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CICLO XXIV

PERIOPERATIVE BLOOD LOSS AND TRANSFUSIONS IN LIVER TRANSPLANT: RISK FACTORS AND IMPACT ON TRANSPLANT OUTCOME- A SINGLE EUROPEAN CENTRE EXPERIENCE.

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To my Family

"O frati", dissi "che per cento milia
perigli siete giunti a l'occidente,
a questa tanto picciola vigilia

d'i nostri sensi ch'è del rimanente,
non vogliate negar l'esperienza,
di retro al sol, del mondo sanza gente.

Considerate la vostra semenza:
fatti non foste a viver come bruti,
ma per seguir virtute e canoscenza".

(Dante Alighieri, Divina Commedia, Inferno, Canto XXVI, vv.112-120)

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LIST OF ABBREVIATIONS USED IN THE TEXT

ALAT	Alanintransaminas
ALI	Acute Lung Injury
APTT	Activated Partial Thromboplastin Time
ASAT	Aspartate Aminotransferases
ATIII	A Antithrombin III
BMI	Body Mass Index
CIT	Cold Ischemia Time
CRRT	Continuous Renal Replacement Therapy
CVP	Central Venous Pressure
DMII	Diabetes Mellitus Type II
EGDS	Esophagastroduodenoscopy
EKVATOR	Elektroniskt KVAlitetsregister för Transplantation Och Resektion
ELTR	European Liver Transplant Registry
FAP	Familial Amyloidotic Polyneuropathy
FFP	Fresh Frozen Plasma
GFR	Glomeruar Filtration Rate
GVHD	Graft Versus Host Disease
Hb / S-Hb	Haemoglobin / Serum haemoglobin
HBc	Hepatitis B Virus – core
HCV	Hepatitis C Virus
HD	Hemodialysis
HPS	Hepatopulmonary Syndrome
HRS	Hepatorenal Syndrome
HTK	Histidine-Tryptophan-Ketoglutarate
ICU	Intensive Care Unit
INR	International Normalized Ratio
LMWH	Low Molecular Weight Heparin
MARS®	Molecular Adsorbent Recirculating System
MELD	Model for End Stage Liver Disease

MOF	Multiple Organ Failure
Na/ S-Na	Natremia / Serum-Natremia
OLT	Orthotopic Liver Transplantation
PLT	Platelets
PT	Prothrombin Time
RBC	Red Blood Cells
TACE	Trans-Arterial - Chemo-Embolization
TEG	Thromboelastogram/ Thromboelastography
TIPS	Transjugular Intrahepatic Portosystemic Shunt
tPA	Tissue Plasminogen Activator
TRALI	Transfusion-Related-Acute Lung Injury
UW	University of Wisconsin
vWF	von Willebrand Factor
WIT	Warm Ischemia Time
WL	Waiting List

ABSTRACT

Introduction

Intraoperative blood transfusions are associated with adverse liver transplantation outcomes and lower patient survival rates. Standard recommendations and guidelines on blood transfusions in liver transplantation are lacking, and a large part of the literature tried to identify risk factors of intraoperative bleeding and blood loss. In the present study a retrospective analysis of the factors correlated to blood loss and transfusion requirements during liver transplanation was performed.

Materials and Methods

Pre, intra and post operative recipient variables and donor data were reviewed in relation to intraoperative blood loss and blood transfusions in 227 liver transplantations performed between 2005-2009 at Karolinska University Hospital, Huddinge, Stockholm in adult patients.

Results

A major indication for liver transplantation was cirrhosis (35%), followed by tumours (27%) and cholestatic diseases (15%). Transplants for Familial Amyloid Polineuropathy were performed in 13% of cases. There was no difference over the years in relation to intraoperative blood loss and the need for blood transfusions. The percentage of liver transplants performed with cava preservation (piggyback technique) increased significantly over the years. Parallel to this, the use of intraoperative veno-venous bypass has been progressively reduced over time. None of these variables is clearly correlated to intraoperative bleeding and transfusion requirements. Warm Ischemia Time (overall median 55 ± 44 minutes; range 20-605 minutes, n=200) showed a significant decrease over time. A significant reduction of post-transplant diabetes mellitus and postoperative Red Blood Cells (RBC) unit transfusions was observed.

In univariate analysis a shorter waiting list time, the Child-Pugh score, the MELD (Model for End Stage Liver Disease) score, the duration of surgery, the Cold Ischemia Time (CIT), lower preoperative haemoglobin value, lower preoperative platelets count, higher preoperative INR value, higher preoperative bilirubin level, higher preoperative urea and creatinine value and lower preoperative albumin levels are correlated to intraoperative blood loss and blood transfusions. Intraoperative bleeding and RBC/Plasma transfusions are associated with longer stays in the intensive care unit (ICU) and longer post-transplant hospitalization, the need for post-transplant transfusions and episodes of post-transplant gastrointestinal bleeding. Intraoperative blood loss and transfusions are correlated to platelets transfusions at 24 hours and during the first month post-transplant.

In the multivariate analysis only cold ischemia time, low preoperative haemoglobin level, low preoperative platelets count, low preoperative albumin, high preoperative INR value and preoperative creatinine are correlated to intraoperative bleeding and transfusion requirements. Child-Pugh score and MELD score are not predictive factors of intraoperative bleeding and transfusions.

Anamnesis of previous bleeding and pre-transplant hospitalization are good predictors of higher intraoperative blood loss and of the need for blood product transfusions.
No correlation was found between previous abdominal surgery and intraoperative bleeding. Among pre-transplant patients' characteristics only the presence of hepatorenal syndrome is associated with intraoperative blood loss and transfusion requirements.
Patients with intraoperative bleeding of more than 5 litres have a survival rate of 70% at 7 years post-transplant, whilst patients with a intraoperative bleeding inferior to 5 litres have a survival rate of 84% ($p<0,05$). Patients transfused with more than 12 Red Blood Cells Units have a survival rate of 67% at 7 years post-transplant, whilst patients receiving an amount of less than 12 Red Blood Cells Units have a survival rate of 81% ($p<0,05$).

