schwarz, Klaus et al. 2015). However, there is still not enough evidence to suggest all liver transections should be done using stapling devices- cost analyses have not been undertaken in these preliminary trials either.

3.4.3 **Intra-operative vascular control**

In order to reduce blood loss, various methods of hepatic inflow, or simultaneous in- and outflow occlusion techniques are applied. The type of occlusive technique employed should reflect the reason for resection (for example donor hepatectomy *versus* oncological surgery), tumour location, the presence of underlying liver disease and the cardiovascular status of the patient (Abdalla, Noun et al. 2004). Different intra operative vascular control techniques are discussed below:

3.4.3.1 Continuous Pringle Manoeuvre

The Pringle manoeuvre is a surgical technique where a large haemostat is used to clamp the hepatoduodenal ligament and decrease blood flow to the liver, via the portal vein and hepatic artery (Gurusamy, Sheth et al. 2009). It can be used in a continuously or in an interrupted manner

Once the lesser omentum is opened a blunt dissector is passed through the foramen of Winslow and the hepatoduodenal ligament can be encircled with umbilical tape *en masse* (Chouillard, Gumbs et al. 2010). A tourniquet or vascular clamp is applied until the hepatic arterial pulse disappears distally. (Lau, Lai et al. 2010)

The Pringle manoeuvre results in a 10% increase in mean arterial pressure, a 40% increase in systemic vascular resistance, a 5% decrease in pulmonary arterial pressure and a 10% decrease in cardiac index (Belghiti, Marty et al. 1998). Pedicle clamping is well tolerated as the caval flow is not interrupted. Splanchnic congestion from portal clamping tends to be mild, especially with intermittent clamping (Lau, Lai et al. 2010). Persistent bleeding during transection is caused by incomplete inflow occlusion or back-bleeding from the hepatic veins. Incomplete inflow occlusion can be minimised by application of the pedicle clamp until pulsation in the distal hepatic artery has disappeared and ensuring that any accessory hepatic arterial systems have also been clamped. Backflow bleeding can be reduced by lowering the CVP to less than 5cm H_2O, or total or partial clamping of suprahepatic veins or inferior vena cava (Lau, Lai et al. 2010).

3.4.3.2 Intermittent Pringle Manoeuvre (IPM)

It remains unclear if a single, prolonged period of ischaemia followed by reperfusion is more harmful than intermittent clamping results in multiple periods of ischaemia (van Gulik, de Graaf et al. 2007). Similar to continuous Pringle manoeuvre, IPM was shown to reduce blood loss and operating time when compared with no vascular clamping without increasing postoperative complication or mortality rates and was shown to be oncologically equivalent (Man, Fan et al. 1997, Capussotti, Muratore et al. 2006, Wong, Hamady et al. 2008, Tralhao, Hoti et al. 2009, van den Broek, Bloemen et al. 2011, Lee, Cheung et al. 2012, Park, Joh et al. 2012).

Intermittent and continuous hepatic pedicle clamping were compared directly in a few trials only. Although a reduced incidence of IRI using IPM was shown in the animal model (Isozaki, Adam et al. 1992), this has not been reproduced in patients (Isozaki, Adam et al. 1992, Isozaki, Okajima et al. 1995, Chiappa, Makuuchi et al. 2001, Capussotti, Nuzzo et al. 2003, Ozmen, Oruc et al. 2003, van Gulik, de Graaf et al. 2007). Both techniques are effective in reducing intra-operative blood loss, whilst proportions of patients requiring blood transfusions were comparable in both groups. Complications and mortality were also found to be comparable between the two groups.

There is a paucity of trials comparing IPM with continuous clamping but accounting for the animal studies and the theoretical risk of increased Ischaemia Reperfusion Injury (IRI, see below) in the continuous clamping group, IPM continues to be preferred by most liver surgeons due to the theoretical decrease in the development of IRI.

3.4.3.3 Hemi-hepatic Vascular Clamping

Hemi-hepatic vascular occlusion (or half-Pringle manoeuvre) selectively interrupts the arterial and venous inflow to the right or left liver lobe. Currently, there is conflicting evidence regarding benefits of hemi-hepatic occlusion compared to the Pringle manoeuvre. The results of a recent randomised trial suggest hemi-hepatic vascular occlusion to be superior in terms of post-operative complication rates and liver function (Ni, Lau et al. 2013).

However, evidence on reduction of intra-operative blood loss is conflicting, some suggesting this technique to be beneficial (Wu, Yeh et al. 2002, Wen, Miao et al. 2009), whilst others suggest that there is no difference in blood loss (Figueras, Llado et al. 2005, Liang, Wen et al. 2009).

A trial by Fu et al compared hemi-hepatic clamping, the Pringle manoeuvre and isolated portal vein clamping. This study showed all three techniques to be safe and comparable in terms of post-operative complications (Fu, Lau et al. 2011). However, there was an increased incidence of liver dysfunction in the Pringle only group. The study was performed in China and the majority of patients had HCC with cirrhosis. These findings led us to investigate the feasibility of looking at inflow control during liver surgery in a western cohort of patients (see Ch 6). Our trial simply looked at Pringle versus isolated portal vein clamping as hemi-hepatic clamping is not really feasible in a western cohort where the majority of resections are performed for CRLM and hence re-resections are often undertaken.

3.4.3.4 Total Hepatic Vascular Exclusion

THVE combines total vascular inflow and outflow occlusion, thereby isolating the liver from the systemic circulation. It is suggested to be useful where backflow bleeding from the hepatic veins causes significant blood loss (Abdalla, Noun et al. 2004).

THVE is indicated for tumours either in close proximity to the major hepatic veins or IVC, or tumours that have penetrated these vessels. In instances where thrombus is present within the vessel, TVHE may also prevent thrombus migration, whilst facilitating vessel reconstruction (Lau, Lai et al. 2010).

THVE is associated with major haemodynamic changes. Cessation of the IVC flow causes a marked decrease in cardiac output and therefore, an increase of approximately 80% in systemic vascular resistance (SVR) and a 50% increase in heart rate. There is also a 10-15% decrease in the mean arterial pressure, as well a 40-50% drop in the cardiac index. A decrease of more than 50% with regard to cardiac output, or a decrease in SVR of more than 30% in a euvolaemic patient, equates to patient intolerance to TVHE. This occurs in approximately 10-20% of patients due to adrenergic cardiovascular reflexes to increase cardiac output in the absence of IVC inflow. Prior to clamp placement, the patient is volume loaded with fluid to a CVP of approximately 12-15mmHg to prevent intolerance. A trial exclusion can be performed to assess response however the large fluid volumes loaded may cause post-operative renal, liver and pulmonary dysfunction (Lau, Lai et al. 2010).

There have been only a few trials conducted comparing TVHE against other occlusive techniques. Belghiti *et al* (Belghiti, Noun et al. 1996) were the first group to perform an RCT comparing TVHE *versus* Pringle. They found blood loss to be similar between groups, though post-operative morbidity was higher in the TVHE. However, Chen *et al* (Chen, Zhang et al. 2006) found significantly more blood loss ($p=0.046$) and transfusion requirements ($p=$

0.041) in the Pringle only group without any difference in the post-operative morbidity between the two groups.

Given that TVHE is associated with unpredictable haemodynamic changes and increased post-operative complications, it is only be used in certain selected cases where there is caval and/or hepatic vein involvement of the tumour.

3.4.3.5 Selective Hepatic Vascular Exclusion

SHVE combines inflow occlusion with extra-parenchymal control of the hepatic veins, without interruption of caval flow, a strategy that can avoid the haemodynamic instabilities caused by TVHE, but retaining the benefit of controlling backflow bleeding from the hepatic veins. This approach is however more technically challenging than TVHE (Lau, Lai et al. 2010).

There have been two retrospective series and one randomised control trial comparing SVHE to a Pringle manoeuvre (Smyrniotis, Kostopanagiotou et al. 2003, Zhou, Li et al. 2008, Zhang, Lai et al. 2012). The studies each demonstrate blood loss and post-operative complications to be higher in the Pringle trial group. However, SVHE takes considerably longer and is felt to be most applicable to centrally located tumours with vascular involvement. In addition, total anaesthetic time is likely to be longer in patients undergoing SVHE and the long term oncological impact of this approach remains unclear. Further larger trials are needed to confirm the beneficial effects of SVHE.

3.4.3.6 Summary

The Pringle manoeuvre is the established method of inflow control. Particularly in the setting of chronic liver disease, it is best to use it intermittently. However, the role of selective vascular inflow occlusion still needs to be addressed.

Total hepatic vascular exclusion and selective hepatic vascular exclusion techniques are useful for centrally located tumours and tumours where there is vascular involvement. However, they should not be employed routinely.

Chapter 4

Systematic Review of Non-Surgical Techniques used to minimise blood loss in liver surgery.

4 Systematic Review of Non-Surgical Techniques

The role of surgical techniques such as vascular occlusion, parenchymal transection technique and use of sealants to decrease blood loss in liver surgery have been extensively reviewed previously (Gurusamy, Sheth et al. 2009, Pamecha, Gurusamy et al. 2009, Wang, Yang et al. 2011, Ding, Yuan et al. 2013, Sanjay, Ong et al. 2013)and the types of intervention have been summarised in Chapter 3.

In contrast, only a few non-surgical interventions to reduce bleeding during and after liver surgery have been have been analysed in the past (Gurusamy, Li et al. 2009, Gurusamy, Li et al. 2009). Gurusamy et al have previously summarised cardiopulmonary interventions (low CVP, autologous blood transfusions and haemodilution before) and pharmacological interventions (Gurusamy, Li et al. 2009, Gurusamy, Li et al. 2012) and hence there is an overlap in terms of our included studies and theirs. Some of the techniques are based on decreasing hepatic perfusion intra-operatively, potentially resulting in ischaemia-reperfusion injury(IRI) which can increase the risk for postoperative liver failure (Lesurtel, Lehmann et al. 2009) . A number of recent trials of non-surgical (anaesthetic and pharmacological) interventions have emerged and the major difference between this review and the work previously done by Gurusamy et al is the inclusion of anaesthetic agents which may confer

protection to the liver from Ischaemia-reperfusion injuries . The evidence in this area has not been summarised.

This chapter will describe a systematic review of the available evidence regarding non-surgical (anaesthetic and pharmacological) interventions used to minimise blood loss during liver surgery.

4.1 Methods

4.1.1 Search Strategy

The review was undertaken according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidance (Liberati, Altman et al. 2009). An electronic search of PubMed, Cochrane Library (1995-2013), CINAHL (1990-2013) and Google scholar was conducted by two independent researchers (SP and AH[1]). This was undertaken using a sensitive search strategy incorporating the following MeSH search terms:

[("Blood Loss" OR "Bleeding" OR "Hemmorhage", "Haemorrhage" OR "Haemostasis" OR "Hemostasis" OR "Blood Transfusion") AND ("Liver" OR "Hepatic" OR "Hepato" OR "Resection" OR "Segmentectomy" OR "Hepatectomy") AND ("Randomised Control Trial" OR "Randomized Control Trial" OR "Controlled Clinical Trial")].

Bibliographies of relevant studies supplemented by the "related articles" link in PubMed were used to identify additional studies. Studies published in abstract form only or unpublished reports were excluded from the analysis. Each citation and associated abstracts

were screened independently by the two researchers (SP/AH[1]). The searches were completed and review closed on the 18th October 2013.

[1] SP denotes myself and AH is a research fellow within the HpB department

4.1.2 **Inclusion Criteria**

Included studies analysed the effect of anaesthetic or pharmacological methods to decrease blood loss in liver surgery. Only randomised control trials (RCT) were considered for inclusion. The studies were carefully evaluated for duplication or overlapping of data. Only studies in English language after 1990 were considered, as hepatic surgery developed as a sub-speciality during the 1990s.

4.1.3 **Exclusion Criteria**

Animal studies, case reports, letters and editorials were excluded. Studies looking at vascular clamping techniques, parenchymal transection technique, liver transplantation, use of low central venous pressure (CVP) anaesthesia versus vascular clamping techniques, fibrin sealants and paediatric populations were also excluded.

4.1.4 **Outcome Measures**

The primary outcomes of interest were overall blood loss (measured in mL) and transfusion requirements (measured in mL or units). Secondary outcome of interest was the risk of ischaemia-reperfusion injury (measured by peak ALT levels). Other clinical outcomes such

as length of ITU/hospital stay, long term survival outcomes and complications were not included as the majority of studies did not report these.

4.1.5 **Study Selection**

Two authors (SP and AH) independently performed the search strategy. Both the authors reviewed the abstracts identified by the search to exclude those that did not meet our inclusion criteria. When no abstract was available or the abstract details were inadequate, the full article was reviewed. Differences between the two authors (SP and AH) in selection of the studies were resolved by consensus with the senior author (DM [2]). If the selection of the study was still not resolved by consensus between the three authors, the lead author's (DM) decision was considered as final.

[2] denotes Danilo Miskovic.

4.1.6 **Data Extraction**

Extraction of data was done by the two authors (SP and AH) independently using a standardised proforma and any disagreement resolved by consensus with the senior author (DM). The following demographic and clinical parameters were recorded: study characteristics (first author, year of publication), population characteristics and outcomes of interest.

4.1.7 **Quality Assessment**

Randomised control trials were assessed using the Cochrane risk of bias tool by two authors (SP and AH) (Turner, Shamseer et al. 2012)

4.2 Results

A total of 17 studies met the inclusion criteria. There were 8 studies on pharmacological approaches constituting 894 patients, and another 9 studies on anaesthetic techniques, including 679 patients. The study quality was variable (Table 1). 8 studies (Lentschener, Benhamou et al. 1997, Hasegawa, Takayama et al. 2002, Matot, Scheinin et al. 2002, Wong, Irwin et al. 2003, Lodge, Jonas et al. 2005, Wu, Ho et al. 2006, Hashimoto, Kokudo et al. 2007, Ryu, Nahm et al. 2010) were judged to be of low risk of bias with 5 studies ((Jarnagin, Gonen et al. 2002, Wang, Liang et al. 2006, Pulitano, Aldrighetti et al. 2007, Beck-Schimmer, Breitenstein et al. 2012, Toprak, Sahin et al. 2012)) at low risk of randomisation bias without explicit details of allocation concealment or blinding of outcomes assessment. Randomisation details were unclear in 4 studies ((Shimada, Matsumata et al. 1994, Inagaki, Nonami et al. 1999, Shao, Yang et al. 2006, Guo, Jin et al. 2013))

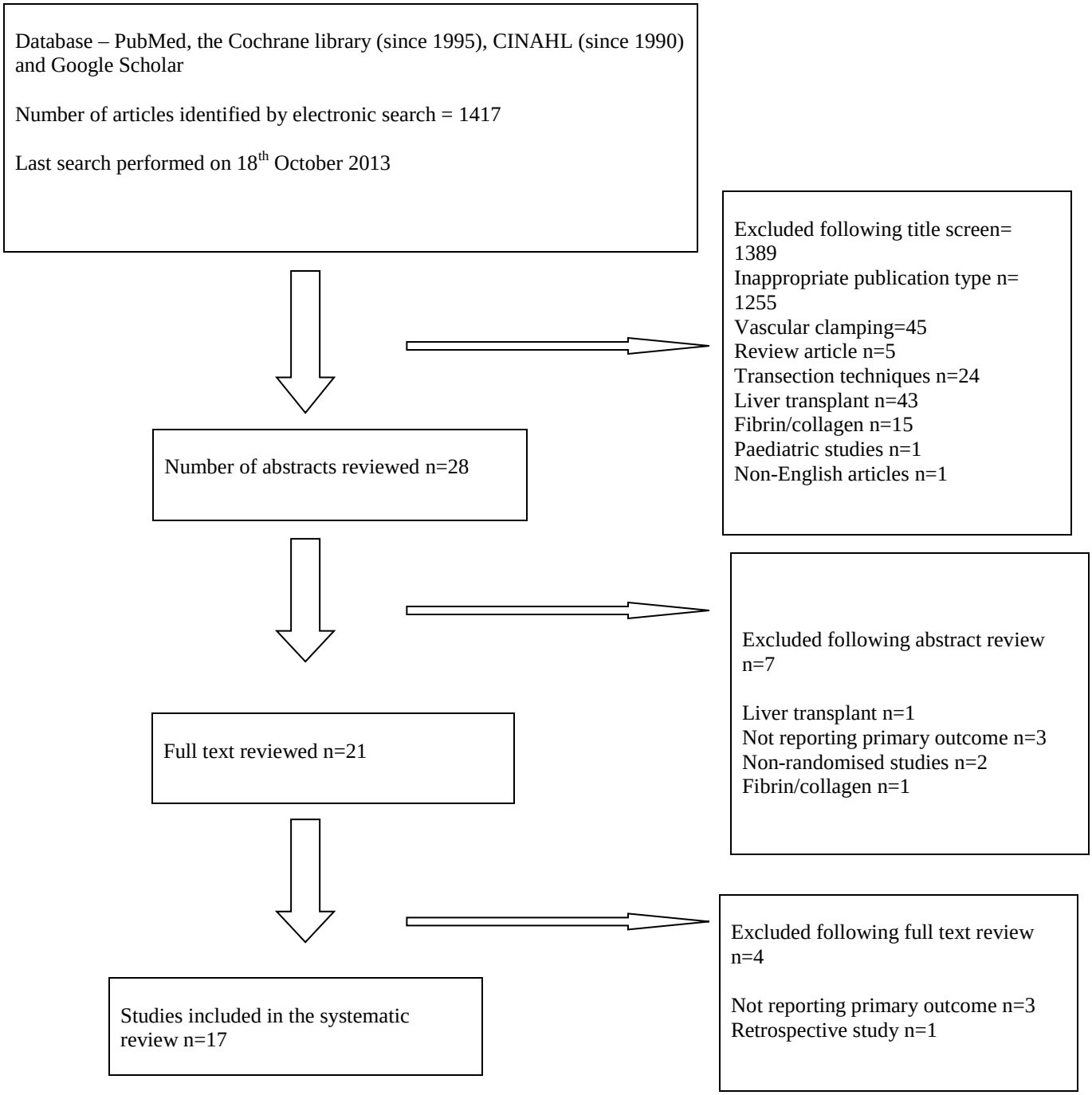


Figure 1 : PRISMA Flowchart depicting the search strategy and selection of articles for the review

Study	Jadad criteria							Total Score
	Described as randomised (+1)	Method of randomisation appropriate (+1)	Inappropriate randomisation method (-1)	Described as double blind (+1)	Method of blinding appropriate (+1)	Inappropriate blinding method (-1)	Description of withdrawals/ dropouts described (+1)	
PHARMACOLOGICAL								
Wu et al	1	1	0	1	1	0	1	5
Pulitano et al	1	1	0	0	0	0	1	3
Lodge et al	1	1	0	0	0	0	1	3
Wong et al	1	1	0	1	1	0	1	5
Inagaki et al	1	0	0	0	0	0	1	2
Lentschener et al	1	1	0	1	1	0	1	5
Shimada et al	1	0	0	0	0	0	1	2
Shao et al	1	0	0	0	0	0	1	2
ANAESTHETIC								
Beck-Schimmer et al	1	1	0	0	0	0	1	3
Toprak et al	1	1	0	0	0	0	1	3
Ryu et al	1	1	0	1	1	0	1	5
Matot et al	1	0	0	0	0	0	1	2
Jarnagin et al	1	0	0	0	0	0	1	2
Guo et al	1	0	0	0	0	0	0	1
Hashimoto et al	1	1	0	1	1	0	1	5
Hasegawa et al	1	1	0	0	0	0	1	3
Wang et al	1	1	0	0	0	0	1	3

Table 1: Assessment of study quality using JADAD score

4.2.1 **Pharmacological Approaches to minimise blood loss after liver surgery**

Of the eight studies, five studies, describing pharmacological methods did not show an effect on blood loss (see Table 2). In three studies, an effect was found. In two trials (Lodge, Jonas et al. 2005, Shao, Yang et al. 2006) the authors investigated potential benefits of the recombinant coagulation factor (RCF) VIIa and concluded that there was no effect to decrease blood loss during liver surgery. Pulitano *et al* (Pulitano, Aldrighetti et al. 2007) concluded that use of methylprednisolone did not decrease blood loss intra-operatively. Similarly, there was no beneficial effect of anti-thrombin III concentrates (Shimada, Matsumata et al. 1994) or desmopressin (Wong, Irwin et al. 2003).

Potentially beneficial effects were identified for the following three agents:

- *Tranexamic Acid (TA)*:

Wu *et al* (Wu, Ho et al. 2006) performed a double-blinded, placebo-controlled randomised trial comparing use of TA *versus* placebo recruiting 212 patients undergoing liver surgery for benign and malignant indications. Patients in the intervention group received 500mg of TA immediately prior to the operation and then 250 mg QDS for 3 days; patients in the control group had a similar volume of normal saline administered. The two groups were comparable, as expected, in terms of parenchymal transection method, use of intermittent in-flow occlusion, background quality of the liver and extent of resection. Patients in the intervention group had significantly less blood loss (overall blood loss 300 [30-2100] ml *versus* 600 [40-3410] ml, p= 0.0001), a lower blood transfusion rate ($p<0.0001$), shorter operative time ($p=0.003$) and hospital stay. It should also be noted that a relatively high proportion (51.8%) of patients in this sample had evidence of cirrhosis or chronic hepatitis.

- *Nafamostat Mesilate*:

10 patients received the serine-protease inhibitor Nafamostat Mesilate (NM) and were compared to a similar sized control group in a randomised controlled trial (Inagaki, Nonami et al. 1999). The 10 patients in the intervention group were given a continuous intravenous administration of 2mg/kg/day of NM for 7 days starting from the day of the operation. Details of randomisation and placebo administration were not provided. The intra-operative blood loss was lower in the intervention group though this did not reach statistical significance (1610 ± 1756 ml *versus* 957 ± 458 ml). Further statistical details were not provided. As secondary outcomes, Natural Killer cell activity and helper/suppressor ratios of T lymphocytes were found to be significantly increased compared to the control group. This may be of oncological importance, particularly when coupled with less intra-operative blood loss.

- *Aprotinin*:

Lentschener *et al* (Lentschener, Benhamou et al. 1997) performed a double-blind RCT looking at the use of aprotinin (protease inhibitor) *versus* placebo in 97 patients undergoing elective liver resection. The intervention group received a loading dose of $2x\ 10^6$ kallikrein inactivator units (KIU) of aprotinin, followed by a continuous infusion of $5x\ 10^5$ KIU per hour administered until skin closure. An additional bolus of $5x10^5$ KIU was administered for every 3 transfused red blood cell units. Patients in the control group received a similar volume of 0.9% Normal Saline placebo. The two groups, as anticipated, were comparable in terms of parenchymal transection method, use of intermittent inflow occlusion, background quality of the liver and extent of resection. The mean blood loss and transfusion requirements were significantly lower (1217 ± 966 *versus* 1653 ± 1221 ml, p=0.048) in the aprotinin group.

Author	Year	Population	Intervention	No. patients	Blood Loss (mL)	Statistical significance	Transfusion requirements [units or mL] (intra-/post op)	No. of patients transfused	Statistical significance
Wu et al	2006	Liver resection-All	Tranexamic Acid	108	300 (30-2100)	P=0.0001	-	0	P<0.0001
			Placebo	106	600 (40-3410)		-	17	
Pulitano et al	2007	Liver Resection-All	Methylprednisolone	36	621 (350-720)	P=0.382	0.54 +/- 0.6	-	P= 0.089
			Placebo	37	662 (300-800)		1.32 +/-0.5	-	
Shao et al	2006	Liver Resection-All	Recombinant Factor VIIa (RCF) 50uL/kg	71	800 (50-7000)	P=0.77	-	-	-
			RCF VIIa 100ul/kg	74	500 (70-6500)		-	-	-
			Placebo	76	500 (40-4700)		-	-	-
Lodge et al	2005	Liver Resection-All	RCF VIIa 20ul/kg	63	1372+/-1301	P=0.07	1354 +/- 989		P=0.78
			RCF VIIa 80ul/kg	59	1073+/- 997		1036 +/- 904		
			Placebo	63	1422 +/- 1271		1024 +/- 1001		
Wong et al	2002	Liver Resection-All	Desmopressin	30	832.5 (350-2955)	P=0.93		3	P=0.45
			Placebo	30	800 (250-7128)			5	
Inagaki et al	1999	Liver Resection (HCC/mets)	Nafamostat Mesilate	10	957+/-458	NS	-	-	
			Control	10	1610 +/- 1756		-	-	
Lenthschener et al	1997	Liver Resection-All	Aprotinin	48	1217 +/- 966	P=0.048	30		P=0.015
			Placebo	49	1653 +/- 1221		77		
Shimada et al	1993	HCC	Antithrombin III concentrate	13	1393 +/- 181	NS	4.8 +/-1.6		NS
			Placebo	11	1856 +/-360		4.4 +/- 1.4		

Table 2: A summary of RCTs investigating pharmacological interventions to reduce blood loss in liver surgery. (Underline indicates p<0.05)

4.2.2 **Anaesthetic Techniques**

Nine trials (Table 3) were included in the analysis that recruited 679 patients. There was a range in the quality of the studies as assessed by the Jadad score (Table 1). The studies described interventions into three main groups: (a) anaesthetic agents, (b) manipulation of physiological parameters and (c) haematological control.

4.2.2.1 a) Anaesthetic Agents

- *Pharmacological Post-conditioning (Sevoflurane):*

Beck-Schimmer *et al* (Beck-Schimmer, Breitenstein et al. 2012) performed a 3-group RCT comparing patients undergoing pharmacological post-conditioning with sevoflurane (n=48), intermittent Pringle manoeuvre (n=50) and continuous Pringle manoeuvre [control](n=17) in patients undergoing liver resection. In the control and intermittent clamping group, propofol was applied continuously until the end of surgery. In the post-conditioning group, propofol infusion was stopped after reperfusion and replaced by sevoflurane 3.2% end-tidal concentration for 5 minutes, followed by the post conditioning phase for 10 minutes and subsequent washout for 15 minutes. Propofol anaesthesia was re-started after this. Blood loss was similar between all three groups. However, post-conditioning (p=0.044) and intermittent clamping (p=0.015) reduced the transaminase levels significantly compared to the control group. Furthermore both groups also had a lower risk of complications and shorter hospital stay when compared with the control group.

- *Desflurane versus Isoflurane during Donor Hepatectomy:*

Toprak *et al* (Toprak, Sahin et al. 2012) compared two inhalation anaesthetic agents, Desflurane (n=40) and isoflurane (n=40) in patients who were all ASA I or II undergoing liver donor hepatectomy in a randomised controlled trial. All patients underwent a standard anaesthetic technique. Anaesthesia was maintained using either desflurane or isoflurane. The two groups were comparable demographically. The blood loss was smaller in the isoflurane group though no statistical analysis was undertaken. However, transaminases and INR were higher in the isoflurane group when compared to the desflurane group.

4.2.2.2 Manipulation of Physiological Parameters

- *Hypoventilation:*

Hasegawa *et al* (Hasegawa, Takayama et al. 2002) assessed whether reducing the tidal volume during surgery would be effective in reducing intra-operative blood loss, via a randomised controlled trial where patients were allocated to either normoventilation or hypoventilation. During liver transection, the tidal volume was 10mL/kg and the respiratory rate was 10/minute. In the hypoventilation group, the tidal volume was reduced to 4mL/kg and the respiratory rate was 15/minute. They found that hypoventilation did not decrease blood loss or peri-operative transfusion requirements.

- *Low Central Venous Pressure:*

Low CVP anaesthesia is an established technique employed to reduce blood loss during liver resection. Wang *et al* (Wang, Liang et al. 2006) randomised 50 patients with hepatocellular carcinoma to either low CVP or control groups. The low CVP group had a markedly lower volume of intra-operative blood loss than the control group (903.9 ml ± 180.8mL *versus* 2329.4 ± 2538.4 mL, $p<0.01$). Furthermore, operative time, hospital stay and post-operative liver function tests were significantly improved via lowering the CVP intra-operatively.

In another study, Ryu *et al* (Ryu, Nahm et al. 2010) randomised adult liver donors to milrinone induced low CVP (n=19) or low CVP via fluid restriction (n=19). Milrinone is a phosphodiesterase 3 inhibitor which works to increase the contractility of the heart (Rieg, Suleiman et al. 2014). During low CVP hepatic surgery, the preload is reduced which carries potential risks such as end organ ischaemia and haemodynamic instability. There was significantly less bleeding in the milrinone only group ($p<0.001$). Interestingly the milrinone only group also had better post-operative aminotransferases on days 1-3. No patients had any significant side effects from milrinone use.

4.2.2.3 Haematological Control

- *Acute Normovolaemic Haemodilution versus Control:*

There were a total of 238 patients undergoing liver resection that were randomised across 3 trials to haemodilution (n=117) or control (n=121). The number of participants in each trial was 78 (Matot *et al*), 130 (Jarnagin *et al*) and 30 (Guo *et al*). In each of the studies the

volume of blood to be removed was calculated pre-operatively. The haemodilution process began after intubation with blood being removed and stored in standard citrate-phosphate-dextrose storage bags. The volume of blood removed was replaced by either 6% medium-molecular weight hydroxyethyl starch or a combination of crystalloid and colloid. The majority of the patients underwent major (> 3 segment resection) liver resections. The proportions of cirrhotic/steatotic livers were not described. The number of patients requiring blood transfusion was significantly lower in the haemodilution group. However, the operative blood loss did not differ between groups.

-Intra-operative Blood Salvage:

Hashimoto *et al* (Hashimoto, Kokudo et al. 2007) randomised living donors intra-operatively to a blood salvage group [BS] (n=40) or control group (n=39). In the BS group a blood volume equivalent to 0.7% of the patient‘s blood volume was withdrawn prior to the start of parenchymal transection. Blood loss during hepatic parenchymal transection was significantly lower (p=0.034) in the BS group compared to control. However, overall blood loss and transfusion requirements between the two groups were similar. It should be noted that the CVP was lower in the BS group and hence the slightly lower overall blood loss may in fact be attributed to the lower CVP rather than BS.

Author	Year	Population	Intervention	No Patients	Blood Loss (ml)	Statistical Significance	Transfusion requirements [units or ml] (intra-/post op)	No. of patients transfused	Statistical significance	Peak AST (IRI)	Statistical Significance
Beck-Schimmer et al	2012	Liver Resection- All	Pharmacological post-conditioning	48	200 (100-325)	-	0	0	-	443	P=0.015
			Intermittent Clamping	50	225 (150-350)	-	0	0	-	438	
			Control	17	200 (100-500)	-	0	0	-	631	
Toprak et al	2012	Living Donor Hepatectomy	Isoflurane	40	503 +/- 203	-	0	0	-	235	
			Desflurane	40	542 +/- 227	-	0	0	-	136	P< 0.05
Ryu et al	2009	Living Donor Hepatectomy	Milrinone	18	142 +/- 129	P< 0.05	-	-	-	-	-
			Control	19	378 +/- 167		-	-	-	-	-
Jarnagin et al (	2008	Liver Resection- All	Acute Normovolaemic Haemodilution (ANH)	63	800 (100-3200)	P= 0.42	28	8	P=0.067		
			Control	67	700 (100-4000)		47	17			
Hashimoto et al	2007	Living Donor Hepatectomy	Blood Salvage	40	403 (120-1240)	P= 0.257		3	P= 0.115		
			Control	39	440 (130-1230)			9			
Wang et al	2006	HCC	Low CVP	25	903.9 +/- 190.8	P<0.01	525.00 +/- 237.57	8	P<0.05	561	P>0.05
			Control	25	2329.4 +/- 2538.4		1285.71 +/- 1162.13	14		700	
Guo et al	2004	HCC	ANH	15	710.9 +/- 75.9	NS	350.5 +/- 70.7		P< 0.01		
			Control	15	734.7 +/- 83.1		457.8 +/-181.3				
Matot	2002	Liver Resection- All	ANH	39	750 (100-7000)	NS		4	P= 0.014		
			Control	39	890 (100-7500)			14			
Hasegawa et al	2002	Liver Resection-Tumours	Hypoventilation	40	630 (72-3600)	NS		4	NS		
			Control	40	630 (120-3520)			3			

Table 3: A summary of RCTs investigating anaesthetic interventions to reduce blood loss in liver surgery. (Underline indicates p<0.05)

4.3 Discussion

Hepatic surgery has become significantly safer with advancements in surgical, radiological and anaesthetic techniques (Jarnagin, Gonen et al. 2002, Chan, Chiang et al. 2011). It has been well documented that both short and long-term outcomes are dependent on the avoidance of blood transfusions (Parrott, Lennard et al. 1986, Little, Wu et al. 1990, Tartter 1992). The purpose of this systematic review was to summarise the evidence of the effects of non-surgical interventions on the intra-operative blood loss during liver resections.

In this review, three categories of anaesthetic support to decrease blood loss were identified. Lowering the CVP state, whilst maintaining adequate organ perfusion is a key anaesthetic concept. As shown, there are also pharmacological agents to achieve this such as the use of Milrinone (Ryu, Nahm et al. 2010). However, most patients undergoing a hepatectomy receive an epidural catheter which decreases analgesic requirements, reduces the stress response and also gives rise to a sympathetic autonomic blockade. This decreases the systemic vascular resistance and lowers the CVP (Feltracco, Brezzi et al. 2008). Intravenous fluid administration or restriction is also guided by various methods of invasive and non-invasive methods of goal-directed fluid therapy (Bundgaard-Nielsen, Holte et al. 2007). Therefore milrinone is seldom required and has not been widely adopted. Nevertheless, it has been shown in animal experiments that Milrinone, injected intravenously immediately after reperfusion, may reduce the ischaemia-reperfusion

injury (Toyoda, Tosaka et al. 2013). The use of milrinone is liver surgery should be investigated further to assess effectiveness in decreasing the ischaemia-reperfusion injury.

Pharmacokinetic knowledge of anaesthetic agents may also play a role in reducing the extent of IRI seen in hepatic surgery. All volatile inhalational drugs are partly metabolised by the liver and may affect hepatic blood flow, as well as having other cardiovascular side-effects (Wissing, Kuhn et al. 2000). Sevoflurane and desflurane appear to reduce the degree of IRI which may be clinically relevant if the functional liver remnant is small. However, only two randomised trials have been performed examining the role of anaesthetic agents in reducing blood loss and the degree of IRI in liver surgery and hence further trials are needed before recommendations can be provided.

Blood preservation techniques such as intra-operative blood salvage and acute normovolaemic haemodilution demonstrate a tendency towards lower blood loss and transfusion requirements. Autologous blood transfusion techniques such as blood salvage avoids the risk of homologous blood transfusion and has been shown to be cost effective in major spinal surgery (Kumar, Chen et al. 2012). Concerns have been raised regarding use of autologous blood donation in oncological surgery due to a perceived risk of recurrent cancer, however, evidence suggests that it is safe to employ (Fujimoto, Okamoto et al. 1993, Bower, Ellis et al. 2011, Kumar, Chen et al. 2014). Other issues with autologous blood donation include a high discard rate and a higher risk of ischaemic events (Goodnough 2004, Ubee, Kumar et al. 2011).

The health economic aspects of routinely using such strategies in liver surgery need to be defined. In the interim period till such analyses occur, acute normovolaemic haemodilution and intra-operative blood salvage should be considered for major liver resections or in cases where blood loss is expected to be greater than normal.

Considering pharmacological approaches, Tranexamic Acid and aprotinin both decrease intraoperative blood loss (table 2). Aprotinin was withdrawn from the market due to concerns about increased rates of thrombosis, renal dysfunction and death (McMullan and Alston 2013) which may explain why there has been only one RCT. Both TA and aprotinin have been shown to successfully decrease blood loss and transfusion requirements in patients undergoing a hepatectomy. Further trials, within liver surgery however, are required to validate these findings and justify routine use of tranexamic acid, though some advocates may feel these agents be used routinely in high risk cases such as re-resections which are being undertaken increasingly nowadays with the trend towards parenchyma-sparing surgery. Nafamostat Mesilate (NM) was shown to decrease blood loss and heighten the immune response in one trial which may be of particular importance in oncology. However, the trial numbers were low and insufficient to draw any firm conclusions.

Most studies included in this review had methodological weaknesses. As demonstrated in Table 1, study quality was variable. Although all included studies were randomised control trials, randomisation details were not provided in all cases. There is also significant heterogeneity in

the populations studied. Though 17 RCTs were included, most of them examined different strategies to reduce intra-operative blood loss and transfusion requirements and most were also underpowered, without a formal sample size calculation. Therefore, pooling of results and meta-analyses was not possible.

Furthermore, it is accepted that blood transfusion requirements do not necessarily directly correlate to blood loss as different centres will have varying trigger points for transfusion and that blood loss is dependent on several patient and physician-based factors. Additionally, the methodology of estimating blood loss was not given in most papers and this would be subject to inter-observer variability. Additionally, most papers used ALT to measure ischaemia-reperfusion injury although the effect of post-operative ALT on short- or long-term outcome has not been previously been published.

Patients are currently assessed and counselled with regard to fitness to surgery via pre-operative assessment and cardiopulmonary exercise testing (Lentschener and Ozier 2002). However, the risk of bleeding is not formerly assessed despite the availability of pre-operative radiological images and knowledge of patient factors which may predispose to bleeding. It may be appropriate to formerly assess the risk of intra-operative blood loss prior to surgery and then ensure certain pathways are followed once a certain trigger figure is reached (e.g. 500ml blood loss). A parallel may be drawn to obstetric medicine where WOMAN- an international, randomised doubled blinded placebo controlled trial is examining the role of TA use in post-partum haemorrhage once a certain amount of blood loss has occurred. Similarly we propose a

multi-faceted approach to pre-operative bleeding risk assessment in liver surgery and initiation of management strategies once a certain amount of blood loss has occurred.

Future trials should examine optimal use of anaesthetic agents, both for anaesthesia maintenance and pharmacological post-conditioning. Although, anaesthetic agents may not significantly reduce blood loss there is evidence to suggest reduced IRI. Milrinone may also facilitate maintenance of a low CVP intra-operatively whilst simultaneously reducing the IRI and blood loss during liver surgery. There is a paucity of trials examining the use of TA in Liver surgery given the positive findings of the two RCTs. Nafamostat Mesilate also reduced blood loss whilst increasing NK cell activity. Well-designed studies could help optimise usage of these drugs in the future. Future efforts should also be directed towards producing guidelines for assessing the risk of intra-operative blood loss and subsequent strategies to deal with bleeding once a threshold has been passed.

Chapter 5

The effect of peri-operative blood loss and blood transfusion on long term survival following liver surgery for CRLM

5 The effect of peri-operative blood loss and blood transfusion on long term survival following liver surgery for CRLM

5.1 Introduction

Recent developments in surgical technique, anaesthesia and peri-operative care have resulted in lower morbidity and mortality rates after liver resections for the treatment of CRLM (Jarnagin, Gonen et al. 2002, Chan, Chiang et al. 2011). Furthermore, Neoadjuvant and adjuvant chemotherapy protocols have led to an increase in the proportion of patients eligible for curative resections (Adam, Delvart et al. 2004, Falcone, Ricci et al. 2007, Folprecht, Gruenberger et al. 2010, Lam, Spiro et al. 2012). However, intra-operative blood loss remains a significant concern as blood transfusions are known to predispose to poorer short- and long-term outcomes (Parrott, Lennard et al. 1986, Little, Wu et al. 1990, Tartter 1992, Panagopoulos, Karakantza et al. 2008). Increasing evidence from other surgical subspecialties (including breast and oesophago-gastric

surgery) indicates that cancer patients who received blood transfusions had poorer long-term survival rates (Parrott, Lennard et al. 1986, Little, Wu et al. 1990, Tartter 1992, Herman and Kolodziejski 1993, Amato and Pescatori 2006, Panagopoulos, Karakantza et al. 2008, Sun, Wang et al. 2015). In resected primary colorectal cancer a link between transfusions, earlier recurrence and decreased survival has been established (Burrows and Tartter 1982, Foster, Costanza et al. 1985, Miki, Hiro et al. 2006). There is limited data regarding the impact of red blood cell transfusion (RBCT) on long-term impact in resectional surgery for CRLM. The majority of published studies on this topic for CRLM resections are analyses of single centre experiences using uni- and multivariate analyses, with conflicting evidence regarding the effect of RBCT and long term outcome (Younes, Rogatko et al. 1991, Gruttadauria, Saint Georges Chaumet et al. 2011, Jiang, Fang et al. 2013, Hallet, Tsang et al. 2015, Schiergens, Rentsch et al. 2015). There remains debate about whether there is a real causal relationship between blood transfusion and long-term outcome, or whether the poorer long-term outcome is due to the more complicated surgery (larger tumour volume/number) and associated risks. Previous studies looking at long term outcomes are largely historical and therefore, given the changes in chemotherapy and reduced transfusion requirements over time, it would be useful to re-evaluate this relationship, especially in the context of expanding indications for more complex and recurrent resections.

5.2 Aim

The primary aim of this chapter is to examine the cause and effects of blood loss and blood transfusions on long-term outcomes after hepatectomy for colorectal liver metastases, in a contemporary series.

5.3 Methods

A retrospective cohort study was performed, using the prospectively maintained database of all patients undergoing liver resections at The Leeds Teaching Hospitals, between 2005-2012

5.3.1 Inclusion Criteria

Patients were included in the final analysis if they underwent surgery for CRLM. Patients undergoing simultaneous resections, two-stage procedures, re-resections and concurrent ablative techniques were excluded, as these patients represent a very different risk group profile.

5.3.2 Outcome Measures

The primary outcomes were defined as (1) overall survival (OS) with ‘death’ as the end point in relation to blood transfusion and (2) relapse–free survival (RFS) with ‘recurrence’ as the end point in relation to blood loss and blood transfusion. Secondary outcome measures were factors associated with RBCT and OS. The relationship between OS and complications was also examined, via the Clavien-Dindo Score.

5.3.3 Definitions

The hospital blood transfusion database was cross-checked. A major hepatectomy was defined as resection of 3 or more contiguous or non-contiguous hepatic segments according to the Couinaud classification (Mullen, Ribero et al. 2007). Recurrence was defined as a radiological diagnosis on cross-sectional imaging. For death as an end-point, OS computed from date of surgery to date of death and for recurrence as an end point, RFS is computed from date of surgery to date of recurrence End of follow up for OS was defined as date of death or 4th June 2014. End of follow up for RFS was defined as date of recurrence, date of death or 4th June 2014.