Conclusions

Low preoperative haemoglobin value is the strongest predictor of intraoperative blood loss and transfusions. Anamnesis of previous bleeding, hepatorenal syndrome, pre-transplant hospitalization, cold ischemia time, low preoperative platelets count, high preoperative INR value, preoperative bilirubin, creatinine and albumin are good predictors of intraoperative bleeding and transfusions. Only intraoperative bleeding has an impact on patient survival and a cut-off level of intraoperative bleeding of 5 litres and 12 RBC units transfusions was shown to have an impact on patients' survival.
As a result of the study local recommendations for blood transfusions in liver transplantation at Karolinska University Hospital will be discussed.

ABSTRACT

Introduzione
Trasfusioni intraoperatorie di derivati del sangue sono associate ad un peggiore esito del trapianto epatico e a una ridotta sopravvivenza dei pazienti. In assenza di raccomandazioni standard e di linee guida riguardanti le trasfusioni di derivati del sangue in corso di trapianto epatico, una gran parte della letteratura ha cercato di identificare i fattori di rischio di perdite ematiche e sanguinamento intraoperatorio. Nel presente studio è stata eseguita una analisi retrospettiva dei fattori correlati al sanguinamento intraoperatorio e alla richiesta transfusionale in corso di trapianto epatico.

Materiali e Metodi
Variabili pre, intra e post operatorie relative al ricevente e variabili relative al donatore sono state raccolte e analizzate in relazione alle perdite ematiche intraoperatorie e alle trasfusioni di derivati del sangue in 227 trapianti epatici eseguiti tra il 2005 e il 2009 in pazienti adulti presso l'Ospedale Universitario Karolinska, Huddinge, Stoccolma.

Risultati
La principale indicazione per il trapianto epatico è stata la cirrosi epatica (35%), seguita dai tumori (27%) e dalle patologie colestatiche (15%). I trapianti a causa della Polineuropatia Familiare Amiloidotica (FAP) sono stati eseguiti nel 13% dei casi.
Non sono state osservate differenze in relazione alle perdite ematiche intraoperatorie e alla necessità di trasfusioni ematiche.
La percentuale dei trapianti epatici eseguiti con la tecnica di preservazione cavale (tecnica piggyback) è aumentata significativamente nel corso degli anni.
Di riflesso, l'utilizzo del by-pass venoso intraoperatorio si è progressivamente ridotto nel tempo. Nessuna di queste variabili è tuttavia correlata all'entità delle perdite ematiche intraoperatorie né alle necessità trasfusionali.
Il tempo di ischemia calda (WIT) (mediana, complessiva, 55 ± 44 minuti; intervallo 20-605 minuti, n=200) ha registrato una significativa riduzione nel tempo.
Una significativa riduzione è stata inoltre identificata nella diagnosi di diabete mellito post-trapianto e nella trasfusione di emazie concentrate nel post-operatorio.
Nell'analisi univariata sono stati trovati essere correlati all'entità delle perdite ematiche intraoperatorie e delle trasfusioni ematiche un ridotto tempo di attesa in lista per il trapianto epatico, il punteggio Child-Pugh, il punteggio MELD (Model for End Stage Liver Disease), la durata dell' intervento chirurgico, il tempo di ischemia fredda (CIT), un ridotto livello pre-operatorio di emoglobina, una ridotta conta piastrinica preoperatoria, un livello preoperatorio di INR più alto, un valore preoperatorio di bilirubina più alto, un maggiore valore preoperatorio di urea e creatinina e un ridotto valore preoperatorio di albumina.
Le perdite ematiche intraoperatorie e le trasfusioni di emazie concentrate e di plasma sono associate ad un prolungato ricovero post-operatorio in rianimazione e ad una più prolungata ospedalizzazione post-trapianto, alla necessità di trasfusioni ematiche post-operatorie e ad episodi di sanguinamento del tratto gastrointestinale.

Le perdite ematiche intraoperatorie e le necessità trasfusionali sono correlate alla trasfusione post-operatoria di concentrati piastrinici nelle 24 ore e durante i primi 30 giorni post-trapianto.
Nell'analisi multivariata, solo il tempo di ischemia fredda, un basso livello preoperatorio di emoglobina, una ridotta conta piastrinica preoperatoria, un basso livello preoperatorio di albumina, un elevato INR e un alto valore preoperatorio di creatinina sono correlati alle perdite ematiche intraoperatorie e alle necessità trasfusionali.
I punteggi di Child-Pugh e MELD non sono fattori predittivi di sanguinamento intraoperatorio e delle trasfusioni intraoperatorie.
Un'anamnesi positiva per un precedente sanguinamento, come pure l'ospedalizzazione pre-trapianto, sono buoni fattori predittivi di maggiori perdite ematiche e della conseguente necessità di trasfusioni ematiche intraoperatorie.
Nessuna correlazione è stata trovata tra pregressa chirurgia addominale e perdite ematiche intraoperatorie.
Tra le caratteristiche pre-operatorie dei riceventi, solo la presenza di sindrome epatorenale è associata al sanguinamento intraoperatorio e alla necessità di trasfusioni ematiche.
Pazienti con un sanguinamento intraoperatorio maggiore di 5 litri hanno una sopravvivenza del 70% a 7 anni post-trapianto, mentre pazienti con un sanguinamento inferiore ai 5 litri hanno una sopravvivenza dell'84%.
Pazienti trasfusi con più di 12 unità di emazie concentrate hanno una sopravvivenza del 67% a 7 anni dal trapianto, mentre pazienti che siano stati trasfusi con meno di 12 unità di emazie concentrate hanno una sopravvivenza dell'81%.

Conclusioni

Un basso valore di emoglobina pre-trapianto è il fattore predittivo più forte del sanguinamento intraoperatorio e della necessità di trasfusioni ematiche intraoperatorie.
Un'anamnesi di pregressi episodi di sanguinamento, sindrome epatorenale, ospedalizzazione pretrapianto, il tempo di ischemia fredda, una ridotta conta piastrinica preoperatoria, un elevato valore di INR preoperatorio come pure alti livelli di bilirubina, creatinina e urea e bassi valori di albumina sono buoni fattori predittivi delle perdite ematiche intraoperatorie e della necessità di trasfusioni ematiche intraoperatorie.
Solo le perdite ematiche intraoperatorie hanno un impatto sulla sopravvivenza dei pazienti e un limite di 5 litri e 12 unità di emazie concentrate transfuse ha dimostrato di avere un impatto sulla sopravvivenza dei pazienti.
Come risultato dello studio, all' Ospedale Universitario Karolinska saranno discusse linee guida locali riguardanti le trasfusioni ematiche in corso di trapianto epatico.