Perioperative transfusion was defined as transfusion of one or more units of allogenic RBC within 7 days of surgery. As actual Intra-operative blood loss (IBL) was not routinely collected in the retrospective data an estimated IBL was computed using the following formula (Gross 1983):

$$\boldsymbol{IBL = EBV\ x\ \frac{(Ho - Hf)}{\frac{1}{2}(Ho + Hf)}}$$

EBV: estimated Blood volume (10 units x 500ml), *Ho*: preoperative Hb, *Hf*: postoperative Hb. IBL was then estimated using another formula (Bourke and Smith 1974) which applied the natural Logarithm function:

$$\boldsymbol{IBL = EBV\ x\ In\frac{Ho}{Hf}}$$

The estimates corresponded well (Kappa= 0.8) Maximum discrepancy between the estimates was < 5%, mainly when estimating very high IBL.

Adjuvant or neoadjuvant therapy was used in patients who had not had any chemotherapy within 12 months of hepatic surgery, or in those who had a positive resection margin.

The Clavien-Dindo score is widely used throughout surgery for grading adverse events which occur as a result of surgery (Dindo, Demartines et al. 2004)

Fong et al published the Clinical Risk Score which used various prognostic criteria to identify patients most likely to benefit from surgery (Fong, Fortner et al. 1999). Clinical Risk Score (CRS) was computed for each patient based on ; CEA > 200 dg/L, number of liver lesions, size of largest liver lesion, nodal status of the primary tumour and disease free interval < 12 months (Fong, Fortner et al. 1999, Hallet, Tsang et al. 2015) . In this study, CRS variable was used as a binary variable; CRS =0 (and CRS > 0) **.**

5.3.4 **Data Extraction**

Data extraction was undertaken by three researchers (SP, AAD, FK) using a pre-designed data extraction form. Data was extracted on baseline patients demographics, pre-operative clinical characteristics (use of neoadjuvant chemotherapy, co-morbidities, size and number of tumours, presence of extra-hepatic disease, tumour markers and routine pre-operative bloods), intra-operative characteristics (use of hepatic inflow occlusion, type and extent of resection) and post-operative features (pathology, post-operative complications, post-operative chemotherapy, recurrence, date of death). Post operatively patients were followed up clinically and

radiologically with computed tomography of the chest, abdomen and pelvis every 3-6 months for the first two years and then yearly until 10 years postoperatively.

5.3.5 **Statistical Analysis**

Data were analysed using SPSS statistical software (IBM SPSS Statistics USA, Version 21, 2012) for uni- and multivariate analysis and STATA (StataCorp Texas USA, Version 11.1, 2009) for survival analysis.

5.3.5.1 Univariate Analysis

Descriptive analysis described characteristics of patients who required transfusion and compared them to those who did not. For categorical data, analysis included the use of cross tabulation, Odds ratios & Chi square to test the difference/ association between study groups. Fisher Exact test was used when indicated The Pearson chi-square test of association was used to examine the relationship between each variable and outcome. Statistical assumptions required for a valid application of the chi-square test were examined and found to be justified. The magnitude of the effect was quantified using the odds ratio (OR) with 95% Confidence interval. Both parameteric

as well as non parametric tests and GLM ANOVA were used to assess the difference in means for continuous and numerical variables between the transfused and non-transfused.

5.3.5.2 Multivariate Analysis

Joint and conditional multivariate association between variables and outcome was assessed using binary logistic regression. All variables that were shown to be significant in the univariate analysis at $p < 0.05$ were included in a multivariate analysis. Logistic regression was used to adjust simultaneously for study variables seen as potential confounders and examined possible interaction terms:

$$\text{Log}_{\text{Probability Intra-operative bleeding}} \textbf{(Log OR) = Constant+ } B_1X_1 + B_2X_2 + \ldots\ldots B_nX_n$$

whereby X is the variable to be explored and B is its regression coefficient. Odds ratio for individual variables is computed from the regression equation as $OR = e^B$, adjusted for all other variables in the model. Variables not included in the final model are those variables which did not provide a statistically significant multivariate contribution to the prediction of intra-operative bleeding.

The appropriateness of the logistic link and model specification was assessed, the data was screened for potentially influential observations, and the extent of multicollinearity amongst predictor variables was examined using variance inflation factors (VIF). These model diagnostics (VIF < 2, tolerance .02) indicated that a logistic specification was appropriate and the predictors in the model did not suffer from problems associated with information overlap. The

sample size is sufficiently large to ensure stable logistic regression parameter estimates were obtained which are not suspect on accuracy or precision

5.3.5.3 Prediction Analysis

A prediction score was calculated from all predicting factors in the multivariate analysis and the linear function for the binary logistic regression model:

B1x1+b2x2.....+ Constant = predictive score

The prediction validity was assessed using ROC curves

5.3.5.4 Survival Analysis

Survival analysis was performed using STATA version 8. The Kaplan -Meier method with Log-rank test was applied to compare survival curves in the compared groups. Cox proportional hazard regression assessed the effect transfusion on the probability of survival during the follow up period. Variables identified as significantly associated with transfusion on univariate analysis ($p<.05$) were included in the regression model. Hazard ratios HR was calculated with 95% confidence intervals. Hazard Ratios were interpreted as the instantaneous relative risk of death any time between 2005 and 2014 for patients who underwent hepatectomy at St James

University Hospital, Leeds between 2005 and 2012. Patients were followed up until June 2014. Kaplan -Meier curve was used to estimate the survival outcomes

5.4 Results

5.4.1 Patient Characteristics

Six Hundred and Ninety patients who underwent hepatectomy between 2005 and 2012 were included and follow up was undertaken until June 2014 (Table 4). Of these, 461 (66.8%) patients were males and 229 (33.2%) females with a mean age of 67.2 years [standard deviation (SD) =10] range 25 to 82 years [median 66, interquartile range (IQR) 13 years].

Two hundred and fifty patients (36%) had preoperative anaemia with a haemoglobin of less than 130 g/L (NICE 2013). Three hundred and twenty seven patients (47%) had multiple liver metastases with a maximal tumour number of 21 (median 3, IQR 2) and a mean tumour size of 41 mm [range: 9-120, standard deviation (SD) =21]. There were 337 of the 690 patients (49%) underwent a major liver resection (i.e. resection of 3 or more segments), and in 238 cases (35%) an anatomical hepatectomy was performed.

Sixty-four (9.3%) patients required peri-operative RBCT. Recurrences were reported in 436 (63%) patients (of which 237 were intra hepatic) during the follow up period. The median follow

up was 33 months (IQR 28.5)**.** At final follow-up, 352 (51%) patients were alive and 338 (49%) died.

Table 4: Characteristics of patients undergoing hepatectomy for CRLM

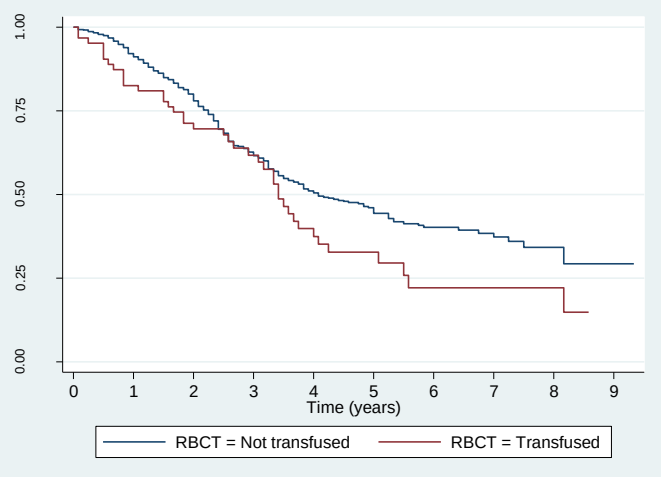
	Transfused patients N=64	**Not transfused patients N =626**	***P* value**
Male gender	38 (59%)	422 (67%)	0.124
Pre-operative anaemia <130g/L	48 (75%)	202 (32%)	**<.001**
Cardiac disease	15(26%)	91(16%)	0.047
Vascular disease	9(16%)	49 (9%)	0.081
Diabetes mellitus	9 (16%)	61 (11%)	0.198
Hypertension	18 (31%)	165 (30%)	0.458
Primary tumour location (colon)	42(71%)	312 (54%)	**0.009**
Pre-operative Chemotherapy	17(29%)	154(27%)	0.412
Extra hepatic spread	6 (9%)	82(13%)	0.264
Major liver resection	40(63%)	297(47%)	**0.015**
Type of hepatectomy (anatomical resection)	29(45%)	209(33%)	**0.021**
Resection Margin status:			
R0	34(53%)	352(57%)	0.323
R1	30(47%)	265(43%)	0.314
R2	1(2%)	4 (1%)	0.390
Post-operative complications	23(36%)	138(22%)	**0.012**
Clinical Risk Score CRS >0	3 (5%)	65 (10%)	0.12
Age (years) Mean (sd)	68.6 (12)	65.6 (10)	**0.034**
Size of large tumours in mm	Median 35 IQR 60	Median 35 IQR 33	0.83
Pre-operative CEA	Median 6 IQR 30	Median 8.50 IQR 30	0.93
Pre-operative CRP	Median 6.40 IQR 30	Median 5 IQR 6	0.64
Number of tumours	Median 3 IQR 4	Median 2 IQR 3	0.97

5.4.2 **Long Term Outcomes**

5.4.2.1 Overall Survival and RBCT

The median follow up was 33 months (IQR 28.5). The median overall survival was 46 months (95% CI 39-53). For those who received RBCT median survival was significantly lower at 41 months (95% CI 41-57) compared to 49 months (95% CI 36-46) in those who did not receive RBCT (p=0.036) (Figure 2). After 1, 3 and 5 years, overall survival rates were 83%, 54% and 30% respectively for patients who were transfused compared to 91%, 62% and 43% for non-transfused patients. RBCT was associated with poorer OS.

Figure 2 : OS and Transfusion status

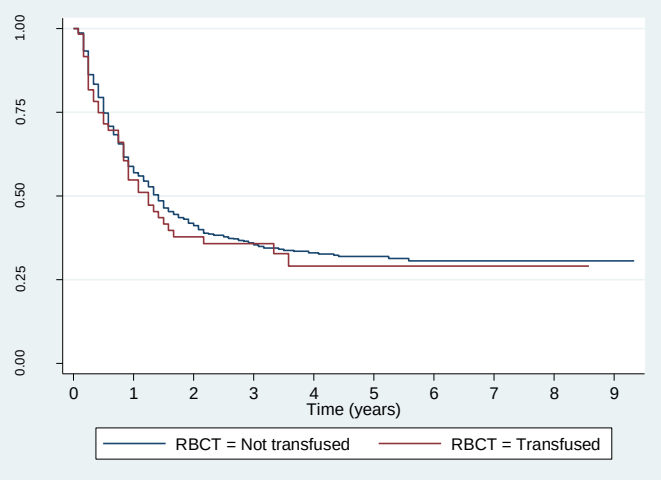


Log rank test p=.036

5.4.2.2 Relapse Free Survival and RBCT

The median RFS survival was 16 months (95% CI 13.8-18.2). For those who received RBCT median survival was 15 months (95% CI 9.7- 20.2) compared to 17 months (95% CI 14.4- 19.6) for those who did not receive RBCT but this difference was not significant (p=0.28). At 1 year, 3 years and 5 years RFS rates were 58%, 35% and 30% for those who had RBCT and 55%, 35% and 29% for non-transfused patients respectively (Figure 3).

Figure 3 : RFS and transfusion status

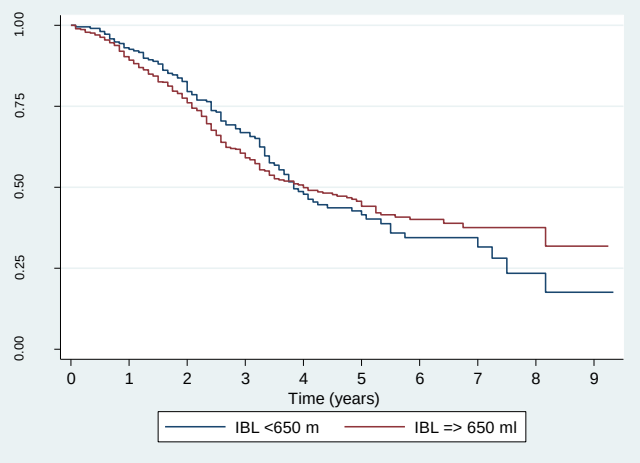


Log rank test p= 0.28

5.4.2.3 Overall Survival (OS), Relapse Free Survival (RFS) and Blood Loss

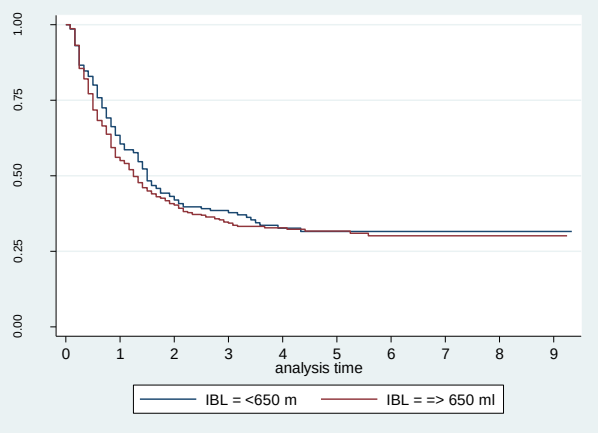
IBL was not significantly related with either OS or RFS (Figures 4 and 5). For high IBL median overall survival was 3.8 years (95% CI 3.1-4.8) compared to 4 years (95% CI 3.4-4.1) for those had a lower IBL (Log rank test p=0.28). Median RFS for high volume IBL was 1.33 years (95% CI 1.1-1.7) compared to 1.4 years (95% CI 1.2-1.7) for those with less IBL (p=.82)

Figure 4: OS and Estimated Blood Loss



Log rank test p= 0.28

Figure 5: RFS and Intraoperative Blood Loss



Log rank test p= 0.82

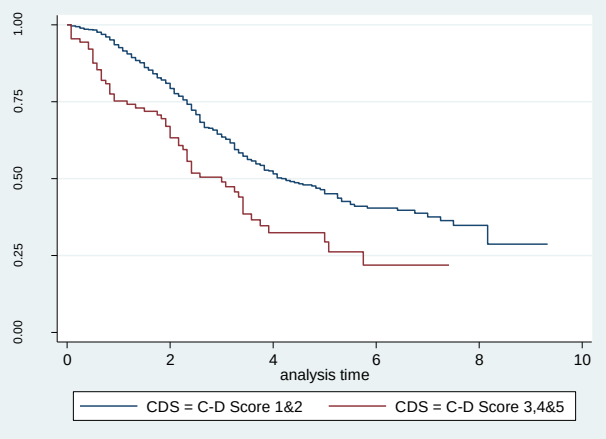
5.4.2.4 Overall Survival (OS) and Postoperative Complications.

The median survival for patients with minor post operative complications based on Clavien-Dindo scores 1 and 2 was 4.2 years (95% CI 3.5-4.8) versus 2.4 years (95% CI 1.6-3.2) for patients with scores >2 (Log Rank test X^2 19 df 1 p=0.0001) [Figure 6]

Patients who had Clavien-Dindo scores >2 and received RBCT had a median survival of 1.2 years (95% CI 0-4.9) compared to median survival of 2.4 years (95% CI 0.6 -3.2) for those with the same CD score who did not have RBCT (Figure 7). The median survival for those who had CD Score 1 and 2 and who received RBCT was 3.8 years (95% CI 2.8-4.6) compared to 4.4

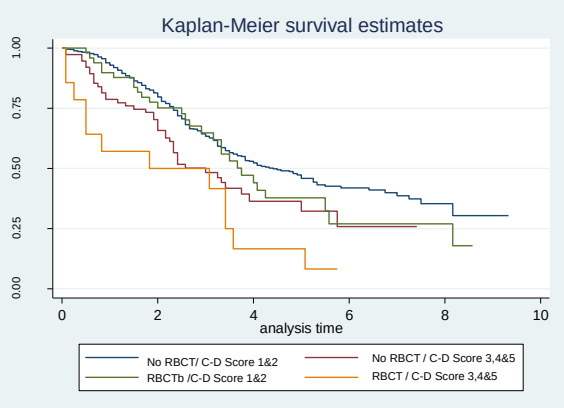
median survival years (95% CI 3.7-5.1) for those with same CD score but who did not receive RBCT (p=0.001) (Figure 7)

Figure 6 : Overall Survival by Clavien Dindo Scores



Log rank test p= 0.0001

Figure 7: Comparison of OS for patients with high and low Clavien-Dindo Scores by transfusion status



Log rank test p= 0.01

5.4.3 **Factors Associated with OS and RFS**

Factors associated with OS and RFS in Cox regression analysis are presented in Table 5**.** In addition to RBCT, age at surgery, sex, pre-operative chemotherapy, post-operative complications and CRS (derived from size and number of liver lesions, CEA level >200 dg/L and nodal status of primary lesion) were included in the model. Pre-operative chemotherapy, post-operative

complications and CRS were independently associated with reduced overall survival, though RBCT was not. RBCT was not associated with RFS

Table 5: Cox regression Analysis of factors predisposing to OS and RFS

	Overall Survival			**Relapse free survival**		
	Hazard ratio	**95.0% CI for HR**	***P* value**	**Hazard ratio**	**95.0% CI for HR**	***P* value**
Age	1.008	0.996-1.019	0.187	1.001	0.99 - 1.01	0.798
Male gender	1.001	0.782-1.283	0.991	1.043	0.84 - 1.29	0.696
Pre operative chemotherapy	1.780	1.383-2.291	**0.000**	1.709	1.37 – 2.13	**0.000**
Clinical Risk Score >0	2.362	1.875-2.974	**0.000**	2.541	2.07 - 3.12	**0.000**
Clavien-Dindo Score	1.566	1.153-2.126	**0.004**	1.026	0.762-1.38	0.865
RBCT	1.360	0.962-1.924	0.082	1.271	0.904 – 1.78	0.168

5.4.4 **Factors Associated with RBCT**

5.4.4.1 Univariate Analysis

Table 4 summarises the univariate analysis of variables associated with the need for RBCT. Significant factors included major liver resections [OR 1.8 (95% CI 1.1-3,1), p=0.015)], anatomical resections [OR 2.1 (95% CI 1.3-3.8), p=0.02], size of largest tumour [OR 1.8 (85% CI 1.1-3,1), p=0.04), age [OR 1.03 (95% CI 1.02-1.16), p= 0.034], post-operative complications [OR 1.97 (95% CI 1.14-3.33), p= 0.012], Charlson scores of 1 and 2 (X^2 12.3, p=0.015), preoperative anaemia (OR of 6.3 (95% CI 3.5-11.3, p <0.001) and colonic primary tumours [OR 1.9 (95% CI 1.13 - 3.32), p=0.009] . Patients who received RBCT were older than those who did not (p=0.034)

5.4.4.2 Multivariate Analysis

Three co-variates (Table 6) were shown to be significant in predicting the need for RBCT: (1) pre-operative anaemia [OR 7.97 (95% CI 4.1- 15.6, p<.001), (2) the size of the largest tumour [OR 1.01 (95%CI 1.00-1.02, p=.001) and the primary tumour location [OR 2.4 (95%CI 1.3 - 4.7, p=0.008). A drop of postoperative Hb did not achieve significance as a predictor (OR 1, p=0.25).

Table 6: Logistic regression Analysis of factors predisposing to RBCT

	RBCT		
	Hazard ratio	**95.0% CI for HR**	***P* value**
Pre operative anaemia	7.968	4.063-15.626	**0.01**
Primary tumour Location in the colon	2.424	1.262-4.653	**0.008**
Size of largest tumours	1.011	1.003-1.020	**0.011**

5.4.4.3 Prediction Modelling for RBCT

A prediction score was calculated from all predicting factors in the multivariate analysis is:

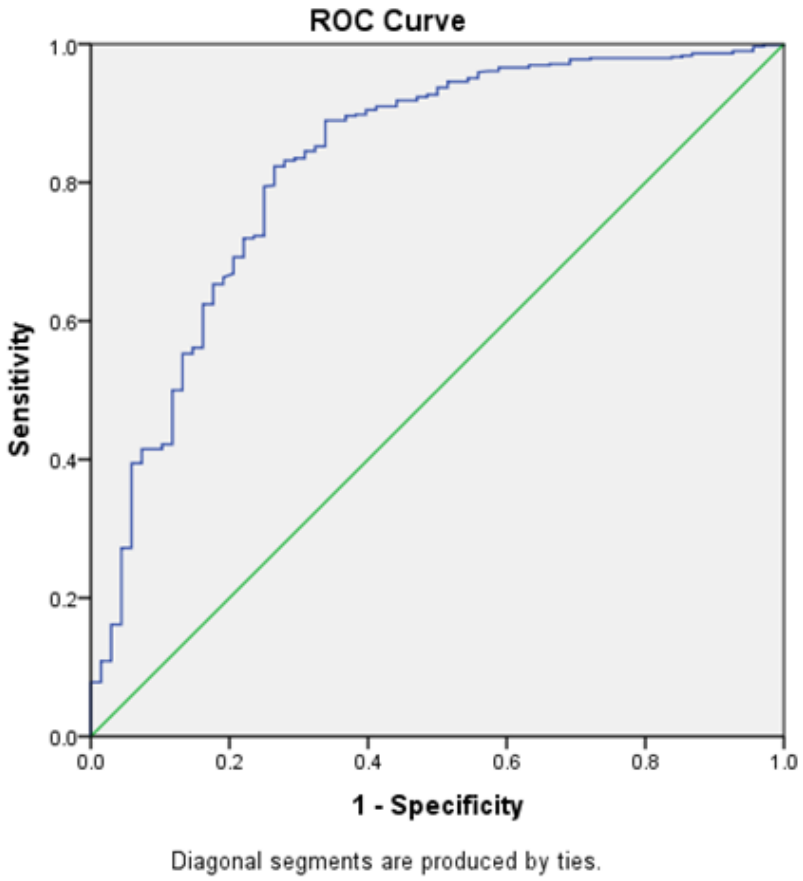
- 0.012 × Size of largest tumour + 2.07x pre operative anaemia + 0.885x primary tumour location - 4.278= score

The model has good predictive properties with an area under the ROC curve (AUC) of 0.845 (Figure 8).