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DEVELOPMENT OF THE DOCTORAL THESIS

PhD Student Paola Violi, MD, has spent part of her Doctoral School Course as a guest doctor and researcher at the Division of Transplantation Surgery, Karolinska University Hospital, Huddinge, Stockholm, Sweden during the period between January 2010 – June 2011 for an effective total amount of 18 months.

Her stay at Karolinska University Hospital had the approval of the PhD School, University of Padova and of the PhD Student´s supervisor Professor Umberto Cillo, Head of Liver Transplant and Hepatobiliary Surgery Unit, University of Padova.

During her stay at the Division of Transplantation, Karolinska University Hospital, Dr Violi's research activity was mainly focused on the identification of risk factors of intraoperative bleeding and blood transfusions in liver transplantation and on the evaluation of the impact of blood transfusions on liver transplant outcome. Dr Violi carried out a retrospective study under the supervision of Professor Bo-Göran Ericzon and Associate Professor Greg Nowak, Division of Transplantation, Karolinska University Hospital, Huddinge, Stockholm. The results from this study constitute the Doctoral thesis.

The study involved other collaborators during its development and their specific roles in the study development are:

Paola Violi, MD

- Study concept and design
- Planning of the study
- Writing of the study protocol
- Critical revisions of the study
- Presentation of the study protocol to the Ethical Committee
- Ideation and construction of the database
- Data collection
- Data analysis
- Critical analysis of the results
- Writing of the thesis

Bo-Göran Ericzon, MD, PhD, Professor

- Study concept
- Critical revision of the study

Greg Nowak, MD, PhD, Associate Professor

- Critical revision of the study
- Data analysis
- Critical analysis of the results

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- Critical revision of the study
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Part of the data from this Thesis was presented as a poster at the international meeting: "The Scandinavian Transplantation Society XXVI Congres", Reykjavik, Iceland May 9th-11th, 2012.

Factors influencing perioperative blood loss in Liver Transplantation

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Introduction

Intra-operative blood transfusions are associated with adverse Liver Transplant outcome. A retrospective analysis of the factors correlated to blood loss during Liver Transplant was performed.

Materials and Methods

Pre and intra operative variables were reviewed in relation to intra-operative blood loss and blood transfusions in 160 Liver Transplantations performed in adult patients between 2006-2009 at Karolinska University Hospital, Huddinge, Stockholm. Split liver transplants, size reduced liver transplants, combined solid organ transplants and transplants performed for Familial Amyloidotic Polineuropathy were excluded from the analysis. The total amount of the Red Blood Cell (RBC) units transfused is comprehensive of Blood Bank RBC units and Cell-saver Units. Intraoperative veno-venous bypass was used in 64 (40%) liver transplantations.

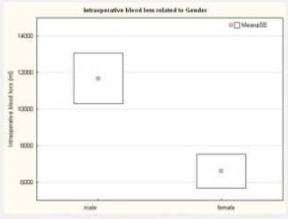
Results

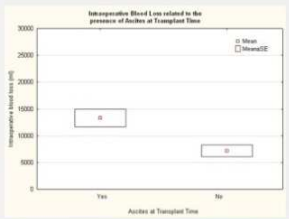
There was no difference over years in relation to intra-operative blood loss and need for blood transfusions. Data are expressed as Mean ± Standard Error. A higher intraoperative blood loss (11682 ± 1384 vs 6604 ± 930; p< 0,05) and need for RBC (21,7 ± 2,7 vs 13,3 ± 1,8; p< 0,05) and Plasma (22 ± 2,3 vs 13,7 ± 1,8; p< 0,05) units transfusions were observed in men than in women. A higher intra-operative blood loss and need for blood transfusions were correlated to the presence of ascites at transplant (p< 0,01), to pre-transplant hospitalization (p<0,01) and to the duration of the operation (p< 0,05). Previous esophageal variceal bleeding was associated with increased amount of Blood Bank RBC (p< 0,05) and Plasma (p<0,05) units transfused. Preoperative hemodialysis necessitated significantly increased intra-operative platelets units transfusions (2, 4 ± 0,7 vs 0,9 ± 0,15; p< 0,05).

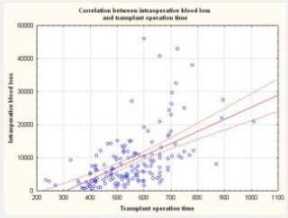
	Ascites at Transplant Time		
	Yes	No	
Intraoperative blood loss (ml)	13330 ± 1644	7167 ± 1102	p=0,002
Total RBC Units Transfused (nr)	25,3 ± 3,1	13,24 ± 2,2	p=0,001

	Pre - Transplant Hospitalization		
	Yes	No	
Intraoperative blood loss (ml)	15956 ± 3237	8406 ± 873	p=0.002
Total RBC Units Transfused (nr)	32,3 ± 6,2	15,4 ± 1,7	p=0.0003

	Previous esophageal variceal bleeding		
	Yes	No	
Intraoperative blood loss (ml)	12342 ± 1879	8588 ± 1092	p=0,06
Total RBC Units Transfused (nr)	22,7 ± 3,6	16,6 ± 2,2	p=0,12
Blood Bank Units Transfused (nr)	21,0 ± 3,5	12,7 ± 1,4	p=0,01
Plasma Units Transfused (nr)	24,1 ± 3,5	16,3 ± 1,6	p=0,02
Platelets Units Transfused (nr)	1,3 ± 0,2	0,8 ± 0,2	p=0,2







Conclusions

Male gender, the presence of ascites at transplant, pre-transplant hospitalization and the duration of the operation are correlated to blood loss and blood transfusions during Liver Transplantation. Previous variceal bleeding is correlated to increased Blood Bank RBC and Plasma transfusions. Preoperative hemodialysis is correlated to increased intraoperative Platelets transfusions.

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INTRODUCTION

Liver transplantation is the treatment of choice for patients with End Stage Liver Disease. Improvements in technique and patient management have led to a considerable improvement in patient survival[1]. Actual patient survival rates after liver transplantation, considering all of the indications, is 82% at 1 year, 71% at 5 years and 61% at 10 years, but considering only patients who survive beyond the first six months the survival rate is higher: 96% at 1 year, 83% at 5 years, 71% at 10 years[2]. The advances achieved in the amelioration of patient and graft survival since the early ages of liver transplantation reflect the improvements in surgical, medical, anesthesiologic and pharmacologic fields.