In the above formula the variables take the values of 0 or 1 depending on whether there was pre operative anaemia (1 = Yes, 0 = No) and primary tumour location (1 =Colon and 0=Rectum). If the value obtained from calculating the formula is negative (or zero) the model predicts that there no transfusions will be required. If the formula is positive then the need for transfusions can be predicted.

Figure 8: ROC curve assessing predictive accuracy of the logistic model predicting RBCT

Area Under Curve (AUC) for predicting RBCT p=0.845



5.4.5 **Factors Associated with Blood Loss**

The median IBL was 833ml (IQR 590 ml, range 0-2605 ml), the median Hb drop was 2.3 g/dL (IQR 1.7 g/dL, range 0-8.9 g/dL) and 64 (9.3%) patients received a transfusion. An IBL volume of 650 ml (Hb drop of 1.4 g /dL) corresponded to the maximum joint sensitivity on the ROC plot AUC 0.81 (95% CI 0.76-88). Univariate analysis identified the following variables to have significant associations with a drop in pre-operative Hb (IBL) ≥1.4 g/dL (based on ROC curve):

1. Pre-operative chemotherapy and Hb drop [OR 0.7 (95% CI .44-.98, p=.028)]

2. RBCT and Hb drop [OR 3.5 (95% CI 2.2-5.9, p> 0.001)]

3. Size of the largest tumour (mean diameter 44.2 mm in higher IBL *versus* 38.5mm in lower level IBL [OR 1.01 (95% CI 1-1.14, p =.034)]

4. Closest resection (5mm in high IBL *versus* 3.8mm in lower IBL [OR .97 (95% CI .95- .99, p=0.036)]

However, multivariate analysis confirmed that only the size of the largest tumour > 3.5 cm (p>0.001) as the main predictor of high IBL.

5.5 Discussion

To date, this is the largest and most contemporary study on the effects of blood transfusions on survival after hepatectomy for colorectal liver metastases. In the current study, 690 patients underwent liver resection, of whom 64 (9.3%) needed a blood transfusion. Our transfusion rate was lower than most published series- this is likely to be secondary to improved surgical and anaesthetic advancements recently, in comparison to older series. Transfusion was associated with a decreased overall survival. However, there was no association between blood transfusion and earlier disease recurrence. Furthermore, on multivariate regression RBCT was not independently associated with OS, although post-operative complications, pre-operative chemotherapy and CRS were. Multi-variate analysis found pre-operative anaemia, location of primary tumour and size of liver metastases to be predictive of the likelihood of RBCT. A predictive score was developed, although this will need to be validated on other cohorts.

Our findings suggest that the poorer OS seen in patients who underwent RBCT was likely to be multifactorial and due to peri-operative complications, rather than due to earlier disease recurrence per se. Patients who had a major complication had a significantly reduced overall survival and this impact was exaggerated if the patient also had a blood transfusion. It is feasible that peri-operative transfusion is a surrogate marker for larger volume disease (major liver resection, anatomical resection and size of largest tumour all associated with RBCT on univariate

analysis) and hence the poorer OS was multifactorial, linked to more extensive surgery and the associated complications.

Previous studies of patients undergoing colectomies for primary colorectal cancer have demonstrated a link between blood transfusion and earlier disease recurrence (Burrows and Tartter 1982, Foster, Costanza et al. 1985, Miki, Hiro et al. 2006). Although mechanisms for this observation are not fully understood, it is believed that micrometastatic cells evade immune surveillance due to decreased tumour surveillance as the transfusion modifies the immune response by a decrease in the natural killer cell activity, phagocytic activity and an increase in suppressor T-cell activity with inhibition on interleukin 2 (Amato and Pescatori 2006, Kneuertz, Patel et al. 2011, Schiergens, Rentsch et al. 2015). This leads to transfusion-related host immunosuppression causing decreased tumour surveillance and early recurrence (Kooby, Stockman et al. 2003).

Our study did not find an independent association between liver resection, blood transfusion and earlier disease recurrence, although there was a negative association between transfusion and overall survival. It may be feasible that stage IV disease represents a different disease entity and hence the immune response to RBCT is different. It is also plausible that better systemic and multi-modal therapies keep micro-metastatic disease well controlled.

A small number of previous studies have also examined the effect of RBCT on patients undergoing a hepatectomy for CRLM (Jiang, Fang et al. 2013, Hallet, Tsang et al. 2015, Schiergens, Rentsch et al. 2015). However, the majority of these studies are limited by the

relatively low patient numbers. A few studies have found an association between RBCT and decreased overall and disease free survival (Stephenson, Steinberg et al. 1988, Rosen, Nagorney et al. 1992, Hallet, Tsang et al. 2015). However, other investigators have found transfusion to be associated with poorer short term outcomes but not necessarily long-term outcomes (Kooby, Stockman et al. 2003, Cannon, Brown et al. 2013). Recently Postlewait et al (Postlewait, Squires et al. 2016) also found that RBCT was associated with increased complications but not disease-specific survival. In their study, only patients who underwent a major hepatectomy were included. Our study includes all liver resections as significant blood loss may also be encountered when performing non-anatomical resections for large liver metastases. The relatively high R1 rates in our series are due to the fact that large non-anatomical resections were often undertaken.

Currently there is considerable variation in use of blood products and more restrictive transfusion strategies should be used (Carson, Terrin et al. 2011). However, this study would support judicious use of blood transfusion during the peri-operative period, ideally within the context of a guideline.

A recent review identified that strategies to inform risk of perioperative bleeding were not routinely performed (Pathak, Hakeem et al. 2015). Certain pharmacological agents such as tranexamic acid and aprotinin were also shown to decrease blood loss during liver surgery but are yet to be widely adopted due to a paucity of evidence (Pathak, Hakeem et al. 2015). Although transfusion rates in this study were low, patients with large tumours and pre-operative anaemia

were more likely to receive a transfusion. Identifying risk factors predisposing to transfusion may further reduce transfusion requirements.

This study is limited by its retrospective design and inherent biases due to the long study period. It is also likely that the finding suggesting that location of primary tumour as a prognostic indicator of transfusion requirements is a retrospective bias although previous research has found it to be an independent predictor of long-term survival (Younes, Rogatko et al. 1991, Adam, de Haas et al. 2011). There is emerging evidence suggesting that the prognosis between left sided and right sided colon cancers may vary, which may also influence outcome in hepatic surgery (von Einem, Heinemann et al. 2014, Brule, Jonker et al. 2015).

Our findings suggest that transfusion is not associated with poorer long term outcomes and that it should be used judiciously in the peri-operative period, ideally within the context of a guideline.

Chapter 6

Pilot Randomised Controlled Trial: A study to compare the impact or The Pringle Manoeuvre or Isolated Portal Vein Clamping on blood loss during liver surgery

6 Randomised Controlled Trial: The Pringle Manoeuvre versus Isolated Portal Vein Clamping (RfPB Funded Trial)

6.1 Background

The portal vein and the hepatic artery supply 75% and 25% of the liver's blood supply respectively, with each vessel contributing 50% of the liver's oxygen requirement (Rahbari, Wente et al. 2008). The Pringle manoeuvre is commonly used to prevent blood loss at the time of liver resection by clamping the vascular pedicle containing the hepatic artery and the portal vein together during liver resection surgery (Figure 9). During clamping a deprivation of oxygenation at the cellular level occurs. Cellular damage occurs when the 'starved' cells are reperfused, resulting in ischaemia reperfusion injury (IRI) (see chapter 1.4) (Montalvo-Jave, Escalante-Tattersfield et al. 2008). It has been suggested that undertaking isolated control of the hepatic portal vein and not clamping the hepatic artery allows a continued supply of energy substrates and oxygen to the hepatic cells, which could potentially reduce the damage caused by IRI whilst ensuring adequate vascular control intra-operatively. An RCT in China compared

these two techniques along with a different technique and found that portal vein clamping in isolation resulted in faster recovery of liver functions (Fu, Lau et al. 2011). Choice of anaesthetic agents may also play a role here as animal studies have shown hepatic arterial inflow to be increased using sevoflurane (Ko, Gwak et al. 2010) .

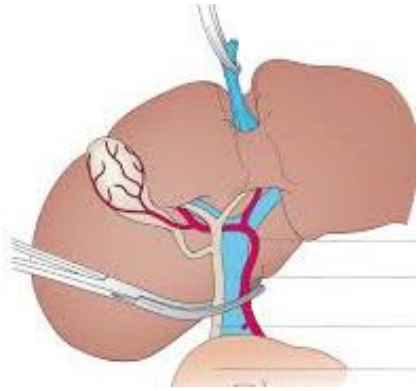


Figure 9: A Diagram of the Pringle Manoeuvre (http://www.surgicalcore.org/popup/43202)

6.2 Aims and Objectives of RCT

6.2.1 **Aims**

The primary aim of this pilot RCT was to test whether the components of a larger multi-centre trial can work together. Specifically:

1) The ability to recruit and randomise patients into the study
2) The ability to conduct portal vein clamping surgical procedure
3) The appropriateness of the follow-up assessments

6.2.2 **Secondary Objectives**

The secondary objectives were to collect information with regard to the following:

1. Incidence of septic complications in portal vein clamping versus Pringle manoeuvre
2. Procedural outcomes including intra-operative blood loss, length of clamping time, length of time transecting the liver and duration of the operation
4. Post-operative events including bleeding, post- operative transfusion requirements, non- infective cardio- respiratory complications and 30- day mortality rate

6.3 Methods

6.3.1 **Ethical Approval**

Ethical approval was sought from the Leeds East NRES committee and approval 13/YH/0195 ;(30.7.2013 ; see Appendix 8.2) was granted. Leeds Teaching Hospitals NHS trust was the sponsor.

6.3.2 **Study Design**

This was a prospective, parallel group, single blind, randomised controlled pilot study. A total of 80 patients were recruited. The first 20 patients were part of a learning curve development (non-randomised) for portal vein clamping. The subsequent 60 patients randomised and enrolled into the study. This was a pilot study and therefore the sample size was deemed suitable to inform feasibility of performing a larger, multi-centre trial investigating Pringle manoeuvre *versus* isolated portal vein clamping in liver surgery.

6.3.3 **Inclusion Criteria**

The inclusion criteria were as below:

(a) At least 18 years of age

(b) Undergoing open liver resection surgery for CRLM

(c) Portal venous clamping is considered appropriate

(d) Participating patients should understand the study objectives and be able and willing to provide written informed consent

(e) Able to complete study questionnaire

6.3.4 **Exclusion Criteria**

The exclusion criteria were as below:

(a) Patients having simultaneous bowel and hepatic surgery

(b) Patients participating in other trials that could impact the outcomes measures being recorded

(c) Pregnancy at the time of surgery

(d) Patients taking immunosuppressive medication within 3 months of surgery

(e) Patients undergoing laparoscopic surgery

(f) Patients who have previously undergone portal vein embolisation

6.3.5 **Blinding**

Patients were blinded to the surgical procedure allocated to minimise the reporting bias. However, blinding of surgeons performing the surgical procedure was not possible.

6.3.6 **Recruitment**

Patients who were diagnosed with resectable CRLM and who met the inclusion criteria were deemed eligible for the trial.

Patients who met the eligibility criteria were invited to take part by the consultant surgeon during the clinic appointment. Patients were given information about the study, involving both verbal information and Patient Information Leaflets (PIL) which included detailed information about the rationale, design and personal implications of the study. After receiving PIL, patients had at least 24 hours to consider participation. The patient was given the opportunity to discuss the study with their family and healthcare professionals before they were asked whether they would be willing to take part in the study.

Three QoL questionnaires (EORTC QLQC30, QLQ-LM21 and EQ-5D-3L) were posted to the patients and information was provided on how to fill them in. Patients were asked to fill the questionnaires within the last week before the operation and handover to the surgical team on the day of procedure. Patients also received a phone call in the week prior to surgery to remind them to complete the questionnaires. Participating research staff members were required to complete a log of all patients screened for eligibility. Anonymous information was collected including:

- Age
- Gender

- Ethnicity

Screened patients who were not randomised either because they are ineligible or because they decline participation will also have the following information recorded:

- The reason(s) for ineligibility for study participation OR
- The reason declined (if the patient was eligible for study participation).

The screening data (baseline demographic data, as outlined above) was obtained via a Case Report Form (CRFs) and collated via a secure database.

6.3.7 **Randomisation**

Patients were randomised centrally using a secure automated system provided by the Leeds Clinical Trials Research Unit (CTRU). The CTRU were telephoned on the morning of surgery to provide treatment allocation and the surgeon informed regarding the chosen procedure pre-operatively.

The following information was required at randomisation:

- Participant details, including initials, gender and date of birth
- Site code for research site
- Name of person making the randomisation

- Name of treating surgeon
- Confirmation of eligibility
- Confirmation and date of written informed consent
- Stratification factors (see below)

Participants were randomised on a 1:1 basis to have either the Pringle manoeuvre (***'Pringle'*** *group*) or portal vein clamping (***'PVC only'*** *group*) used as the method of clamping during their operation. Each participant was allocated a unique study number. The randomisation was stratified using stratified random permuted blocks to ensure that treatment groups are well balanced for the following stratification factors, details of which will be required at randomisation:

- Body Mass Index (BMI)
    - Less than 30
    - Greater than or equal to 30
- Previous chemotherapy exposure within the last 3 months

6.3.8 **Surgical Procedure**

At the time of surgery, the standard procedure for operative dissection planned for that patient was undertaken. Surgery was performed through a reverse L shaped incision. During surgery, a

careful search of the abdominal cavity was performed to determine the extent of the local disease, extra hepatic metastasis and seeding within the peritoneal cavity. After mobilisation of the liver, intra operative ultrasound scan was performed to assess the number, size of the lesions and the relation of the tumour to major blood vessels. When ready to proceed with liver resection the surgeon achieved portal inflow control using the method allocated by the randomisation process.

Details of intervention by group

Intervention Group:

In the main portal vein occlusion group, the portal vein was isolated and occluded with a clamp.

Control Group: In the Pringle manoeuvre group, the entire hepato-duodenal ligament containing both hepatic artery and portal vein, was occluded using standard vascular clamp.

In both the groups, intermittent vascular occlusion was performed with cycles of 15 minute inflow occlusion followed by 5 minutes of reperfusion.

6.3.9 Postoperative Care:

The postoperative management will be identical in both the groups and will involve Intensive Care Unit (ITU) or High Dependency Unit (HDU) stay for the first 24-48 hours, or longer if required. Stable patients will be transferred to the ward with routine care involving daily ward rounds and monitoring with bloods. Any untoward complication will be managed appropriately by the clinical team managing the patient and the details will be recorded in the clinical notes. The Clavien-Dindo score was used to grade adverse events occurring post-operatively and is the standard classification system used for all surgical specialities.

6.4 Results

6.4.1 Patient Characteristics

The sample included 80 patients (20 in the learning phase and 60 in the trial phase, see Figure 10). Patients were stratified based on their BMI and whether they had received pre-operative chemotherapy within the last 3 months or not (see Table 7 and 8).

In the learning phase (see Table 9) there were 9 male patients and 11 females. The mean age was 58.4 years (standard deviation [SD]= 10.4) ranging from 27 years of age to 74 years. The mean BMI was 28.2 (SD=7.6), ranging from 17.8 to 51.7. Patient co-morbidities can be seen in Table 9. Nine patients underwent neoadjuvant chemotherapy.

Seven patients underwent major resection, whilst 12 had minor resections in the learning phase group. Unfortunately, one patient was not resectable due to advanced disease. Six patients had an anatomical resection, whereas 11 had a non-anatomical resection (see Table 11). Two patients had both an anatomical and a non-anatomical resection.

There were no eligibility violations or withdrawals in the learning phase group.

Table 7: Recruitment Stratification Factors for Learning Phase

	PVC (n=15)	No Inflow Occlusion (n=5)	Total (n=20)
BMI			
< 30	11 (55%)	3 (15%)	14 (70%)
>=30	4 (20%)	2 (10%)	6 (30%)
Previous chemotherapy in last 3 months			
Yes	4 (20%)	2 (10%)	6 (30%)
No	11 (55%)	3 (15%)	14 (70%)

Table 8: Recruitment Stratification for Trial Phase

	PVC (n=30)	Pringle (n=30)	Total (n=60)
BMI			
< 30	19 (63%)	17 (57%)	36 (60%)
>=30	11 (27%)	13 (43%)	24 (40%)
Previous chemotherapy in last 3 months			
Yes	4 (13%)	5 (17%)	9 (15%)
No	26 (87%)	25 (83%)	51 (85%)

Figure 10: A Consort Flow Diagram of patient participation in trial

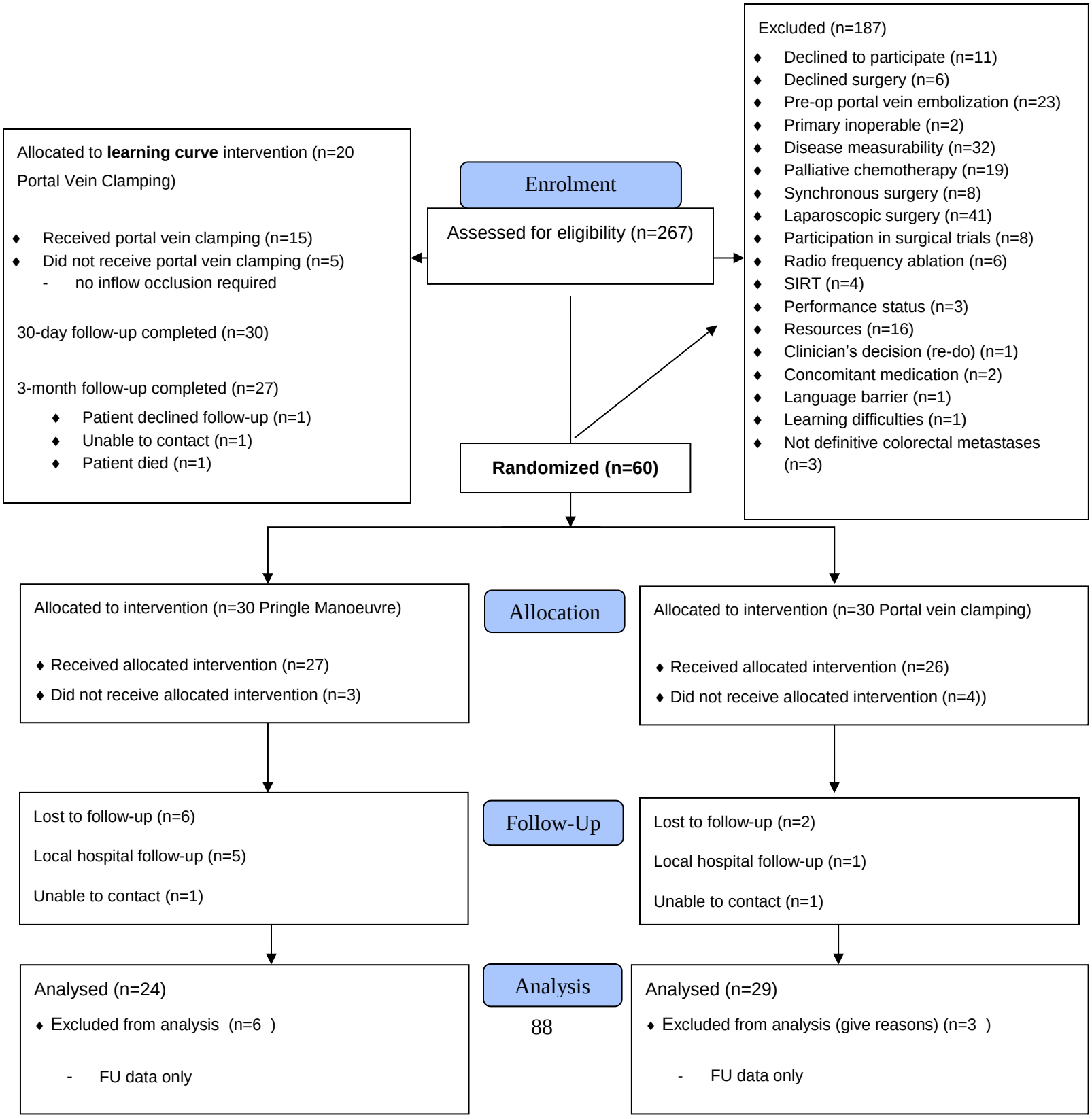


Table 9: Patient Demographics for Learning Phase

	PVC (n=15)	No Inflow Occlusion (n=5)	Total (n=20)
Age at referral			
Mean (SD)	57.5 (11.6)	61.0 (5.4)	58.4 (10.4)
Median (IQR)	61.0 (52,65)	60.0 (56.5, 66)	61.0 (54.3, 64.8)
Range	(27,74)	(55,69)	(27,74)
Gender			
Male	8 (40%)	1 (5%)	9 (45%)
Female	7 (35 %)	4 (20%)	11 (55%)
BMI (kg/m^2)			
Mean (SD)	28.7 (8.4)	26.7 (4.4)	28.2 (7.6)
Median (IQR)	26.2 (23.9, 33.3)	29.1 (21.9, 30.3)	26.6 (22.7, 30.4)
Range	(17.8,51.7)	(17.8, 51.7)	(17.8, 51.7)
Co-morbidities (presence)			
Smoker	3 (15%)	0 (0%)	3 (15%)
Cardiovascular Disease	2 (10%)	0 (0%)	2 (10%)
PVD	2 (10%)	0 (0%)	2 (10%)
Respiratory Disease	3 (15%)	0 (0%)	3 (15%)
Diabetes	1 (5%)	0 (0%)	1 (5%)
Hypertension	2 (10%)	0 (0%)	2 (10%)
Chemotherapy	7 (35%)	2 (10%)	9 (45%)
Pathological classification of primary tumour (pT)			
N missing	1	1	2
pT1	0 (0%)	0 (0%)	0 (0%)
pT2	1 (5%)	0 (0%)	1 (5%)
pT3	8 (40%)	4 (20%)	12 (60%)
pT4	5 (25%)	0 (0%)	5 (25%)
Pathological classification of primary tumour (pN)			
N missing	2	1	3
pN0	2 (10%)	0 (0%)	2 (10%)
pN1	7 (35%)	2 (10%)	9 (45%)
pN2	4 (20%)	2 (10%)	6 (30%)

In the main trial group there were 31 male patients and 29 female patients (see Table 10). The mean age was 62.3 years (SD= 12.5 years), ranging from 31 to 82 years of age. The mean BMI was 28.3 (SD=5.4), ranging from 20.0 to 42.2. Eleven out of 30 patients underwent some form of neoadjuvant chemotherapy.