Liver transplantation is historically associated with major blood loss and blood products transfusions. However, since the beginning of the liver transplant era the need for intra and perioperative blood transfusions has been decreased thanks to a better comprehension of the physiopathology of the liver diseases, a better understanding of the coagulation process, and an improvement in anesthesiologic and surgical techniques[3].

These efforts resulted in a reduction in peri-operative blood transfusions in most transplant centres[4]. The use of blood products is considerably lower now than in the past, yet nevertheless the need for transfusions is still one of the most substantial landmarks of the procedure and an emerging part of the medical literature started to show the detrimental effects of blood transfusions on liver transplant outcome and patient survival[5, 6].

A substantial body of literature tried to identify predictive factors for intraoperative blood loss in order to minimize their impact and identify patients at higher risk of bleeding in order to provide them with more effective anesthesiological support and surgical treatment. At the same time, large efforts are being made to reduce intra and perioperative blood transfusions in order to minimize complications and improve patient survival rates. There is still no consensus on transfusion guidelines during liver transplantation[7]. Regarding intraoperative blood products administration, there is a wide variability among not only different centres, but also among different anaesthetists in the same centre[8]. Nevertheless, the actual trend in the international transplant community is to practice a more rational use of intraoperative blood transfusions[9].

SCIENTIFIC BACKGROUND

Intraoperative bleeding and transfusions need in Liver Transplantation

Liver transplantation is historically associated with major blood loss and the need for transfusions of blood products. The first liver transplant was attempted by Thomas Starzl in 1963, but the patient, along with the next four, died from bleeding. The operative technique had been defined on animal experiments, but the surgical team was not prepared for the technical complications related to portal hypertension[10]. It was only in 1967 that the first successful human liver transplantation was performed[11, 12].

During the first years of transplant activity, transfusions exceeding 100 red blood cells (RBC) units were common, with most centres presenting an average of 20 units for patient[13, 14]. Since then a steady decrease of intraoperative blood loss and the need for blood products transfusions during liver transplantation has been observed, reflecting an overall improvement[4, 15]. In the early 90s at Birmingham Liver Unit, the median of blood units transfused was 11,5 (range 0-14)[16]. Today, many centres have an average of around 2 units of red blood cells transfusions[5, 15] and a significant percentage of patients undergo liver transplantation without any transfusion requirement[17, 18] The possibility to perform a liver transplantation without the use of blood products is no longer an anecdotal report, but is in fact a common reality with centres reporting up to 75% of patients transplanted without any blood product transfusion[18].

Massicotte[19] et al. published a recent report on transfusion rates in 500 consecutive liver transplants performed at their centre. The authors reported that 79,6% of the liver transplantations did not receive any blood product. They also reported that 7,6% of patients received only 1 RBC unit and 8.2% received 2 RBC units transfusions. Transfusion of more than 5 RBC units was only necessary in 6 patients. The explanation of the amelioration achieved in blood transfusions management is multi-factorial, residing in improvements in anaesthetic management, evolution of surgical technique and a better understanding of coagulation disorders[15, 18, 20]. Despite the improvements achieved during the last few decades, blood loss still remains a major concern during the liver transplantation procedure.

Table 1. Blood Products Transfusions in Liver Transplantation (modified from Dalmau[27] et al.)

	RBC	FFP	PLT	No RBC	Coagulation Monitoring	Initial Hb (g/L)	Final Hb (g(L)
deBoer[21] (n=433)	7 (0-105)*	9 (0-51)*	0 (0-4)*	26% (1997-2004)	Blood Tests, TEG	Not described	Not described
Dalmau[21] (n=122)	2,9±2,9^	1,6±2,6^	7,9±8,3^	34%	Blood Tests, TEG	112±24	105±34
Massicotte[23] (n=206)	2,8±3,5^	4,1±4,1^	0,4±1,9^	32% (19,42% no transfusions)	Not described	105±22	87±14
Dalmau[24] (n=127)	2,3±2,7^	1,25±2,28^	5,06±5,9^	38%	Blood tests	115±21	102±15
Mangus[25] (n=526)	3	7	6	17,5%	Not described	12	Not described
Massicotte[26] (n=200)	0,3±0,8	0	0	81,5%	Not described	10,78±2,3	9,12±1,5

*Median and range

^Mean ± SD

Predictive factors for bleeding in Liver Transplantation

Many studies have focused on the identification of predictive variables for massive blood transfusions in liver transplant recipients[17, 18, 23-26]. Other studies tried to identify a predictive index to calculate the risk of intraoperative bleeding and transfusions[28, 29]. Results for the studies are often controversial, probably due to different transfusion practices and perioperative management among different centres.

In an early study conducted by Deakin[16] et al. on liver transplantations performed in Birmingham between 1982-1990, only low platelets count and high serum urea levels were associated to a higher amount of blood transfusions, while no association was found with cause of liver disease, severity of liver disease, or coagulation tests values.

McCluskey[28] et al. identified MELD score, renal function, preoperative hematocrit, Child-Pugh score, cold ischemia time (CIT) and surgical technique as independent risk factors for bleeding in liver transplantation. Similar results have been reported by Boin[30] et al.: in their analysis higher Child-Pugh and MELD scores, recipient weight, ischemia times and surgery times were associated to a higher rate of transfusions (≥ 6 Units).

Xia[31] et al. showed that MELD score is a strong predictor of the need for intraoperative transfusions. These authors performed a retrospective study, finding that patients with a higher MELD score (≥30) required a higher amount of red blood cells, plasma, platelets and cryoprecipitate transfusions compared to patients with MELD < 30. Moreover, patients with a higher MELD had lower baseline haematocrit, lower fibrinogen levels, and a higher need for respiratory and vasopressors support. These characteristics are indirect signs of poor clinical conditions, requiring a higher need for transfusions, medical support and more complex surgery.

Studies from Massicotte[32] et al. showed different results. In their analysis, MELD score was not associated with blood transfusions in multivariate analysis. In the same study, the authors identified the preoperative haemoglobin level as the strongest predictor of intraoperative blood transfusions. In another study, the same group[18] showed that there was a correlation in the multivariate analysis between preoperative platelets count, INR value and surgery time and the number of RBC units transfused.

In a retrospective study conducted by Hendriks[33] et al. on 164 patients who underwent liver transplantation, a higher requirement for red blood cell transfusions was observed in men than in women, and in patients with Child B or C than in patients with Child A.

Transfusion rates increased with the duration of cold ischemia time and were correlated to the use of a Cell-saver. In the multivariate analysis, the authors identified gender, Child-Pugh classification, serum urea level, year of transplantation, cold ischemia time and use of Cell-saver as factors associated to RBC transfusion rates in liver transplantation.

However, Massicotte[34] et al. showed that the need of blood transfusions did not change after introduction of Cell-saver use in their centre, despite longer surgery duration and an increase in blood loss. In fact, 80% of patients did not receive any blood product transfusions.

The authors specified that since its introduction the Cell-saver was used in every liver transplant, but there was only enough blood to re-transfuse in 65% of cases, with a mean volume of 338 ± 339 ml (range 40-200 ml). These data suggest that the use of the device is only useful in cases of massive intraoperative bleeding.

In a study conducted by Mangus[25] et al., the principal predictors of intraoperative blood loss were higher MELD score, previous surgery, preoperative higher INR, lower platelets count, lower preoperative haemoglobin level, elevated preoperative creatinine and elevated initial Central Venous Pressure (CVP). These findings can be interpreted as markers of poorer clinical conditions and more advanced liver disease.

Anaemia is an important factor that contributes to bleeding in patients with liver disease. Preoperative haemoglobin value is a variable with a strong association with intraoperative transfusions need. In the prospective study conducted by Ramos[5] et al., the preoperative haemoglobin level was the only factor to predict the transfusion of one or more red blood cells units. Ozier [8] et al., in a multicentric study conducted among French transplant centres, identified factors such as preoperative serum creatinine, preoperative PT, preoperative haemoglobin level, duration of surgery and previous abdominal surgery as factors associated to a higher rate of RBC transfusions. Higher fresh frozen plasma (FFP) and platelets (PLTs) transfusions were found to be associated to the duration of surgery, PT and preoperative serum creatinine. Preoperative ascites was correlated to fresh frozen plasma transfusions, while preoperative platelets count was associated with platelets transfusions. In the same study, the high variability among different centres in terms of transfusions, perioperative care and anaesthesia management was pointed out. In fact, the high variability among centres is probably one of the reasons why results vary so much among studies and centres.

The observations are not univocal, and other studies showed different results.

In fact, another study demonstrated no association between MELD score and intraoperative bleeding or blood products requirements[32].

A recent abstract[35] presented at the American Transplant Congress 2012, Boston, MA, June 2-6 2012 from the Transplantation group at the University of California, Los Angeles (UCLA), put the question in an interesting perspective. They analyzed variables predicting the blood loss during the hepatectomy performed by a single experienced surgeon, eliminating in this case the bias related to different surgical expertise. The results showed that only preoperative bilirubin value $\geq$ 14,7 mg/dl was a significant predictor of transfusion requirements in a multivariate analysis.

Donor characteristics have also been investigated as possible risk factors for intraoperative bleeding, but they did not show any correlation to intraoperative bleeding and transfusions[5].

Table 2. Predictive factors for intraoperative blood loss and blood transfusions

Deakin [16] et al.	Preoperative low platelets count Preoperative elevated serum urea level
McCluskey[28]	MELD score Renal function Preoperative hematocrit Child-Pugh score Cold Ischemia Time Surgical technique
Boin[30] et al.	Child-Pugh score MELD score Recipient weight Ischemia times Surgery times
Xia[31] et al.	MELD ≥ 30
Massicotte[32]	Preoperative haemoglobin level
Hendriks[33] et al.	Gender (male) Child-Pugh score (B or C vs A) Preoperative serum urea level Year of transplantation Cold Ischemia Time Intraoperative Cell-saver use
Mangus[25] et al.	Higher MELD score Previous surgery Preoperative high INR, low platelets count, low haemoglobin level, elevated creatinine value Elevated initial Central Venous Pressure (CVP)
Ramos[5] et al.	Preoperative Hb level
Ozier [8] et al.	Preoperative serum creatinine value Preoperative PT Preoperative haemoglobin level Duration of surgery Previous abdominal surgery
Rana AA[35] et al.	Preoperative bilirubin value ≥ 14,7 mg/dl

Impact of transfusions on Liver Transplant outcome

The impact of blood products administration on liver transplant outcome has been extensively investigated, demonstrating that blood transfusions are associated with increased morbidity and mortality. Intraoperative blood transfusion has been associated to poorer liver transplant outcome in terms of patients survival[6, 21, 36]. A higher need for perioperative blood transfusions can certainly be considered as a surrogate marker for poorer clinical conditions, more complex surgery and more serious diseases, but the role of blood transfusions on liver transplant outcome has been found to be independent from other predictors of intraoperative blood loss and post-transplant survival[5,6].

A retrospective study conducted by Cacciarelli[6] et al. on 334 ortothopic liver transplants demonstrated that recipients who underwent liver transplantation without RBC transfusions had a superior patient survival rate when compared to patients who received RBC transfusions. Moreover, in the same study it was shown that even if the liver transplant outcome was affected by many other factors such as age, male sex, and medical condition at the time of transplant, an increased RBC transfusion requirement was independently associated with patient and graft survival. Even a small amount of red blood cells transfusions is associated to a longer hospital stay and to a decreased survival rate [5, 6].

In a prospective study conducted by Ramos[5] et al., in a series of 122 liver transplantations the transfusion of more than three red blood cells units was associated with prolonged post-transplant hospitalization in the multivariate analysis. In their study, the mean of RBC units transfused was 2,9 ± 2,9 (SD) with a median of 2 units (range 0-14); in 42 (34%) of the patients no RBC unit transfusion was necessary. In the same study, excluding peroperative deaths, the transfusion of more than 6 RBC units was statistically significant for the patients survival rates.

A retrospective analysis on 526 liver transplants performed using the piggyback hepatectomy technique showed that a high intraoperative transfusion requirement was associated to a longer hospital stay and to a shorter 3-month and to 1-year patient survival[25].

In many other studies, red blood cell[21, 23], platelets[21, 36, 38] and fresh frozen plasma[23] transfusions have been found to be independent risk factors for the patients survival rates after liver transplantation.

A study conducted by Markmann[36] et al. identified three major factors predicting a poor liver transplant outcome: lack of intraoperative bile production, transfusion of more than 20 platelets units and a low intraoperative urine production (≤ 2 ml/Kg/min).