Thirty one patients underwent a major resection, whilst 27 had a minor resection. Two patients were not resectable. Twenty-nine patients had an anatomical resection, whereas 22 had a non-anatomical resection. Seven patients had a combination of anatomical and non-anatomical resections (see Table 12).

There were no eligibility violations or withdrawals in the trial phase group either.

Table 10: Patient Demographics for Trial Phase

	PVC (n=30)	Pringle (n=30)	Total (n=60)
Age at referral			
N missing	0	0	0
Mean (SD)	61.4 (13.5)	63.1 (11.6)	62.3 (12.5)
Median (IQR)	63.0 (53,72)	65.0 (55,75)	64.0 (53.5, 73.0)
Range	(31,82)	(37,81)	(31,82)
Gender			
Male	18 (60%)	13 (43%)	31 (52%)
Female	12 (40%)	17 (57%)	29 (48%)
BMI (kg/m^2)			
N missing	0	0	0
Mean (SD)	28.4 (4.8)	28.2 (6.1)	28.3 (5.4)
Median (IQR)	28.7 (25.0,30.7)	26.6 (24.0, 32.0)	28.0 (24.3,31.2)
Range	(20.0-38.2)	(20.0,42.2)	(20.0,42.2)
Co-morbidities (presence)			
Smoker	2 (7%)	4 (13%)	6 (10%)
Cardiovascular Disease	12 (40%)	17 (57%)	28 (47%)
PVD	4 (13%)	2 (7%)	6 (10%)
Respiratory Disease	7 (23%)	9 (33%)	16 (27%)
Diabetes	5 (17%)	5 (17%)	10 (16%)
Hypertension	11 (37%)	9 (30%)	20 (33%)
Chemotherapy	6 (20%)	5 (17%)	11 (18%)
Pathological classification of primary tumour (pT)			
N missing	3	1	4
pT1	3 (10%)	0 (0%)	3 (5%)
pT2	1 (3%)	2 (7%)	3 (5%)
pT3	16 (53%)	15 (50%)	31 (52%)
pT4	7 (23%)	12 (40%)	19 (32%)
Pathological classification of primary tumour (pN)			
N missing	4	1	5 (8%)
pN0	9 (30%)	7 (23%)	16 (27%)
pN1	7 (23%)	10 (33%)	17 (28%)
pN2	10 (33%)	12 (40%)	22 (37%)

6.4.2 **Procedural Outcomes**

In the learning phase group, five patients were deemed to be not suitable for any form of inflow occlusion. Generally the operating time was shorter for those patients requiring no occlusion (mean 145.5 minutes (SD=37.5) *versus* 84.0 (SD=54.5), though blood loss slightly greater (mean 790 ml (SD=1238.1) *versus* mean 410 (SD=224.6). The mean total clamping time in the PVC only group was 29.4 minutes (SD=10.7). The mean total time transecting the liver was 53.0 minutes (SD= 21.9 minutes) in the PVC group and 47.5 minutes (SD=31.8 minutes) in the no clamping group. The mean total operating time was 145.5 minutes (SD=37.5 minutes) versus 84.0 minutes (SD=54.5 minutes) in the no clamping group (see Table 11). No patients in the learning phase required a blood transfusion.

Table 11: Learning Phase Procedural Outcomes

	PVC (n=15)	No Inflow Occlusion (n=5)	Total (n=20)
Total operative time (mins)			
Mean (SD)	145.5 (37.5)	84.0 (54.5)	133.9 (46.2)
Median (IQR)	142.0 (115,176)	83.0 (56.5,111.0)	137.5(92.5, 165.8)
Range	(88, 198)	(30,139)	(30,198)
Total clamping time (mins)			
Mean (SD)	29.4 (10.7)	NA	29.4 (10.7)
Median (IQR)	30.0 (25,35)	NA	30.0 (25,35)
Range	(10.0,48.0)	NA	(10.0,48.0)
Total time transecting the liver (mins)			
Mean (SD)	53.0 (21.9)	47.5 (31.8)	52.3 (22.1)
Median (IQR)	46.0 (35.5,60.5)	47.5 (25.0,70.0)	46.0(35.0,61.0)
Range	(29.0,98.0)	(25.0, 70.0)	(25.0,98.0)
Estimated blood loss (ml)			
Mean (SD)	410 (224.6)	790 (1238.1)	505 (623.2)
Median (IQR)	500(225,550)	300 (-,-)	400 (212.5, 500.0)
Range	(50,800)	(100,NA)	(50,800)
Number of transfusion units			
0	15 (100%)	5 (100%)	20 (100%)
ASA grade			
1	2 (13%)	2 (40%)	4 (20%)
2	11 (73%)	1 (20%)	12 (60%)
3	1 (7%)	1 (20%)	2 (10%)
Number of metastases			
N missing	9	2	11
0	0 (0%)	0 (0%)	0 (0%)
1	3 (20%)	2 (40%)	5 (25%)
2	1 (7%)	0 (0%)	1 (5%)
3	1 (7%)	1 (20%)	2 (10%)
6	1 (7%)	0 (0%)	1 (5%)
…			
Size of largest metastases (cm)			
N	1	1	2
N missing	14	4	18
Mean (SD)	7.4 (-)	0.5 (-)	4.0 (4.9)
Median (IQR)	7.4 (-)	0.5 (-)	4.0 (0.5,7.4)
Range	(7.4)	(0.5)	(0.5,7.4)
Was intra-operative ultrasound performed?			
N missing	0	0	0
Yes	7 (47%)	2 (40%)	9 (45%)
No	8 (53%)	3 (60%)	11 (55%)
Was the resection:			
Major	7 (47%)	0 (0%)	7 (35%)
Minor	8 (53%)	4 (80%)	12 (60%)
Not applicable	0 (0%)	1 (20%)	1 (5%)
Anatomical	6(40%)	0 (0%)	6 (30%)
Non-anatomical	7(47%)	4 (20%)	11 (55%)
Both	2(13%)	0 (0%)	2 (10%)
Not applicable	0 (0%)	1 (20%)	1 (5%)
N/A	0 (0%)	1 (5%)	1 (5%)

In the trial phase group (by treatment received), twenty-six patients underwent PVC only, 31 patients underwent the Pringle manoeuvre for inflow control whilst three patients were deemed to be not suitable for any form of inflow occlusion by the operating surgeon. Generally the operating time was shorter for those patients requiring no occlusion (mean 101.5 minutes [SD=82.7] *versus* 132.8 [SD=34.3] for the Pringle group and 128.4 [SD=51.9] for the PVC only group) . The intra-operative blood loss was slightly greater in the PVC only group (mean 436.5 ml (SD=289.7) *versus* mean 311.6 (SD=199.6). The mean total clamping time in the PVC only group was 36.3 minutes (SD=14.0) compared with a mean total clamping time of 34.9 minutes (SD=17.4) in the Pringle group. The mean total time transecting the liver was 53.6 minutes (SD= 34.6 minutes) in the PVC group *versus* 49.6 minutes (SD=24.6) in the Pringle group. The mean total operating time in the PVC only group was 128.4 minutes (SD=51.9 minutes) versus 132.8 minutes (SD=34.3 minutes) and 101.5 minutes (SD=82.7) in the no clamping group (see table 12).

No patients in the trial phase required an intra-operative blood transfusion.

Table 12: Procedural Characteristics Trial Phase

	PVC (n=26)	Pringle (n=31)	No Inflow Occlusion (n=3)	Total (n=60)
Total operative time (mins)				
N	25	29	2	56
N missing	1	2	1	4
Mean (SD)	128.4 (51.9)	132.8 (34.3)	101.5 (82.7)	129.5 (44.0)
Median (IQR)	120.0 (94.0, 149.0)	125.0 (107.0, 159.0)	101.5 (43.0, 160.0)	123.5 (96.5, 156.5)
Range	(66,268)	(76,194)	(43, 160)	(43,268)
Total clamping time (mins)				
N	26	31	NA	57
Mean (SD)	36.3 (14.0)	34.9 (17.4)	NA	35.5 (15.8)
Median (IQR)	34.5 (29.5, 42.5)	32.0 (25.0, 43.0)	NA	33.0 (28.0, 44.0)
Range	(8.0, 73.0)	(11.0, 97.0)	NA	(8.0,89.0)
Total time transecting the liver (mins)				
N	25	30	0	55
N missing	1	1	3	5
Mean (SD)	53.6 (34.6)	49.6 (24.6)	-	51.4 (29.4)
Median (IQR)	49.0 (33.5, 64.0)	45.5 (32.8, 71.2)	-	47.0 (34.0, 65.5)
Range	(7.0, 182.0)	(11.0, 103.0)	-	(7.0, 182.0)
Estimated blood loss (ml)				
N	26	31	1	58
N missing	0	0	2	2
Mean (SD)	436.5 (289.7)	311.6 (199.6)	-	363.1 (251.8)
Median (IQR)	400.0 (200.0, 500.0)	300.0 (180.0, 400.0)	-	300.0 (200.0, 500.0)
Range	(50.0, 1500.0)	(30.0, 800.0)	-	(30.0, 1500.0)
…				
ASA grade				
1	8 (31%)	10 (32%)	1 (33%)	19 (32%)
2	17 (65%)	20 (65%)	2 (67%)	39 (65%)
3	0 (0%)	1 (3%)	0 (0%)	1 (2%)
Number of metastases				
N missing	6	12	1	19
0	0 (0%)	1 (3%)	0 (0%)	1 (2%)
1	8 (31%)	5 (16%)	1 (33%)	14 (23%)
2	3 (12%)	5 (16%)	1 (33%)	9 (15%)
3	4 (15%)	2 (65%)	0 (0%)	6 (10%)
4	2 (77%)	3 (10%)	0 (0%)	5 (8%)
5	1 (4%)	1 (3%)	0 (0%)	2 (3%)
6	1 (4%)	1 (3%)	0 (0%)	2 (3%)
7	1 (4%)	0 (0%)	0 (0%)	1 (2%)
11	0 (0%)	1 (3%)	0 (0%)	1 (2%)
Size of largest metastases (cm)				
N	5	5	0	10
N missing	21	26	3	50
Mean (SD)	2.6 (1.5)	3.7 (2.6)	-	3.2 (2.1)
Median (IQR)	2.5 (1.5,2.6)	3.4 (2.5, 4.8)	-	2.6 (1.5,4.8)
Range	(1,5)	(1,8)	-	(1,8)
Was the resection:				
Major	14 (54%)	17 (55%)	0 (0%)	31 (52%)
Minor	12 (46%)	14 (45%)	1 (33%)	27 (45%)
Not applicable	0 (0%)	0 (0%)	2 (67%)	2 (3%)
Anatomical	13 (50%)	16 (52%)	0 (0%)	29 (48%)

Non-anatomical	9 (35%)	12 (39%)	1 (33%)	22 (37%)
Both	4 (15%)	3 (10%)	0 (0%)	7 (12%)
Not applicable	0 (0%)	0 (0%)	2 (67%)	2 (3%)

6.4.3 **Post-operative Outcomes- Liver Function Tests**

In the learning phase the mean peak bilirubin for patients who underwent PVC clamping was 21.2 µmol/L (SD=12.5) versus 17.2 µmol/L (SD=9.9) in the no clamping group (see supplement 8.8). There was a trend towards a higher mean peak ALT in the PVC group (312.5 IU/L [SD=138.0] versus 207.6 IU/L [SD=194.0] in the no clamping group, see Table 13).

Table 13: Peak Post-operative Bilirubin and ALT: Learning Phase

	PVC (n=15)	No Inflow Occlusion (n=5)	Total (n=20)
Post surgery liver function test A: mean (SD)			
N missing	1	0	1
Peak Bilirubin	21.2 (12.5)	17.2 (9.9)	20.2 (11.7)
Post surgery liver function test B: mean (SD)			
N missing	1	0	1
Peak ALT	312.5 (138.0)	207.6 (194.0)	284.9 (156.2)

In the trial phase (treatment received), the mean peak bilirubin for patients who underwent PVC clamping was 28.8 µmol/L (SD=20.5) versus 26.7 µmol/L (SD=21.2) for those who underwent Pringle and 14.3 µmol/L (SD=6.7) for those who had no clamping. The mean peak ALT in the PVC only group was 327.0 IU/L (SD=201.2) compared to 336.3 IU/L (SD=426.0) in the Pringle group and 591.7 IU/L (SD=825.2) in the no clamping group (see table 14).

Table 14: Peak Post-operative Bilirubin and ALT: Trial Phase

	PVC (n=26)	Pringle (n=31)	No Inflow Occlusion (n=3)	Total (n=60)
Post surgery Peak Bilirubin: mean (SD)				
N Missing	2	0	0	2
Peak Bilirubin	28.8 (20.5)	26.7 (21.2)	14.3 (6.7)	27.0 (20.4)
Post surgery Peak ALT: mean (SD)				
N Missing	2	0	0	2
Peak ALT	327.0 (201.2)	336.3 (426.0)	591.7 (825.2)	345.6 (373.0)

6.4.4 **Post operative Follow Up**

In the learning group all the 30 day follow ups were completed during outpatient clinic appointments. The mean time to be seen was 32.4 days (SD=6.7) with a range from 23 to 45 days. At 3 months 12 patients were followed up in the outpatient clinic and five by telephone. Three patients were lost to follow up.

In the trial phase, 51 patients were seen in clinic for the 30 day follow ups. Nine patients were lost to follow up. Thirty nine patients were seen in clinic and 21 were followed up by telephone

6.4.5 **Post operative Complications and Blood Transfusion Requirements**

In the learning phase five patients in the PVC only group suffered minor complications. Four patients had wound infections, whilst one patient had pneumonia (all Clavien Dindo II). No patients required any subsequent surgery and no patients required a post-operative blood transfusion.

In the trial phase (by treatment received), six patients had one complication in the PVC only group compared to four patients who had one complication and three that had two complications in the Pringle group (see Table 11)

One patient in the Pringle group had to return to theatre due to wound dehiscence and one patient also required a transfusion between surgery and 30 days post-operatively.

Table 15: Complications in Trial Phase

Number of complications	**PVC (n=26)**	**Pringle (n=31)**	**No Inflow Occlusion (n=3)**	**Total (n=60)**
0	20 (77%)	24 (77%)	2 (67%)	46 (76%)
1	6 (23%)	4 (13%)	0 (0%)	10 (17%)
2	0 (0%)	3 (10%)	1 (33%)	4 (7%)
…				
Type of complication (not mutually exclusive)				
Wound infection	1 (4%)	4 (13%)	0 (0%)	5 (8%)
Intra-abdominal infection	1 (4%)	1 (3%)	1 (33%)	3 (5%)
Need for re-operation (open surgery within one month)	0 (0%)	1 (3%)	0 (0%)	1 (2%)
Urinary infection	1 (4%)	1 (3%)	0 (0%)	2 (3%)
Pulmonary embolism	0 (0%)	0 (0%)	1 (33%)	1 (2%)
Viral Infection	1 (4%)	0 (0%)	0 (0%)	1 (2%)
Increased pain post op	0 (0%)	1 (3%)	0 (0%)	1 (2%)
Pneumonia	1 (4%)	1 (3%)	0 (0%)	2 (3%)
Ileus	1 (4%)	1 (3%)	0 (0%)	0 (0%)
Number of septic complications				
0	21 (81%)	26 (84%)	2 (67%)	49 (82%)
1	5 (19%)	3 (10%)	1 (33%)	9 (15%)
2	0 (0%)	2 (6%)	0 (0%)	2 (3%)
…				
…				
Clavien-Dindo classification of complications				
II	5	8	3	16
IIIa	1	1	0	2
IIIb	0	1	0	1
IVa	0	0	0	0

6.4.6 **Safety Outcomes**

There was one post-operative death in the learning phase group immediately prior to the three month Follow Up period. This was not related to the surgery. There were no other serious complications in either the learning phase or trial phase groups.

6.5 Discussion

Peri-operative blood transfusions may lead to poorer short and long-term outcomes. In order to mitigate intra-operative blood loss the Pringle manoeuvre is often used during partial hepatectomy. However, the Pringle manoeuvre has some adverse effects as well such as ischaemia-reperfusion injury and splanchnic congestion.

A systematic review of 30 studies on liver resection for CRLM using Pringle manoeuvre conducted in 2006, showed septic complications to be as high as 19% (Simmonds, Primrose et al. 2006). The Leeds group have used selective intermittent Pringle manoeuvre (clamping for 15 minutes and releasing for 5 minutes) and demonstrated a septic complication rate of 12%. It has also previously been demonstrated that a post-operative infective complication is an independent predictor of recurrence of cancer in the long term (Farid, Aldouri et al. 2010). Therefore, by reducing liver tissue injury, isolated portal vein clamping has the potential to reduce the post-

operative septic complications thereby improving both short and long-term outcomes. A recent RCT compared these two techniques along with hemi-hepatic vascular occlusion and found that selective inflow control resulted in faster recovery of liver functions (Fu, Lau et al. 2011). Therefore, the primary aim of our pilot study was to assess the feasibility of performing a larger multi-centre trial comparing the Pringle Manoeuvre and isolated portal vein clamping.

Our main findings were that it was technically feasible to perform isolated portal vein clamping in patients. Furthermore, it was possible to recruit patients into the trial and perform short term follow-up on them.. The operating times between the two groups were similar though there was a trend towards more blood loss in the PVC only group. There were no peri-operative transfusion requirements, although one patient in the Pringle group did require a blood transfusion post-operatively.

The limitations of the study included some design faults which could be incorporated when designing the main trial. Post-operative inflammatory markers (such as White Cell Count and CRP) were not measured which may have been useful. Furthermore, pre-operative selection of patients regarding the need for intra-operative inflow occlusion could be more rigorous as almost 10% of patients in the trial underwent no inflow occlusion at all. It may be prudent to only include patients requiring a major hepatectomy in the main trial and/or to randomise once the initial laparotomy and intra-operative ultrasound have been performed

Future work will include completing the quality of life outcomes.

The objectives of the trial have been met; namely to recruit and randomise, to perform PVC clamping and to collect peri-operative data. However, a UK wide consensus would be useful prior to proceeding to a multi-centre trial. Some UK centres do not even routinely use any form of inflow occlusion and the method of determining the benefit of PVC only clamping has not been firmly established. Post-operative liver function tests have been used previously, however it is not known how these affect long term survival and hence their relevance here.

7 Conclusions

The research as outlined in this thesis was aimed towards understanding the role of surgical and non-surgical techniques to decrease peri-operative blood loss in liver surgery and therefore improve patient related outcomes.

The systematic review of non-surgical techniques identified that certain anaesthetic agents (sevoflurane and desflurane) reduced the degree of IRI. Furthermore, pharmacological agents such as tranexamic acid were proven to decrease intra-operative blood loss though there was a paucity of randomised controlled trials in these areas.

The retrospective study demonstrated that patients who underwent a blood transfusion had a poorer long term outcome. However, the poorer OS seen in patients who underwent RBCT was likely to be multifactorial and due to peri-operative complications, rather than due to earlier disease recurrence per se. Nonetheless, factors such as pre-operative anaemia, larger burden of disease and pre-operative chemotherapy predisposed to a greater likelihood of transfusion.

The main findings of the RCT were that it was technically feasible to perform isolated portal vein clamping in patients and to recruit patients into the trial. However, a larger RCT will be needed to obtain definitive evidence on the role of PVC in hepatic resections in the future.