In a retrospective study conducted by deBoer[21] et al., RBC and platelets units transfusions were strong independent risk factors for 1-year patients survival after liver transplantation. In this study, patients receiving platelets transfusions during liver transplantation had a higher MELD score, a lower Karnofsky score, higher blood loss, worse preoperative laboratory values and higher FFP and RBC transfusion rates. To limit the influence of other confounding factors related to blood transfusions and outcome, the authors performed a propensity score-adjusted statistical analysis: the predictive values of RBC and platelets transfusions on outcome are even more significant due to this.

The mechanism that leads to the negative impact of blood products transfusions is not well clarified, but it is likely multifactorial.

Risks associated with blood transfusions

Blood products transfusions are associated with an increased risk of postoperative complications[3, 39, 40] such as red blood cells allo-immunization[41], infections[42, 43] (viral transmission, bacterial sepsis), pulmonary complications[38, 43] like transfusion-related-acute lung injury (TRALI)[44], renal failure, longer hospitalization and a higher rate of reoperations[45].

Some of the more common adverse effects of blood transfusions are listed below:

Table 3. Complications of blood products transfusions

Immediate Adverse Effects
Allergic reactions
Acute haemolytic reactions
Bacterial contamination
Transfusions-Related Acute Lung Injury (TRALI)
Volume overload
Hypothermia

Delayed and Long-Term Adverse Effects
Delayed haemolysis
RBC - alloimunization
Transfusion associated Graft Versus Host Disease (GVHD)
Immunomodulatory effects
Iron accumulation
Infectious disease transmission

Transfusion related pulmonary failure is associated with high morbidity and mortality post-transplant[21]. Pereboom[38, 46] et al. analyzed the causes of death in the group of patients who received platelets transfusions during liver transplantation, finding that platelets transfusions were associated with increased postoperative mortality due to Acute Lung Injury (ALI). Among the early transfusion-related complications, Transfusions-Related Acute Lung Injury (TRALI) is one of the most serious, characterized by a high rate of

mortality[47]. According to the 2004 Consensus Conference definition on TRALI, it is diagnosed as the development of Acute Lung Injury within six hours after the initiation of transfusions[47].

Epidemiological studies showed that patients with chronic liver disease have the greatest individual risk of developing TRALI in comparison with other populations[48, 49].

A recent study demonstrated that in patients who underwent liver transplantation, TRALI is associated with higher hospital mortality rates[44]. In this study, only plasma and platelets (such as blood products containg plasma) transfusions were associated with TRALI, while RBC units transfusions were associated with an increased risk of postoperative infections.

Regarding late blood transfusions complications, the phenomen of RBC allo-immunization deserves a particular mention. The presence of antibodies against RBC makes the selection of blood products more difficult in case of the need for transfusion. Moreover, some studies showed a correlation between the presence of RBC-alloimmunization and post-transplant outcome[41, 50]. Shariatmadar[50] et al., in a retrospective study on 2000 consecutive patients who had undergone liver, intestine or multi-visceral transplantation, showed that the incidence of pre-transplant RBC allo-antibodies was higher in the liver transplant population than in others such as multi-visceral and intestine transplanted patients. This could be due to a higher rate of transfusions in this group of patients[50]. No association was found between pre transplant and de-novo allo-antibodies and graft or patient outcome[50].

However, Boyd[41]et al. found an association between the presence of RBC allo-antibodies and transplant outcome. These authors found that three factors were associated with poorer survival: RBC allo-immunization history, numbers of intraoperative RBC and plasma units transfused and the immunosuppression history. In this series the main causes of mortality after liver transplantation were Multiple Organ Failure (MOF), haemorrhage and sepsis. An interesting observation is that 5 of the 8 deceased patients with RBC allo-immunization had at least one positive culture within the 30 days preceding death, versus only 11 of the 33 deceased patients without RBC allo-antibodies.

Multifactorial bleeding in Liver Transplantation

Impairment of the coagulation profile is a characteristic of patients affected by liver disease. Nevertheless, concepts regarding coagulation abnormalities in patients with end stage liver disease are changing[51, 52]. The historical perspective on a bleeding tendency in cirrhotic patients has been modified during the last few years. Several studies showed that in patients with liver disease there is a "rebalanced homeostasis", in which both hemorrhagic and thrombotic tendency coexist[53-56]. Patients with cirrhosis have defects in primary (thrombocytopenia, alterations in platelets function) and secondary homeostasis (reduced levels of factors V, VII, IX, X, XI and prothrombin; dysfibrinogenemia), nevertheless these defects in the pro-coagulation pathway are balanced by a parallel reduction in anti-coagulant systems[55-58].

The balance between these two pathways that normally exist in physiological conditions is perturbed by stressful conditions such as infections, surgery, and renal failure[54, 55, 56].

Table 4. Alterations of the haemostatic system in patients with liver cirrhosis (modified from Lisman[57] et al.)

Haemostatic changes impairing haemostasis
Low platelet count
Impaired platelet function
Impaired platelet-vessel wall interaction
Increased platelet inhibition by nitric oxide and prostacyclin
Low coagulation factors levels (V, VII, IX, X, XI)
Alterations in fibrinogen level
Alterations in fibrinogen function
Elevated levels of plasmatic tissue activator (t-PA)

Haemostatic changes promoting haemostasis
Elevated levels of factor VIII and vonWillebrand factor
Reduced levels of protein C, protein S, protein Z, α-2-macroglobulin, heparin cofactor II
Low plasminogen levels

In this new perspective, "classical" routine coagulation tests (PT, APT, INR) are not able to properly reflect the real *in vivo* homeostasis[60]. These evidences suggest that the practice to correct haemostatic abnormalities in patients with liver disease before the liver transplant procedure is not effective and the administration of blood products guided only by coagulation tests does not reduce bleeding.

The pathogenesis of intraoperative bleeding in liver transplantation is multifactorial[61, 62]. Surgical technical factors like inadequate surgical homeostasis impact for 75-90% of the intra and early post-operative bleeding, not only on liver surgery or liver transplantation[62].

Table 5. Causes of intraoperative and postoperative bleeding in surgery (modified from Marietta[53] et al.)