It was not possible to identify specific strategies to decrease the degree of IRI. Although certain anaesthetic agents were seen to decrease the IRI further studies are needed in this area. The role of isolated portal vein clamping in decreasing IRI also remains uncertain. Part of the difficulty in driving this forward is that there is no universally agreed biochemical definition (liver function

tests and inflammatory markers) of ischaemia reperfusion injury and it is not feasible to take liver biopsies in a post-operative patient.

Due to logistical difficulties, the Quality of Life data is still incomplete but should be finished within the next 3 months. Specifically, the change in patient quality of life (QOL) outcomes at 3 months post-surgery will be examined as below:

1. The aim of the health economic component (via analysis of the three quality of life questionnaires - EORTC QLQC30, QLQ-LM21 and EQ-5D-3L) was to collect data for a full economic evaluation. Specifically,

a) To test the acceptability and feasibility of health care resource use questionnaires developed in accordance with the existing literature on liver surgery and with the team of clinical experts who will help identify the participants' pathway of care.
b) To assess whether the health-related quality of life questionnaire, EQ-5D-3L, used for obtaining utility weights for constructing quality-adjusted life years (QALYs), is acceptable based on completion rates.

Further research should involve a consensus study to ascertain what constitutes a liver ischaemia-reperfusion injury post-operatively. The systematic review and RCT demonstrated that many investigators use post-operative ALT to measure IRI, without any evidence to suggest its utilisation as a marker for IRI.

A multi-centre RCT looking at isolated portal vein clamping versus Pringle will also help place portal vein clamping in the management algorithm for patients undergoing CRLM surgery, although it may be prudent to get consensus from UK HPB surgeons as to whether they feel this is a research priority. Further trials should also look at the role of tranexamic acid in cases where blood loss risk is high.

Future research areas should include:

- Using a database to retrospectively evaluate the short- and long term effects of peak post-operative ALT. This could be done prospectively as well, via collaboration between HPV units
- An RCT between volatile (desflurane/isoflurane) and non-volatile inhalational anaesthetic agents in patients undergoing liver surgery to ascertain if volatile anaesthetic agents are protective for the liver
- A further RCT in patients at high risk of blood loss utilising tranexamic acid. A consensus will need to be reached as to what defines "high risk of blood loss during liver resection" but it is likely to include patients undergoing major resections, patients who have had recent chemotherapy and those with increased BMI.
- A consensus between UK liver surgeons needs to be reached regarding whether further trials around isolated portal vein clamping are warranted, prior to proceeding to a multi-centre RCT. Many surgeons do not routinely employ any form of inflow control during resections. It is therefore, not currently possible due to the evidence base and

practicalities to recommend stratification of inflow modulation, on the basis of the pilot RCT and evidence review in this thesis.

In summary, it is likely that a combination of approaches will be required in the future to further improve outcomes. For example, use of PVC for inflow control may be appropriate in a patient with BMI >30 who has recently undergone chemotherapy and who needs an extended resection. It may also be prudent to employ other strategies to decrease blood loss in such a patient. However, a patient who requires only a metastectomy in a peripherally located tumour may not require even inflow control. The type of approach should be individually tailored to the patient.

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9 Appendix

9.1 PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	(in paper)
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	NA
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	21
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	21
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	23

Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	22
	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	22
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	23
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	24
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	23
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	25
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	24
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	N/A
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Table1
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 2/3
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 2

			and 3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	25-27
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	26
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	25-27
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

9.2 Approval Letter

Health Research Authority
NRES Committee Yorkshire & The Humber - Leeds East
North East REC Centre
Room 002
TEDCO Business Centre
Viking Industrial Park
Rolling Mill Road
Jarrow
NE32 3DT
Telephone: 0191 4283545

30 July 2013

Mr K Raj Prasad
Consultant HPB and Liver Transplant Surgeon / Clinical Director
St James's University Hospital NHS Trust
Bexley level 3, Beckett Street
Leeds
LS9 7TF

Dear Mr Prasad

Study title:	**Randomised Controlled Trial of Pringle Manoeuvre versus Portal Vein Clamping in Patients undergoing Liver Resection for Colorectal Liver Metastasis - A Pilot Study**
REC reference:	**13/YH/0195**
IRAS project ID:	**123555**

Thank you for your letter of 18th July, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Hayley Jeffries, hayley.jeffries@nhs.net

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter	1.0	28 May 2013
Covering Letter		18 July 2013
GP/Consultant Information Sheets	1.0	28 May 2013

Investigator CV	Raj Prasad	
Letter of invitation to participant	1.0	28 May 2013
Other: CV - Abdul Hakeem		

Other: CV - Giles Toogood		
Other: CV - Samir Pathak		
Other: CV - Rajesh Dey		
Other: CV - Gillian Ivey		
Other: CV - Sarah Brown		
Other: CV - Mark Glen Vero		
Other: Letter from funder		14 November 2012
Participant Consent Form	2.0	18 July 2013
Participant Information Sheet: Randomisation (Tracked changes)	2.0	18 July 2013
Participant Information Sheet: Learning Curve (Tracked Changes)	2.0	18 July 2013
Protocol	1.0	
REC application	1.0	
Response to Request for Further Information		
Summary/Synopsis	part of protocol	12 May 2013

Statement of compliance
The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review
Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/YH/0195 **Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training

With the Committee's best wishes for the success of this

project. Yours sincerely

pp

Dr C E Chu
Chair

Email:hayley.jeffries@nhs.net.

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: Mr Derek Norfolk, R&D, Leeds

Ms Anne Gowing, Leeds Teaching Hospitals NHS Trus

9.3 Research Protocol

Department of Hepatobiliary and Transplant Surgery
St James's University Hospital NHS Trust

Research Protocol

Version 2.0

Date 18.07.2013

Study Short Title: Pringle Manoeuvre *versus* Portal Vein Clamping for Liver Resection

Study Full Title: Randomised Control Trial of Pringle Manoeuvre *versus* Portal Vein Clamping in patients undergoing Liver Resection for Colorectal Liver Metastasis – A Pilot Study

Sponsor Name: Leeds Teaching Hospitals NHS Trust

Funder Name: National Institute for Health Research: Research for Patient Benefit (RfPB)

Funder Reference: PB-PG-0711-25080

Chief Investigator:

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Signatures Page

Pringle Manoeuvre *versus* Portal Vein Clamping for Liver Resection [1.0, 22.04.2013]

Written and approved by the following:

_____	_____
Mr K Raj Prasad [Chief Investigator]	Date
_____	_____
Mr Giles J Toogood [Co-investigator]	Date

Key contacts

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Abbreviations

Abbreviation	Description
CRLM	Colorectal liver metastasis
HDU	High Dependency Unit
HPB	Hepatopancreatobiliary
ICU	Intensive Care Unit
IRI	Ischaemia Reperfusion Injury
LTHT	Leeds Teaching Hospitals NHS Trust
NIHR	National Institute for Health Research
PPI	Patient Public Involvement
QoL	Quality of Life
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RfPB	Research for Patient Benefit
SJUH	St James's University Hospital NHS Trust
TMG	Trial Management Group
TSC	Trial Steering Committee

Table of Contents

1.0 Protocol Summary

GENERAL INFORMATION	
Short Title	Pringle Manoeuvre *versus* Portal Vein Clamping for Liver Resection
Full Title	Randomised Controlled Trial of Pringle Manoeuvre versus Portal Vein Clamping in patients undergoing Liver Resection for Colorectal Liver Metastasis – A Pilot Study
Sponsor	Leeds Teaching Hospital NHS Trust
Sponsor ID	
EudraCT No.	Not applicable
MREC No.	Not applicable
Chief Investigator	Mr K Raj Prasad
Co-ordinating Centre	Department of Hepatobiliary and Transplant Surgery St. James's University Hospital NHS Trust, Leeds
National / International	National
STUDY INFORMATION	
Phase	Pilot Study
Indication	Colorectal liver metastasis (CRLM).
Design	Prospective, parallel group, single blind, randomised controlled pilot study.
Primary Objectives	The primary objective of the pilot randomised controlled trial (RCT) is to test whether the components of a larger multi- centre trial can work together. Specifically, 1. The ability to recruit and randomise patients into the study 2. The ability to conduct portal vein clamping surgical procedure 3. The appropriateness of the follow-up assessments
Secondary Objectives	To collect information on the following outcome measures to further inform the main trial 1. Incidence of septic complications in portal vein clamping versus Pringle manoeuvre 2. Incidence of ischaemia-reperfusion injury in the two arms 3. To measure the regeneration of the remnant liver in the two arms 4. Procedural outcomes including intra-operative blood loss, length of clamping time, length of time transecting the liver and duration of the operation will be recorded 5. Post-operative events including bleeding, post-operative transfusion requirements, non- infective cardio- respiratory complications and 30- day mortality rate will be recorded 6. Change in patient quality of life (QOL) outcomes at 3 months post surgery 7. A 10x10mm biopsy of normal liver will also be taken

	during surgery to assess for immunohistochemical evidence of ischaemia-reperfusion injury The aim of the health economic component is to test the feasibility of collecting data for a full economic evaluation on the main study. Specifically, 8. To test the acceptability and feasibility of health care resource use questionnaires developed in accordance with the existing literature on liver surgery and with the team clinical experts who will help identify the participants' pathway of care. 9. To assess whether the health-related quality of life questionnaire, EQ-5D, used for obtaining utility weights for constructing quality-adjusted life years (QALYs), is acceptable based on completion rates.
Expected start date	01.06.2013
Subject enrolment phase	16 months
Follow-up duration	3 months
End of Trial Definition	The 3 month clinical visit of the last patient recruited to the trial.
Expected completion date	31.12.2015
TRIAL SUBJECT INFORMATION	
Number of trial subjects	80
Age group of trial subjects	18 and above
Inclusion criteria	1. At least 18 years of age 2. Undergoing liver resection surgery for CRLM 3. Portal venous clamping is considered appropriate 4. Willing to provide informed consent 5. Patients able to complete questionnaires
Exclusion criteria	1. Patients having simultaneous bowel and hepatic surgery 2. Patients participating in other trials that could impact the outcomes measures being recorded 3. Patients who are pregnant 4. Patients taking immunosuppressive drugs
INVESTIGATIONAL MEDICINAL PRODUCT	
IMP name(s)	Not applicable
Duration of IMP Treatment	Not applicable
IMP Supplier(s)	Not applicable
Non IMP name(s)	Not applicable

2.0 Introduction

2.1 Background

The portal vein and the hepatic artery supply 75% and 25% of the liver's blood supply respectively, with each vessel contributing 50% of the liver's oxygen requirement [1]. The Pringle manoeuvre is commonly used to prevent blood loss at the time of liver resection. It involves clamping the vascular pedicle containing the hepatic artery and the portal vein together during liver resection surgery. During clamping a deprivation of oxygenation at the cellular level occurs, which is worsened by the fact that surgery will remove some of the functioning volume of the liver. Research has shown that the true damage occurs when the 'starved' cells are reperfused, resulting in ischaemia reperfusion injury (IRI) [2]. Not clamping the hepatic artery allows a continued supply of energy substrates to the cells, potentially reducing the damage caused by IRI. A recent randomised controlled trial (RCT) compared these two techniques along with a different technique and found that portal vein clamping resulted in faster recovery of liver functions [3]. The proposed pilot study will overcome some of the methodological weaknesses in the reference study whilst piloting the approach in the UK.

2.2 Rationale for the Proposed Study

Colorectal cancer is the third most common cancer in the UK with around 110 new cases diagnosed each day [4]. More than half of these patients present with cancer spread to the liver and those with early tumours can potentially be cured by resecting these deposits. Unlike most organs, which have a single blood supply, the liver receives blood from two sources, namely portal vein and hepatic artery supplying 75% and 25% of its blood respectively. Both contribute 50% of the liver's oxygen requirement, with the hepatic artery supplying predominantly oxygen rich blood and portal vein circulating deoxygenated blood from the gut to be cleansed by the liver.

During liver resection surgery, it is standard practice to clamp both these vessels ('Pringle manoeuvre'), so as to avoid bleeding. Though the Pringle manoeuvre is an established technique, it is not without risk. Clamping both the blood vessels leads to ischaemic damage to the liver, which is worsened further by reperfusion of toxic substances when the clamp is released (ischaemia reperfusion injury). This leads to dead non-functioning cells, which can become infestated by microorganisms leading to septic complications.

A systematic review of 30 studies on liver resection for CRLM using Pringle manoeuvre conducted in 2006, showed septic complications to be as high as 19% [5]. Our group have used selective intermittent Pringle manoeuvre (clamping for 15 minutes and releasing for 5 minutes) showing a septic complication rate of 12%. We have also demonstrated that a post-operative infective complication is an independent predictor of recurrence of cancer in the long term [6]. Therefore, by reducing liver tissue injury, isolated portal vein clamping has the potential to reduce the post-operative septic complications thereby improving both short and long-term outcomes. A recent RCT compared these two techniques along with a different technique and found that isolated portal vein clamping resulted in faster recovery of liver functions [3]. Further research is now required to obtain definitive evidence on the benefits of portal vein clamping technique in other clinical outcomes.

3.0 Study Objectives

Primary objectives

Pilot Study

The primary objective of the pilot RCT is to test whether the components of a larger multi-centre trial can work together.

Specifically,

1. The ability to recruit and randomise patients into the study
2. The ability to conduct portal vein clamping surgical procedure
3. The appropriateness of the follow-up assessments

Secondary objectives

To collect information on the following outcome measures to further inform the main trial

3. Incidence of septic complications in portal vein clamping versus Pringle manoeuvre
4. Incidence of ischaemia-reperfusion injury in the two arms
3. To measure the regeneration of the remnant liver in the two arms
4. Procedural outcomes including intra-operative blood loss, length of clamping time, length of time transecting the liver and duration of the operation will be recorded
5. Post-operative events including bleeding, post- operative transfusion requirements, non-infective cardio- respiratory complications and 30- day mortality rate will be recorded
6. Change in patient quality of life (QOL) outcomes at 3 months post surgery
7. A 10x10mm biopsy of normal liver will also be taken during surgery to assess for immunohistochemical evidence of ischaemia-reperfusion injury

The aim of the health economic component is to test the feasibility of collecting data for a full economic evaluation on the main study.

Specifically,

c) To test the acceptability and feasibility of health care resource use questionnaires developed in accordance with the existing literature on liver surgery and with the team clinical experts who will help identify the participants' pathway of care.

d) To assess whether the health-related quality of life questionnaire, EQ-5D, used for obtaining utility weights for constructing quality-adjusted life years (QALYs), is acceptable based on completion rates.

4.0 Study Design

This is a prospective, parallel group, single blind, randomised controlled pilot study. A total of 80 patients will be recruited. The first 20 patients are part of a learning curve development for portal vein clamping

Setting: The project will be carried out at St James's University Hospital NHS Trust (SJUH) which is part of The Leeds Teaching Hospitals NHS Trust (LTHT). Patients attending the Hepatobiliary (HPB) clinic at SJUH who meet the inclusion criteria will be invited to take part during the clinic appointment.

4.1 Blinding

Patients will be blinded to the surgical procedure allocated to minimise the bias in patient reported outcome measures. However, blinding of surgeons performing the surgical procedure is not possible. As the outcome measures are objective there is little risk of assessment bias in the study.

4.2 Endpoints

4.2.1 Primary Endpoint

Pilot Study: The pilot study will be considered as successful, if the following domains run as specified in the protocol.

1. Recruitment of patients into the study in line with the planned recruitment target (5 patients per month on average, with a total of 80 over the 16 month recruitment period)
2. Complete data capture. Should data capture be incomplete, we will further analyse reasons for failure to recruit and/or reasons for conversion from isolated portal vein clamping to standard Pringle manoeuvre

4.2.2 Secondary endpoint(s)

Pilot Study: The following secondary outcome measures will be recorded.

Intra-operative outcomes:

The following parameters will be recorded by the operating surgeon, his assistant or the research fellow (if present in theatre) via a case report from (CRF).

1. Intra- operative blood loss (mls): This is the amount of blood lost intra- operatively during the full length of operation. This will be recorded at the end of the procedure from the suction drains.
2. Length of clamping time (mins): The total duration of clamping time will be recorded
3. Length of time spent on "transecting the liver" (mins) (from start of cutting the liver to liver bed haemostasis)
4. Total length of operation (mins): The length of operation from skin incision to skin closure will be recorded. This will provide information about the difficulty of the procedure and will include the time taken for the initial clamp application.

Immediate postoperative outcomes (within 30 days):
The following parameters will be recorded by the research fellow at 30 days post operative intervention, via a CRF.

5. Postoperative bleeding: This outcome indicates if the patient had any postoperative bleeding causing haemodynamic instability, need for transfusion or need for further laparotomy.
6. Transfusion requirements (number of RBC units transfused).

7. Recovery of liver functions: Liver function tests will routinely be performed immediately following surgery (Day 0/in theatre recovery) and then every morning from Day 1 to Day 7.

8. Non infective cardio respiratory complications: Cardio-respiratory complications including atrial fibrillation, myocardial infarction and pulmonary oedema.

9. 30 day overall mortality rate

10. The development of septic complications by 30 days post procedure. These will be graded using the Clavien-Dindo classification as Grade III– V [7]. This data will be recorded via telephone follow up if the patient has been discharged, or via case note review if the patient is still admitted.

Health Economic endpoints:

The following outcome measures will be recorded by the research fellow at the point of discharge, via a CRF.

11. Length of HDU/ICU stay (days): Number of postoperative days in HDU/ICU will be recorded.

12. Length of hospital stay (defined as day of operation to day of discharge)

The following health economic outcome measures will be recorded by the research fellow at the 3 month out-patient appointment, via a CRF

Secondary, primary, community and social care services that participants may have used after discharge

Delayed Postoperative outcomes (3 months):

The following parameters will be recorded by the research fellow at the 3 month out-patient appointment, via a CRF

13. Liver regeneration: The growth of remnant liver will be assessed by performing Computerised Tomography (CT) scan pre-operatively and at 3 months post surgery. This is routine for any patient undergoing CRLM resection and will enable quantification of the extent of liver regeneration following cancer resection. This will be measured using 3-Dimensional volumetry tools on the pre and post resection CT images [8].

The following parameters will be collected by the Research fellow

14. Quality of life assessment in cancer trials provides a more accurate evaluation of the wellbeing of the patients and of the benefits and side effects that may result from the surgery. The patient's quality of life will be assessed using three standard questionnaires. These questionnaires will be completed both pre- operatively and at 3 months post surgery.

A. European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core30 (EORTC QLQC30,version 3.0): This is a 30point questionnaire and has five functional scales assessing physical, social, emotional, cognitive and overall daily activities. This questionnaire also takes account of the financial burden of cancer accrued to the patients [9].

B. Liver Metastasis Colorectal (QLQLMC21): This is a 21point questionnaire, which is specific for colorectal liver metastasis patients. This questionnaire assesses patients symptoms pertaining to the cancer and the influence of the disease on their day to day activities [10].

C. EuroQol5Dimensional Questionnaire (EQ5D): This questionnaire looks into general health outcome. It questions on 5 dimensions namely: mobility, self care, usual activities,

pain/discomfort and anxiety/depression. The questionnaire is supplemented by a visual analogue scale (VAS) for recording a respondent's current self assessed health on a scale from 0 (worst imaginable health state) to 100 (best imaginable health state) [11].

5.0 Patient Selection

5.1 Eligibility Criteria

Patients will be assessed for eligibility at the surgical out-patient clinic, in the Bexley Wing, of St James's University Hospital.

5.1 Inclusion Criteria

(a) At least 18 years of age
(b) Undergoing liver resection surgery for CRLM
(c) Portal venous clamping is considered appropriate
(d) Participating patients should understand the study objectives and be able and willing to provide written informed consent
(e) Able to complete study questionnaire

5.1.2 Exclusion Criteria

(a) Patients having simultaneous bowel and hepatic surgery
(b) Patients participating in other trials that could impact the outcomes measures being recorded
(c) Patients who are pregnant
(d) Patients taking immunosuppressive medication

Eligibility waivers to inclusion/exclusion criteria are not permitted.

5.2 Recruitment, Consent and Randomisation Processes

The research site will be required to have obtained local ethical and management approvals prior to the start of recruitment onto the study. The recruitment target requires 80 participants over a 16 month period.

5.2.1 Recruitment

Patients who have been diagnosed with resectable colorectal liver metastases and who fit the inclusion/exclusion criteria will be deemed eligible for this study by the Consultant Liver surgeon in charge of their care. To confirm eligibility, radiology images will be reviewed by the consultant surgeon in the clinic to ensure that there are no contraindications to portal vein clamping.

Patients who meet the eligibility criteria will be invited to take part by the consultant surgeon during the clinic appointment. Patients will be given information about the study, which will involve both verbal information and Patient Information Leaflets (PIL). The PIL will include detailed information about the rationale, design and personal implications of the study. After receiving PIL, patients will have at least 24 hours to consider participation. The patient will be given the opportunity to discuss the study with their family and healthcare professionals

before they are asked whether they would be willing to take part in the study. This process will be clearly documented into the patient's medical notes.

Three Quality of Life (QoL) questionnaires will be posted to the patients and information will be provided how to fill them. Patients will be asked to fill the questionnaires within the last week before the operation and handover to the surgical team on the day of procedure. Patients will receive a phone call in the week prior to surgery to remind them to complete the questionnaires. Those who are undecided about participating in the study will be asked to fill the questionnaires on the morning of the procedure.

Participating research staff will be required to complete a log of all patients screened for eligibility. Anonymous information will be collected including:

- Age
- Gender
- Ethnicity

Screened patients who are not randomised either because they are ineligible or because they decline participation will also have the following information recorded:

- The reason(s) for ineligibility for study participation OR
- The reason declined (if the patient was eligible for study participation).