Intraoperative
Technical defects
Hypothermia
Metabolic acidosis
Disseminated intravascular coagulation
Heparin overdose
Hyperfibrinolysis
Early postoperative period (days 0-2)
Technical defects
Thrombocytopenia
Inherited/acquired platelets disorders
Inherited/acquired coagulation disorders
Delayed postoperative period (days 2-7)
Thrombocytopenia
Vitamin K deficiency
Acquired platelets disorders
Multiorgan failure
Antibodies to factors V following use of bovine thrombin in fibrin glue

In liver transplantation these factors combine with others more specific to liver disease and others related to the different phases of the surgical procedure:

Table 6. Causes of bleeding in liver transplantation (modified from Senzolo[53] et al.)

Pre-anhepatic phase
Extensive surgical trauma
Surgical technique
Baseline coagulation status
Etiology and status of liver disease
Anhepatic phase
Hyperfibrinolysis
Reperfusion and post-reperfusion phase
Trapping of PLTs in the graft
Heparin-like activity
Thrombocytopenia
Graft function

During the pre-anhepatic phase, surgical factors such as the technique of hepatectomy, presence of intra-abdominal adherences and/or portal hypertension and the clinical conditions of the patients play a major role. Cirrhotic patients have a circulation characterized by a high cardiac output, low systemic vascular resistance and an abnormally high distribution of blood volume in the splanchnic circulation[63]. Surgical manoeuvres contribute to decreased cardiac output during liver transplantation: a correction of hemodynamic alterations with an infusion of crystalloids or colloids causes a volume overload with increased congestion in the abdominal field and a diluitonal coagulopathy that worsens the efficacy of coagulation process[27, 62]. During the anhepatic phase there is an increased hyperfibrynolysis due to a rise in tissue-Plasminogen Activator (t-PA) activity as a consequence of a lack of hepatic clearance[64]. Moreover, early after reperfusion there is an ulterior increase in t-PA activity due to the release of t-PA by the endothelial cells of the graft[65]. Moreover, the entrapment of platelets in the graft after reperfusion due to ischemia-reperfusion injury causes a relative thrombocytopenia that contributes to coagulation impairment[46]. The graft function and the capacity to produce coagulation factors is also one of the important factors related to perioperative bleeding in liver transplantation.

Measures to reduce intraoperative bleeding

Several approaches have been attempted over time in order to try to reduce intraoperative bleeding in liver transplantation and the need for transfusions. Among the non-pharmacological measures, the reduction of central venous pressure (CVP) obtained by different means (fluid restriction, forced diuresis, venous vasodilatation, phlebotomy) has been extensively performed with controversial results[66, 67, 68].

Massicotte[66] et al. showed that lowering the central venous pressure to an average of 6,4 ± 3,5 mmHg just prior to the clamping of the inferior vena cava using a combined strategy of fluid restriction and pre-operative phlebotomy did not increase the need for post-transplant renal replacement therapy compared to historical controls. In the same study, transfusion rates were a mean of 0,4 red blood cells units per patient, while 79% of the patients did not receive any blood products. These data were confirmed by their experience on 500 consecutive liver transplantations performed at their centre with the same technique[19].

Different results were obtained by Schroeder[68] et al. when comparing liver transplantations performed in two nonrandomized groups of patients, one maintaining CVP < 5 mmHg by fluid restriction, use of adrenalin and/or noradrenalin, venodilators (nitroglycerin), morphine and furosemide and the other one in which no efforts were made to maintain a low CVP. The authors found a higher postoperative creatinine peak, a higher need for postoperative dialysis and a higher mortality at 30 days post transplant. These results can also be affected by the fact that the two groups of transplants were performed in two different centers with different perioperative management processes. Another explanation could be that the CVP lowering protocol was very aggressive, causing a worsening in renal function outcome.

Regarding surgical measures, in a prospective study conducted by Ramos[5] et al. the use of a temporary porto-caval shunt was associated to a significant reduction of intraoperative blood loss, probably related to a reduction in portal vein pressure.

Among pharmacological measures, different drugs have been used to reduce peri-operative bleeding[69]. The administration of recombinant activated factor VII (rFVIIa), studied in different randomized trials did not result in a reduction in intraoperative bleeding or transfusion rates in liver surgery and liver transplantation performed in cirrhotic patients[70, 71].

The only drugs that showed efficacy in reducing intraoperative bleeding in cirrhotic patients are the antifibrinolytics, such as aprotinin[72]. Moreover, the use of aprotinin is not associated with increased postoperative thrombotic complications[73].

Another approach is the use of intraoperative thromboelastography (TEG) to guide the blood transfusions requirements in liver transplantation. Thromboelastography offers the unique characteristic of showing the real thrombus-formation process. It is a functional analysis that can be done directly in the operation room, providing real-time indications on whole blood clot formation. TEG is an effective and promising instrument for performing tailor-made transfusions during liver transplantation[74, 75].

Characteristics of blood products in Sweden

In Sweden, 85% of all blood products are leukocyte depleted in order to reduce potential immuno-modulatory effects and transfusion associated morbidity. In Stockholm, since 1998 100% of all blood products are leukocyte depleted. It is still unclear, however, whether leukocyte depletion prevents negative effects of blood transfusions, particularly in liver transplantation. Furthermore, at Karolinska University Hospital fresh plasma is routinely transfused instead of fresh frozen plasma. Fresh plasma is a Swedish variant of plasma that can be transfused. It is not frozen, but is instead stored up to 14 days from the moment of collection in the refrigerator (4-6 °C).

In the table below, the differences between fresh frozen plasma and fresh plasma are listed. No study, to the best of our knowledge, has been carried out on liver transplant patients transfused with fresh plasma.

	FRESH FROZEN PLASMA	**FRESH PLASMA**
Volume	~ 270 ml	~ 270 ml
Citrate	60 ml	60 ml
Preparation after collection	Thawing	-
Fluid form lasting	Up to 24 hours	Up to 14 days
Not transfused plasma	Can be converted to Fresh Plasma	Can be returned to Blood Bank
Coagulation Proteins	> 70% of the initial values of the fresh plasma	Day 1. - as FFP Days 2-14. -FVIII to 50% - FV to 65-70% Others - F > 70%
Proteins	> 50 g/L	Individual variation
Residual Cells	Erythrocytes: < 6×10^9 /L Platelets: < 50×10^9 /L Leukocytes: < $0,1 \times 10^9$ /Unit	Erythrocytes < 6×10^9 /L Platelets: < 20×10^9 /L Leukocytes: < 1×10^6 /Unit
Tests	Anti HIV 1-2 HBsAg Anti HCV	Anti HIV 1-2 HBsAg Anti HCV

AIM OF THE STUDY

The aim of the study was to identify risk factors associated with intraoperative blood loss and blood products transfusions. Also, we want to evaluate the effect of intraoperative transfusions on the post transplant outcome (patients' survival).