The screening data (baseline demographic data, as outlined above) will be obtained and collated via a secure database.

5.2.2 Consent

Patients will be formally assessed for eligibility and invited to provide informed, written consent. During further clinical appointment or on the morning of surgery, informed written consent will be obtained by either the Consultant Surgeon or the Research Fellow all of whom have undertaken GCP training. Informed, written consent must be obtained prior to being recruited into the study.
The right of the patient to refuse consent without giving reasons will be respected. Further, the patient will remain free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment. A copy of the consent form will be given to the patient, one filed in the Study Master File (the original), one filed in the hospital notes and a fourth copy sent to the Sponsor. The written consent will be taken by a clinician, who has signed / dated the staff authorisation / delegation log. The process of obtaining written consent will be clearly documented in the patient's medical notes.

5.2.3 Randomisation

After the process of informed consent and completion of the baseline questionnaires**,** patients will be randomised centrally using a secure automated system provided by the Leeds Clinical Trials Research Unit (CTRU).

Randomisation will be performed centrally using the CTRU office hours telephone randomisation service (open 9.00 to 17.00 Monday to Friday excluding public/band

holidays, the period between Christmas and New Year, Thursday afternoon before Good Friday and all Tuesdays following a bank holiday except for Mayday and New Year's Day).

The following information will be required at randomisation:

- Participant details, including initials, gender and date of birth
- Site code for research site
- Name of person making the randomisation
- Name of treating surgeon
- Confirmation of eligibility
- Confirmation and date of written informed consent
- Stratification factors (see below)

Participants will be randomised on a 1:1 basis to have either the Pringle manoeuvre or portal vein clamping used as the method of clamping during their operation. Each participant will be allocated a unique study number. The randomisation will be stratified using stratified random permuted blocks to ensure that treatment groups are well balanced for the following stratification factors, details of which will be required at randomisation:

- Body Mass Index (BMI)
    - Less than 30
    - Greater than or equal to 30
- Previous chemotherapy exposure within the last 3 months

5.2.4 Surgical Procedure

Surgical procedure: At the time of surgery, the standard procedure for operative dissection will take place. Surgery will be performed through a reverse L shaped incision. The surgeon will clamp using the allocated method. During surgery, a careful search of the abdominal cavity will be performed to determine the extent of the local disease, extra hepatic metastasis and seeding within the peritoneal cavity. After mobilisation of the liver, intra operative ultrasound scan will be performed to assess the number, size of the lesions and the relation of the tumour to major blood vessels. A wedge liver biopsy (10x10mm) is taken from the unaffected liver (the part of the liver without clamped blood supply) before closure to study the extent of reperfusion injury. This will not be adding additional risk to the patient.

Intervention group: In the main portal vein occlusion group, the portal vein will be isolated and occluded with a clamp.

Control group: In the Pringle manoeuvre group, the entire hepatico-duodenal ligament containing both hepatic artery and portal vein, will be occluded using standard vascular clamp.

In both the groups, intermittent vascular occlusion will be performed with cycles of 15 minute inflow occlusion followed by 5 minutes of reperfusion.

Postoperative care: The postoperative management will be identical in both the groups and will involve Intensive Care Unit (ITU) or High Dependency Unit (HDU) stay for the first 24-48 hours, or longer if required. Stable patients will be transferred to the ward with routine care involving daily ward rounds and monitoring with bloods. Any untoward complication

will be managed appropriately by the clinical team managing the patient and the details will be recorded in the clinical notes.

5.2.5 Patients who Withdraw Consent

If a patient wishes to withdraw, information collected to the point of withdrawal will be included in the analyses. Withdrawal will be documented on the corresponding CRF. The patient will remain free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment. The patient will continue to receive treatment as per standard practice for patients undergoing liver resection for CRLM that is followed in the Liver Unit at SJUH.

5.2.6 Managing / replacing patient who withdraw from the study early

The patients, who withdraw from the study following recruitment or randomization or after the surgical procedure, will continue to receive treatment as per standard practice for patients undergoing liver resection for CRLM that is followed in the Liver unit of SJUH.
The patients will not be replaced in the study and the data from the patient will be used in final analysis on an intention to treat basis. Those who leave the study at any point will have their anonymity maintained and their further treatment/care will not be affected.

5.2.7 Definition for the End of Study

The end of the study is the last clinical visit, corresponding to the third month out-patient visit for the last subject recruited to the study.

6.0 Study Schedule

6.1 Assessments and Procedures

First out-patient appointment:

- Assessment of eligibility for the study by Consultant Surgeon
- Verbal study information and Patient Information Leaflets (PIL) given to eligible patients

Second out-patient appointment or day of surgery:

- Informed consent will be obtained
- Baseline demographic data obtained for eligible patients (sex, date of birth, age, study number, Body mass index (assessed using height and weight), alcohol and smoking history, other comorbidities, primary tumour characteristics, chemotherapy history, medication history, pre-operative bloods and ASA grade)
- QoL questionnaires (EORTC QLQ-C30, EORTC QLQ – LMC21, EQ-5D-3L) will be given to the patients to complete
- Treatment allocation will occur (randomised)
- Surgical procedure carried out as per treatment arm
- Intra-operative parameters will be obtained (Type of incision, adhesions to abdominal wall/liver, surgical procedure received, conversion, reason for conversion,

time of clamping, blood loss, time of operation, time for transection, major or minor resection, anatomical/non-anatomical resection, intra-op blood transfusion, use of platelets/Fresh Frozen Plasma, use of fibrin sealant or pro-thrombotic)

Day 1-7 post-procedure:

- Routine blood tests including full blood count, renal function tests, liver function tests and clotting profile will be measured from day 1 till day 7 post-operatively

Day 30 post-procedure:

- If patient still an in-patient at 30 days post surgery, an assessment of incidence of septic complications will be made
- If the patient has been discharged at 30 days post surgery, a telephone call will be made to assess for incidence of any septic complications
- Data on histology, post-op blood transfusion, ITU and/or HDU stay, use of tranexamic acid, other post-op complications, 30-day mortality

3 months follow-up assessment:

- 3 month volumetric analysis using CT scan
- Recurrence
- Loss to follow-up
- Post-op QOL questionnaires (EORTC QLQ-C30, EORTC QLQ – LMC21, EQ-5D-3L)
- Health care resource use questionnaire

7.0 Safety Reports

For the purpose of this trial the safety reporting term "adverse events" will be referred to as "complications".

7.1 Defining Complications

A complication is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study, and has a causal relationship to the trial. The trial includes the surgical intervention and any trial specific interventions e.g. the consent process and the completion of questionnaires. A complication does include a/an:

a) exacerbation of a pre-existing illness
b) increase in frequency or intensity of a pre-existing episodic event or condition
c) condition detected or diagnosed after the surgical procedure, even though it may have been present prior to the start of the study

A complication does not include a:

a) medical or surgical procedure (eg tooth extraction) but the condition that lead to the procedure may be a complication
b) pre-existing diseases or conditions present or detected at the start of the study that did not worsen
c) situation where an untoward medical occurrence has not occurred (hospitalisation for cosmetic procedure, social and/or convenience admissions)

d) disease or disorder being studies or sign or sympom associated with the disease or disorder unless more severe than expected for the participants condition.

7.2 Defining Serious Complications

A Serious complication is defined as a complication which satisfies at least one of the following:

a) results in death
b) is life-threatening
c) requires or prolongs hospitalisation
d) results in persistent or significant disability or incapacity
e) may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above
f) any other significant clinical event, not falling into any of the criteria above, but which in the opinion of the investigator requires reporting

For the purposes of the trial, pre-planned elective hospital admissions will not be classed as a serious complication

7.3 Defining Unexpected Serious Complications (USCs)

All serious complications assigned by investigators as both *related* to the procedure and *unexpected* are subject to expedited reporting. A complication is *related* if it resulted from administration of any research procedures; note that all complications are *related* to the research procedures by definition (since untoward medical events which are unrelated to the research procedures are not being classed as complications or reported in this trial).A complication is *unexpected* when information is not consistent with the expected risk profile of the procedure.

7.4 Reporting Complications

Information about complications, whether volunteered by the patient or discovered by the investigators via history, physical examination, laboratory testing or radiological investigation will be collected and recorded on the CRF.

Complications will be collected for all participants from time of surgery until the final trial visit of the final trial patient

A summary of all captured complications will be sent to the sponsor if requested

7.5 Reporting Serious Complications

Serious complications will be collected for all participants from time of surgery until the final trial visit.

Serious complications must be reported on a sponsor-approved form and faxed through to the trial manager within 24 hours of any member of the research team becoming aware of a potential serious complication.

7.6 Reporting USCs

All serious complications assigned by investigators (or another suitably qualified delegated clinician)
as both *related* to trial treatment and *unexpected*, will be discussed
with the Principal Investigator (PI) before reporting. If the consensus is that this is unexpected then such serious complications will be reclassified as USCs and will undergo expedited reporting to the Research Ethics Committee (REC).

All USCs occurring whilst on Trial until the final trial out-patient visit, must be reported on a sponsor approved form and faxed through to the trial manager, within 24 hours of any member of the research team becoming aware of a USC. The Research team will inform the REC[(a)] and the sponsor[(b)] within the following timescales:

(a) USCs resulting in Death or are deemed to be life-threatening must be reported to the REC within **7 calendar days** of the PI being aware of the event. Follow-up information must be reported within a further **8 calendar days.**

(a) Any USCs **not** resulting in Death or deemed to be life-threatening must be reported to the REC within **15 calendar days** of the PI being informed of the event. Follow-up information must be reported within a further **8 calendar days**

(b) All USCs must be reported to the sponsor QA office within 24 hours of the event being reported to the PI.

7.7 End of Trial Report

Upon completing the trial, as defined in 5.2.7 above, an end of trial report will be submitted to the REC, sponsor and other regulatory authorities with 90 days. A copy of this end of trial report should also be submitted to the Sponsor's office

8.0 Data Collection, Source Data and Confidentiality

8.1 General

All information collected during the course of the trial will be kept strictly confidential.
Information will be held securely on paper and electronically at the Department of HPB and Transplant Surgery, SJUH campus.
The data storage will comply with all aspects of the Data Protection Act 1998. Operationally this will include:

- consent from patients to record personal details including name, date of birth and hospital ID
- appropriate storage, restricted access and disposal arrangements for patient personal and clinical details
- consent from patients for access to their medical records by responsible individuals from the research staff, the sponsor or from regulatory authorities, where it is relevant to trial participation
- consent from patients for the data collected for the trial to be used to evaluate safety and develop new research.

8.2 Archiving

In line with the principles of GCP / UK Clinical trial Regulations guidelines, at the end of the trial, data will be securely archived at LTHT for a minimum of 15 years. Arrangements for confidential destruction will then be made.Data to the point of withdrawal will still be used. No records will be destroyed without first obtaining written permission from the Sponsor.

9.0 Statistical Considerations

9.1 Sample Size

A total of 80 patients will be recruited into the pilot study. Twenty patients (corresponding to the first 5 patients for each of the 4 surgeons) will undergo portal vein clamping surgical procedure in order to address any learning curve associated with this procedure. In clinical opinion, a sample of five patients is sufficient to address any learning curve.
Sixty patients (30 per group) will be randomised to the surgical procedure. The sample size is based on the recommended number for a pilot study [12].

9.2 Statistical Analysis

The number of patients recruited per month will be presented. Baseline data will be summarized to comparability between groups. The intention-to-treat patient population will be used. Analysis will focus on descriptive statistics and confidence interval estimation rather than formal hypothesis testing. Outcome measures will be summarised and 95% confidence intervals presented by treatment group and overall. The difference in outcomes between the intervention and control groups will also be summarized and 95% confidence intervals presented. Data will be reported in line with the CONSORT statement.

10.0 Data Monitoring and Quality Assurance

10.1 Data Monitoring

All data obtained in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically in the Department of Hepato-pancreatico-biliary (HpB) and Transplant Surgery, St James's Hospital under the provisions of the 1998 Data Protection Act. Anonymity of data will be ensured. The spreadsheets will be password protected and will only be accessible to members of the research team.
The sponsor will permit monitoring audits and will review data collected, including consent forms. Data will be monitored for quality and completeness by the research
fellow. Entries on CRFs will be verified by inspection against source data. A sample of CRFs will be checked on a regular basis for verification of all entries made. In addition the subsequent capture of data on the Trial database will be checked. Where corrections are required these will carry a full audit trail and justification.

10.2 Quality Assurance

The Sponsor has systems in place to ensure that there is reporting and appropriate action taken in respect of:

(a) serious breaches of GCP, the trial protocol and the Clinical Trial Authorisation.
(b) Urgent safety Measures
(c) Protocol violations

A "serious breach" is a breach which is likely to effect to a significant degree:

(a) The safety or physical or mental integrity of the trial participants; or
(b) The scientific value of the trial

Investigators will promptly notify the Sponsor Office of the following within the required time frame, once they become aware of :

(a) Serious breached of GCP, the trial protocol and the clinical Trial Authorisation
(b) Urgent Safety measures
(c) Protocol violations
(d) Any amendments to the trial
(e) Any other issues as stated in the Research Sponsorship Agreement

The study will be conducted in accordance with the principles of GCP and the NHS Research Governance. There will be thorough adherence to departmental standard operating protocols (SOPS).

10.3 Good Clinical Practice (GCP) and Regulatory Compliance

This clinical trial, which does not involve the use of an investigational medicinal product has been designed and will be run in accordance with the Principles of GCP and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004 / 1031) and any subsequent amendments of the clinical trial regulations

10.4 Trial conduct

Approval will be obtained from the Research Ethics Committee (REC) prior to starting the trial. The Chief Investigator will be responsible for the ethical and governance issues. Two committees will be established to govern the conduct of the study – Trial Management Group (TMG) and TSC. The Trial Management Group will comprise the full research team and will have responsibility for project management, monitoring timescales, recruitment, analysis, ethical issues and safety. The TSC will include the research team and members with trial methodology and clinical expertise who are independent of the research team, including an independent chairperson. The PPI co- applicants will be full members of the TSG and TMG and will contribute to the management of the study.

KRP will be responsible for the ethical and governance issues. Clinical and research governance will be monitored by the R&D at SJUH.

11.0 Ethical Considerations

The trial will be performed in accordance with the recommendations guiding ethical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended at the 52nd World Medical Association General Assembly, Edinburgh, Scotland, October 2000. Informed written consent will be obtained from the patients prior to recruitment into the study. The right of a patient to refuse participation without giving reasons will be respected. The patient will remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment.
The study will be submitted to approval by a main REC, prior to entering patients into the study. A copy of the final protocol, patient information leaflets, consent form and all other relevant study documentation will be provided to the main REC.

12.0 Statement of Indemnity

Clinical negligence indemnification will rest with The Leeds Teaching Hospitals NHS Trust under standard NHS arrangements. The sponsor, Leeds Teaching Hospitals NHS trust, will provide insurance and/or indemnity to meet the potential legal liability for harm to patients arising from the management and design of the research.

13.0 Publication Policy

The trial will be registered with an authorised registry, according to the International Committee of Medical Journal Editors (ICMJE) guidelines, prior to the start of recruitment.

The success of the trial depends upon the collaboration of all participants. For this reason, credit for the main results will be given to all those who have collaborated in the trial, through authorship and contributorship. Uniform requirements for authorship for manuscripts submitted to medical journals will guide authorship decisions. These state that authorship credit should be based only on substantial contribution to:

- Conception and design, or acquisition of data, or analysis and interpretation of data
- Drafting the article or revising it critically for important intellectual content
- Final approval of the version to be published

and that all of these conditions must be met (www.icmje.org).

To maintain the scientific integrity of the trial, data will not be released prior to the first publication of the analysis of the primary endpoint, either for trial publication of oral presentation purposes, without the permission of the Trial Steering Committee (TSC).

The results of this trial will be presented at national and international meetings. They will also be submitted for publication in peer-reviewed journals. The investigators will follow International Committee of Medical Journal Editors (ICMJE) guidelines.
Participants who wish to receive information about the results from the Trial can ask their SSP/RN. They will be made aware that this will be some time after their participation in the Trial has finished.

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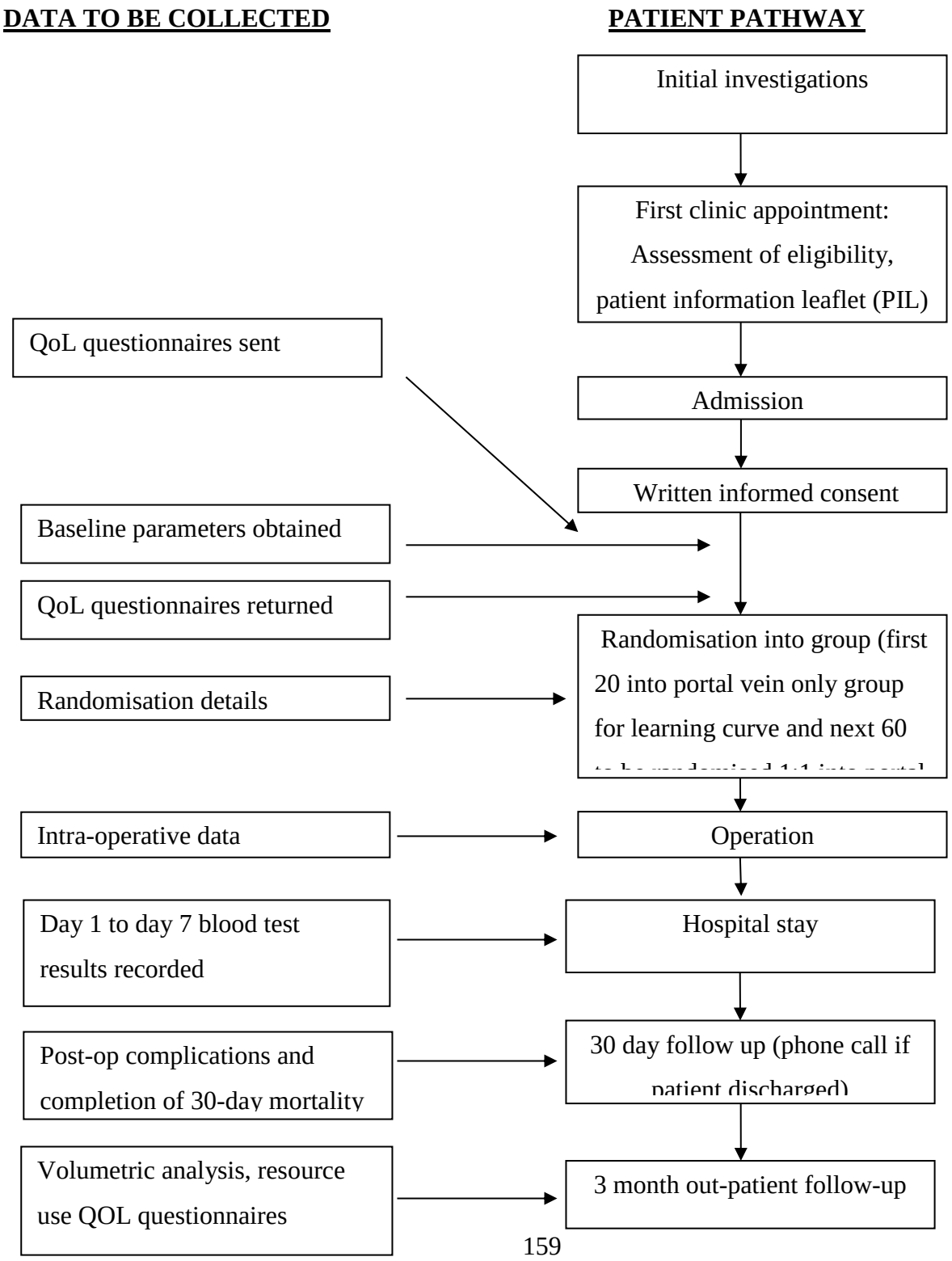
15.0 Appendices

A. Flow diagram of patient pathway
B. Main study objectives and endpoints

A. Flow diagram of patient pathway with schematic representation of when data will be collected



B. Main study (for information only):

Objectives:

Primary objective:

The primary objective of the main study is to demonstrate that portal vein clamping is superior to the Pringle manoeuvre technique in terms of the proportion of patients with septic complications by 30 days post hepatic resection.

Secondary objectives:

1. To demonstrate that portal vein clamping results in reduced ischaemia reperfusion injury to the liver
2. To demonstrate that portal clamping results in better regeneration of the remnant liver
3. To compare the two clamping methods in terms of procedural outcomes including intra-operative blood loss, length of camping time, length of time transecting the liver and duration of the operation
4. To compare the two clamping methods in terms of post-operative events including bleeding, post- operative transfusion requirements, non- infective cardio- respiratory complications and 30- day mortality rate
5. To test the feasibility of collecting data for a full economic evaluation on the main study
6. To compare patient quality of life (QoL) outcomes at 3 months post surgery

9.4 Patient Information Leaflet

The Leeds Teaching Hospitals NHS
NHS Trust
John Goligher Colorectal Unit
Colorectal & HPB Research Office
Ground Floor Lincoln Wing
St James' University Hospital
Beckett Street
Leeds
LS9 7TF
Tel: 0113 2064672 / 0113 2069215
Catherine Moriarty / Louise Clarke

PATIENT INFORMATION SHEET v3.0

(for patients undergoing learning curve part of the study)

Randomised Controlled Trial of Pringle Manoeuvre *versus* Portal Vein Clamping in Patients undergoing Liver Resection for Colorectal Liver Metastasis - A Pilot Study

PART 1

1. Invitation

You are being invited to take part in a research study. Before you to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, and discuss it with others if you wish.

PART 1 tells you the purpose of this study and what will happen if you take part.

PART 2 gives more detailed information about the study methodology.

Your liver surgeon and the study doctor would like to ensure that you completely understand the study and the study requirements. Please contact us if anything is unclear, or if you would like more information.

2. What is the purpose of the study?

As you are aware, you have been diagnosed with liver metastasis (deposits of cancer in the liver) from colorectal (bowel) cancer. Your liver surgeon thinks that the colorectal liver metastasis (CRLM) is operable by resecting the part of the liver with tumour (liver resection). It has been conclusively proven that surgery is the best form of treatment for such CRLM.

The liver has 8 segments and up to 70% of the liver can be safely removed surgically. The remaining liver grows back over a period of 4-6 weeks. St James's University Hospital is one of the leading centres for liver resections in the UK and has an international reputation. The centre currently performs around 270 liver resections per year for CLRM.

The liver is supplied by two blood vessels - the hepatic artery and the portal vein, each carrying about 25% and 75% respectively of the total blood flow to the liver, and 50% each of the total oxygen supply to the liver. During liver resection, it is standard practice to clamp the hepatic artery and the portal vein for short periods during the operation. This is done with the objective of reducing blood loss during surgery. This part of the procedure is called the Pringle manoeuvre.

However, clamping both of the blood vessels to the liver means that the liver will be starved of oxygen and nutrients (ischaemia) for the length of time the clamp is on. Recent studies have shown that the damage occurs when the blood flow to the liver is restored (reperfusion), which leads to flow of accumulated toxic substances into the liver. This has been termed as

ischaemia-reperfusion injury (IRI) and leads to liver damage and thereby increases risk of post-operative infections within the liver.

There has been some recent research suggesting that clamping of the portal vein only during liver resection (instead of Pringle manoeuvre in which the hepatic artery is also clamped) achieves the same effect as the Pringle manoeuvre in terms of reducing blood loss, with possible advantages, as the liver continues to receive blood supply through the hepatic artery. This potentially reduces the degree of injury to the liver cells, and may translate into decreased incidence of infections after the operation with overall improvement in patient outcome but may increase the risks of bleeding

However, as stated, the evidence for this is limited and the initial positive findings still need to be confirmed. Hence, we are performing this study which will randomly allocate patients to either undergo isolated portal vein clamping or the traditional Pringle manoeuvre during the operation. .

The main purpose of the study at this stage is to assess the ability to recruit and randomise patients into the study, assess the appropriateness of follow-up and collect data. .

We shall also look at the occurrence of septic (infectious) complications at 30 days after your surgery as well as:

- Blood loss during surgery
- Duration of surgery and portal vein clamping or Pringle manoeuvre
- Blood transfusion if required and the number of units transfused
- Recovery of liver function which will be assessed by daily liver function tests
- Non-infective complications affecting the heart and/or lungs

Three months after your surgery we will be looking at delayed outcomes such as the rate and extent of liver regeneration via a CT or MRI scan and quality of life after surgery. . The CT scan at 3 months will mean exposure to ionising radiation which carries a minimal risk to you. However, all patients undergoing liver surgery are subjected to this as part of standard care.

During the procedure, we will be taking a small piece (10x10 mm) of your healthy liver, so as to assess ischaemia reperfusion injury. This does not add any further risk to the surgery as the biopsy will be taken from the part of the liver that has already been removed. The rest of the procedure and the post-operative follow up will be performed as similar to those patients who are not part of the study.

3. Why have I been chosen?

Your liver surgeon has advised the research team to approach you for the study. All patients who need liver surgery for CRLM are invited to take part. A total of 80 patients will be recruited as part of this study at St James's University Hospital. The first 20 patients will be enrolled into the learning curve part of the study and next 60 patients randomly allocated to either of the two different clamping techniques (explained in later sections). You are one of the first 20 patients and hence will undergo isolated portal vein clamping.

4. Do I have to take part?

No. Participation is entirely voluntary. If you decide to take part you will be given this information sheet to keep and asked to sign a consent form to confirm that you understand what is involved when taking part in this trial. You are free to leave this trial at any time without giving a reason. This will not affect the quality of care that you receive.

5. What will happen to me if I take part?

The research team will post 3 questionnaires to your home address, which would need to be filled in during the last week before your operation. If you agree to participate you will undergo liver resection surgery for CRLM using the portal vein clamping technique.

The risks and benefits of this technique will be explained to you as per usual hospital practice. The consultant will give additional time to explain the procedure to you during the outpatient visit. You shall have adequate time to think about it before giving consent (either during next outpatient visit or on the day of surgery). The procedure will be is performed according to the well-established standard of practice already being followed in the hospital. In the case of any difficulty faced by the surgeon while performing isolated portal vein clamping, the procedure will be converted to a traditional Pringle manoeuvre.

You shall receive standard post-operative care and follow up as received by all patients undergoing liver surgery for CRLM.

The only additional requirement will be to receive 2 phone calls from a member of the research team just before your admission to remind you of the questionnaire that needs completing and at 30 days after your surgery to assess your recovery. It is most likely that you will have been discharged from hospital at this point and so a telephone follow up will occur. However, if you are still an in-patient, a member of the research team may come and speak to you instead.

A summary of the events, study tests and procedures is provided below:

Before the operation	➢ Information sheet and verbal study

	related information provided ➢ Consent obtained in the clinics or on the day of surgical procedure ➢ Three questionnaires will be posted to you so as to get information on quality of life prior to surgery. You will receive a phone call in the week before your operation to remind you to complete the forms
During the operation	➢ Portal vein clamping performed during the surgical procedure
After the operation and at 3 month follow-up in clinic	➢ Clinical assessment, daily blood tests including liver function tests and CT scan at 3 months after operation (all these tests/investigations are routine as performed for someone not part of the study) A CT scan involves minimal exposure to ionising radiation ➢ Telephone call for follow up at 30 days post surgery (if you have been discharged home) ➢ A further 3 questionnaires will be provided at 3 months period, so as to assess

	your quality of life following surgical procedure.

It is important for you to understand that participation in the study is entirely voluntary. In addition if you agree to participate initially and reconsider at a later date you may decline to continue with the study follow up.

6. What do I have to do?

When you have had the opportunity to read this patient information sheet a member of the research team will contact you to see if you would like to participate. If you do then your consent will be obtained at either a clinic visit or on the morning of surgery. There will be no additional clinic appointments or investigations if you decide to take part. The only additional requirement will be a telephone follow-up call 30 days following surgery by a member of the research team providing you have been discharged.

If you decide not to take part you shall receive the standard of care available to all patients undergoing similar surgery in the Liver Unit at St James' University Hospital.

7. What is the procedure that is being tested?

The research compares two techniques employed during liver surgery to control blood loss – The Pringle manoeuvre and isolated portal vein clamping. The Pringle manoeuvre (currently standard practice) clamps both the portal vein and the hepatic artery. Isolated portal vein clamping allows oxygenated blood to still be delivered to the liver via the hepatic artery while the portal vein is clamped.

At the time of surgery, the standard procedure for operative dissection will take place. Surgery will be performed through a reverse L-shaped incision made on the abdomen. The surgeon will clamp using the allocated method and then proceed to removing the affected part of the liver. A wedge liver biopsy (10x10mm) is taken from the affected liver (the part of the liver with clamped blood supply) to study the extent of reperfusion injury. This will not be adding additional risk to the patient as the biopsy will be taken from the liver that is being resected. The liver biopsy specimen will be sent to the pathology laboratory along with the resected liver specimen for standard histo-pathological examination as is routinely performed in Pathology department. The tumour tissue will be stored in the pathology department as per normal clinical practice but the biopsy taken from the sample will be destroyed after 18 months.

8. What are the alternatives for diagnosis or treatment?

The alternative is to not clamp the blood vessels at all during surgery. However, this is infrequently performed worldwide because of the risk of increased bleeding. If you do not wish to participate in the study, it is likely that a Pringle manoeuvre would still be performed during your operation and you will receive standard post operative care and follow up as per routine for patients undergoing CRLM resection.

9. What are the side effects of any treatment received when taking part?

This study compares two different methods of clamping blood vessels during liver resection surgery. It does not involve any drug treatment, so side effects following the procedure are unlikely.

Portal vein clamping alone is not commonly performed during liver resection surgery. However, there is published literature on the safety of this procedure in this setting. The four liver surgeons at St James's University Hospital are experienced in both liver resection and liver transplantation. It is routine practice in liver transplantation for surgeons to isolate the portal vein during liver transplantation surgery. So as to address any learning curve that the new procedure will have (portal vein isolation may be different in resectional surgery compared to transplant), the first 20 patients in the study will undergo portal vein clamping alone (5 per surgeon). In the event of any difficulty encountered during operation, the procedure (portal vein clamping alone) will be converted to standard Pringle manoeuvre. *You will form part of this 20 patients, who will undergo portal vein clamping alone.*

10. What are the possible benefits of taking part?

It is important for you to understand that the information obtained during this study will be crucial in determining whether isolated portal vein clamping is superior to the Pringle manoeuvre. The information that you provide as a participant in this study will contribute to a body of evidence that will help determine the best direction for future routine practice and therefore benefit other patients in the future. However, there is no direct benefit to you in taking part in this study

11. What happens when the research study stops?

The duration of participation is 3 months from the day of surgery. At the end of the study period, you will continue to receive the same standard of treatment and follow up care as is

practiced in the Liver unit of St James's University Hospital for CRLM (6 monthly follow up for the first two years followed by annual follow up thereafter).

12. What if there is a problem?

If you have a concern about any aspect of this Trial, you should ask to speak with the Screening Practitioner or Research Nurse who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research Trial, whether or not this is due to someone's negligence, you may have grounds for a legal action for compensation but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

13. Contact Details

Chief Investigator

Name: Mr K Raj Prasad Tel. Number: 0113 2066665

Research Fellow

Name : Mr Samir Pathak Tel Number: 07779 607659

Lead Research Nurse

Name : Ms Catherine Moriarty Tel Number: 0113 2064672

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART 2

15. What if new information becomes available?

Sometimes during the course of a clinical Trial, new information becomes available on the topic being studied. If this happens, we will tell you about it and discuss with you whether you want to or should continue in the Trial. If you decide to withdraw, we will make arrangements for your care to continue. If you decide to continue in the Trial, you will be asked to sign an updated consent form.

On receiving new information, we might consider it to be in your best interests to withdraw you from the Trial. If so, we will explain the reasons and arrange for your normal care to continue.

If the Trial is stopped for any other reason, you will be told why and your continuing care will be arranged.

16. What will happen if I don't want to carry on with the study?

You can withdraw from participation at any point of time during the study. Your withdrawal will not affect your treatment and standard of care in any way.

17. Will my part in this study be kept confidential?

If you consent to take part in this Trial, the records obtained while you are in this Trial as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the Sponsor, who is not involved in the Trial. You will be allocated a Trial number, which will be used as a code to identify you in all Trial paperwork and samples.

Your anonymised data will be available to people authorised to work on the Trial but may also need to be made available to people authorised by the Research Sponsor (Leeds Teaching Hospitals NHS Trust), which is the organisation responsible for ensuring that the Trial is carried out correctly. By signing the consent form, you agree to this and for any further research that may be conducted in relation to it, even if you withdraw from the Trial.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority and Independent Ethics Committee; this is to ensure that the Trial is

carried out to the highest possible scientific standards. All will have a duty of confidentiality to you as a research participant.

In line with Good Clinical Practice guidelines, at the end of the Trial, your data will be securely archived for a minimum of 15 years. Arrangements for confidential destruction will then be made.

18. Informing your General Practitioner (GP)

With your permission, your GP will be notified that you are taking part in this study. The letter will explain fully what participation in the trial involves.

19. What will happen to any samples I give?

The samples obtained during the course of study will include blood and tissue from liver (which are taken as per routine practise). The tumour tissue will be stored in the pathology department as per normal clinical practice but the biopsy taken (to assess ischaemia-reperfusion injury) from the sample will be destroyed after 18 months.

The samples will be stored in the Department of Pathology, St James's University Hospital in accordance with the Trust policy of storing and safeguarding of pathological samples.

20. Will any Genetic testing be done?

This study does not involve any genetic testing of samples.

21. What will happen to the results of this clinical trial?

The results of the study will be available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication.

If you wish to receive a copy of these, you should let your study doctor know so that it may be sent to you when it is ready.

22. Who is organising and funding this study?

You will not be paid for participation in this study. The study will be funded by the National Institute of Health Research (NIHR). The routine costs for this operation and post-operative care and follow-up will be billed to the National Health Service as normal. You can ask the study co-ordinator for any additional costs as result of your participation in this study.

23. Who has reviewed the study?

The study has been approved by the local research ethics committee responsible for St James's University Hospital. Their role is to check that the study is acceptable from an ethical and safety point of view in the interests of the patients participating.

This study was given favourable ethical opinion for conduct in the NHS by Yorkshire & The Humber - Leeds East Research Ethics Committee.

24. Contact for further information

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your study doctor, who will be able to provide you with up to date information about the procedure(s) involved. If you wish to read the research on which this study is based, please ask your study doctor. If you require

any further information or have any concerns while taking part in the study please contact one of the following people:

Principal Investigator: Mr K Raj Prasad **(**Tel. Number: 0113 2066665)

Principal Co-Investigator: Mr Samir Pathak (Tel. Number: 07779 607659)

Lead Research Nurse: Ms Catherine Moriarty (Tel Number: 0113 2064672)

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

Thank you for taking the time to read this information sheet and to consider this study.

9.5 Consent Form

The Leeds Teaching Hospitals NHS
NHS Trust

John Goligher Colorectal Unit
Colorectal & HPB Research Office
Ground Floor Lincoln Wing
St James' University Hospital
Beckett Street
Leeds
LS9 7TF
Tel: 0113 2064672 / 0113 2069215
Catherine Moriarty / Louise Clarke

PATIENT CONSENT FORM

Randomised Controlled Trial of Pringle Manoeuvre versus Portal Vein Clamping in Patients undergoing Liver Resection for Colorectal Liver Metastasis - A Pilot Study

Patient ID: …..…….. Initials: …….. Date of Birth: ……...

Patient initials

1. I confirm that I have read and understand the information sheet dated 28/10/2013 for the above study, and have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected. I agree to take part in the study. _____

2. I understand that my medical records may be looked at by authorised individuals from the Sponsor for the study, the UK Regulatory Authority, Independent Ethics Committee or from the NHS Trust in order to check that the study is being carried out correctly. I give _____

permission, provided that strict confidentiality is maintained, for these bodies to have access to my medical records for the above study and any further research that may be conducted in relation to it. I also give permission for a copy of my consent form to be sent to the Sponsor for the study.

3. I understand that even if I withdraw from the above study after the surgical procedure, the data and samples collected from me will be used in analysing the results of the study. I understand that my identity will remain anonymous. _____

4. I consent to the storage including electronic, of personal information for the purposes of this study. I understand that any information that could identify me will be kept strictly confidential and that no personal information will be included in the study report or other publication. _____

5. I consent to receiving a phone call from the research team just before my operation and at 30 days after my operation for purposes of follow up and data collection _____

6. I agree that my GP, or any other doctor treating me, will be notified of my participation in this study. _____

7. I agree that the samples collected for this study will be stored for potential future research. However any future study will be subject to approval by Research Ethics Committee.

_____

(This is optional and should not hinder your participation in the current study)

_____	_____
Name of the patient	Patient's signature and the date the patient signed the Consent form
_____	_____
Name of the Investigator taking written consent	Investigator's signature and date the Investigator signed the consent form

Original to be retained and filed in the site file. 1 copy to patient

9.6 Learning Plan

Please see attached on next page

Annex I www.leeds.ac.uk/rds/assets/word/Forms/training%20plan.doc

UNIVERSITY OF LEEDS

TRAINING PLAN FOR RESEARCH DEGREE STUDENTS

Student's Details:

Surname:	PATHAK	**Forename:**	Samir
School:	Faculty of Medicine	**Full-time / Part-time/ split-site :**	Part time
ID Number:	2 0 0 7 4 3 5 4 8	**Start date:**	July 2012
Programme: (PhP, PGR)	MD		
Main Supervisor:	Raj Prasad		
Additional Supervisors (if applicable)	Giles Toogood Danilo Miskovic Mark Hull		
Area of research/ draft thesis title:	Factors Affecting Blood Loss in Liver Surgery		

Details of Training Plan agreed by the supervisor(s) following discussion with the student within one month of the date of commencement of study:

The original project was terminated (see previous training plan) and the student started a new project was started in May 2013.

It was agreed to gain knowledge about the following topics through courses/ reading:

- - Skills in SPSS, Excel, Word
- - Basic skills in writing/ reading scientific work
- - Systematic review methodology

We agreed to present his plan at the internal research meeting for discussion.

Essential skills (Health and Safety, Safeguarding data and REC requirements) will be obtained by online learning

Please attach an additional sheet

The following issues must also be considered as part of Training Needs Analysis and included in the Training Plan (as appropriate):

- Learning Outcomes for the relevant research degree programme
- Health and Safety (http://www.leeds.ac.uk/safety/
- Safeguarding Data (http://campus.leeds.ac.uk/dpa/code.htm)
- Research Ethics Framework: (http://researchsupport.leeds.ac.uk/index.php/academic_staff/good_practice/university_ethics_policies/)

Signed: ..... Date: 21/5/2013
Supervisor(s)

Signed: ..... Date: 21/5/13
Student

9.7 Published Article

REVIEW ARTICLE

ANZJSurg.com

Anaesthetic and pharmacological techniques to decrease blood loss in liver surgery: a systematic review

Samir Pathak,* Abdul Hakeem,* Thomas Pike,* Giles J. Toogood,* Matthew Simpson,† K. Raj Prasad* and Danilo Miskovic‡

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†Department of Anaesthesia, St James's University Hospital NHS Trust, Leeds, West Yorkshire, UK and
‡John Goligher Colorectal Unit, St James's University Hospital NHS Trust, Leeds, West Yorkshire, UK

Key words
blood loss, blood transfusion, liver surgery.

Correspondence
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S. Pathak BSc, MBChB, MSc, MRCS; **A. Hakeem** MBBS, MRCS; **T. Pike** BSc, MBChB, MRCS; **G. J. Toogood** MBBS, MA, DM, FRCS; **M. Simpson** MBChB, FRCA; **K. R. Prasad** MBBS, MS, FRCS; **D. Miskovic** MD, PhD, FRCS.

This study was presented at the International Surgical Congress of the Association of Surgeons of Great Britain and Ireland, Harrogate, May 2014.

Accepted for publication 28 April 2015.

doi: 10.1111/ans.13195

Abstract

Background: There is increasing evidence that perioperative blood loss and blood transfusions are associated with poorer short- and long-term outcomes in patients undergoing hepatectomy. The aim of this study was to systematically review the literature for non-surgical measures to decrease intraoperative blood loss during liver surgery.

Methods: The literature search was performed using PubMed, Embase, Cochrane Library, CINAHL and Google Scholar databases. The primary outcome measures were perioperative blood loss and transfusion requirements. A secondary outcome measure was development of ischaemia–reperfusion injury.

Results: Seventeen studies met the inclusion criteria and included 1573 patients. All were randomized controlled studies. In eight studies (n = 894), pharmacological methods, and in another nine studies (n = 679), anaesthetic methods to decrease blood loss were investigated. Anti-fibrinolytic drugs, acute normovolaemic haemodilution, autologous blood donation and use of inhalational anaesthetic agent may affect blood loss and post-operative hepatic function.

Conclusions: There is potential for use of non-surgical techniques to decrease perioperative bleeding. However, on the basis of this review alone, due to heterogeneity of randomized trials conducted, no particular strategy can be recommended. Future studies should be conducted looking at pathways to decrease bleeding in liver surgery.

Introduction

Advancements in surgical technique, anaesthesia and perioperative care allow for routine liver resections with acceptable morbidity and mortality rates for both benign and malignant conditions.[1,2] Nevertheless, bleeding from exposed liver parenchyma after resection is common. Liver pathology such as steatosis or cirrhosis and intraoperative conditions such as high venous pressures and intermittent inflow occlusion may increase the risk for bleeding.[3] Intra- and post-operative hepatic bleeding remains an important operative risk with negative impact on short-term outcomes resulting from organ hypoperfusion.[4,5] In addition, there is increasing evidence that the need for blood transfusions also results in poorer long-term survival in surgical oncology.[6–9] Attempts to explain this observation include host immunosuppression due to the transfusion resulting in decreased tumour surveillance and earlier recurrence.[4]

Hence, in addition to short-term morbidity due to acute bleeding, reducing blood loss to a minimum remains a crucial concept for liver surgeons, and several surgical and non-surgical techniques have been developed. The role of surgical techniques such as vascular occlusion, parenchymal transection technique and use of sealants to decrease blood loss in liver surgery has been extensively reviewed,[10–14] but only a few non-surgical interventions have been analysed in the past.[15,16] Some of the techniques are based on decreasing hepatic perfusion intraoperatively, potentially resulting in ischaemia–reperfusion injury (IRI) that can increase the risk for post-operative liver failure.[17] A number of recent trials on non-surgical (anaesthetic and pharmacological) interventions have