SIGNIFICANCE OF THE STUDY

Several studies showed an improvement in transplant outcome with a reduction of blood products transfusions, so the actual trend is to minimize the need for transfusion requirement. There are no widely accepted guidelines regarding blood transfusions during liver transplantation. For this reason, every transplant centre should critically evaluate its own routine in blood products transfusion with the aim to improve that.

This is the first retrospective quality control study performed at Karolinska University Hospital, Huddinge, Stockholm, Sweden, to evaluate intraoperative blood products transfusions in liver transplanted patients.

The aim of the study is to identify risk factors associated to blood loss and blood products transfusions and evaluate the effect of transfusions on the post transplant outcome.

The critical review of the routine of intraoperative blood transfusion can lead us to a better understanding of liver transplant patients needs and improve our clinical practice.

The results of the study will be used to write local guidelines designed to optimize the transfusion management in patients undergoing liver transplantation.

MATERIALS AND METHODS

Study design

The study investigates different variables considered as possible risk factors associated with intraoperative blood loss and blood products transfusions in liver transplant procedures. The liver transplant outcome was evaluated through variables related to the early postoperative period (first 1-3 months post transplant) and through patients survival rates. Variables were chosen based on the literature review. All of the liver transplants performed at the Division of Transplantation Surgery, Karolinska University Hospital, Huddinge, Stockholm, during a period of five years between 1.01.2005 and 31.12.2009 were reviewed. This retrospective study was approved by the Ethical Committee of the Stockholm region (2012/625-31/1) and has been conducted at Karolinska University Hospital, Division of Transplantation Surgery.

Patient selection

The patients included in the study were selected according to the following criteria:

Inclusion Criteria

- ✓ Patients age ≥ 18 years
- ✓ Orthotopic whole organ liver transplantation
- ✓ Domino liver transplantation
- ✓ Retransplantation

Exclusion Criteria

- ✓ Patients age < 18 years
- ✓ Split liver transplantation (deceased donors, living donors)
- ✓ Reduced liver transplantation
- ✓ Combined solid organ transplantation

Methods

The patients were identified by the Swedish Personal Identity Number, a ten digit number assigned to all Swedish citizens and residents, to be searched in the registries and in the electronic archives. The patients have been coded and identified in the database by the progressive number of the liver transplant they underwent.

Every liver transplant performed at the Division of Transplantation Surgery, Karolinska University Hospital, Huddinge, Stockholm is characterized by a progressive number according to the Ekvator archiviation system (EKVATOR - Elektroniskt KVAlitetsregister för Transplantation Och Resektion is the transplant surgery department own IT-based electronic quality registry).

The data were collected from the following sources:

- Ekvator: the transplant surgery department own IT-based quality registry;
- TakeCare: the Stockholms Läns Landsting (Stockholm Country Council) IT-based medical records system that contains all documented information related to the care of the patient, including patient administrative sections in the electronic medical journal system. The access to the registry is restricted to authorized personal requiring an username and a password;
- Patients´ medical journals;
- ProSang: the blood bank data system that contains information on all blood transfusions and analysis (blood groups, pre-transfusion tests and antibody investigations).

All the data collected have been transferred to an electronic database (Excel files) for the statistical analysis. All of the material is stored in the offices at the Division of Transplantation Surgery, Karolinska University Hospital, Huddinge, Stockholm.

Variables selection

DONOR RELATED VARIABLES

Donors related factors
- Age (years) - Gender (Male/Female) - Body Mass Index (BMI) - Cause of death (when applicable) - Duration of Intensive Care Unit (ICU) stay (days) - Use of inotropic agents - Cardiocirculatory arrest - Serum Natremia (Na) (mmol/L) defined as the last recorded value before donation - Presence of antibodies anti Hepatitis B Virus–Anti core (HBc) - Presence of antibodies anti Hepatitis C Virus (HCV) - Type of perfusion solution

RECIPIENT RELATED VARIABLES

Demographic characteristics at the time of transplant
- Age (years) - Gender (Male/Female) - Body Mass Index (BMI)

Waiting List time
- Waiting List time (defined as the time in days from the day in which the patient was active in the waiting list for liver transplantation to the day of the liver transplantation)

Liver disease and related complications
- Diagnosis of liver disease - Presence of liver cirrhosis - Anamnesis for oesophageal varices and/or portal hypertensive gastropathy, diagnosed with esophagastroduodenoscopy (EGDS) - History of previous gastrointestinal bleeding - Anamnesis for portal thrombosis - Presence of Transjugular Intrahepatic Portosystemic Shunt (TIPS) - Presence of spenomegaly, defined as a maximum lenght of the spleen of $\geq$ 12 cm - Presence of Hepatorenal Syndrome (HRS) - Presence of Hepatopulmonary Syndrome (HPS)

Patient medical history and clinical conditions at Waiting List time
- Previous abdominal surgery - Previous upper abdominal surgery - History of previous transfusions - Diabetes Mellitus Type II in treatment with insulin (DMII)

Variables related to recipient clinical status at the time of transplant
- Pre-transplant hospitalization - Duration of the pre-transplant hospitalization period (days) - Child-Pugh Score (A 5-6; B 7-9; C 10-15) - MELD (Model for End Stage Liver Disease) score - Pretransplant hemodialysis, ultrafiltration treatment, Continuous Renal Replacement Therapy (CRRT) - Pre-transplant liver dialysis treatment with the Molecular Adsorbent Recirculating System (MARS®) - Presence of ascites and/or encephalopathy - Preoperative laboratory values: serum Haemoglobin (Hb, g/L), serum Platelets count (PLTs; x 10^9/L), International Normalized Ratio (INR), Activated Partial Thromboplastin Time (APTT, seconds), serum Creatinine (µmol/L), serum Urea (µmol/L), serum Bilirubin (µmol/L), serum Albumin (g/L).

Surgery related variables
- Duration of the operation - Cold Ischemia Time (CIT) defined as the time (minutes) from in situ flush of the donor to the liver being taken out of the ice - Warm Ischemia Time (WIT) defined as the time (minutes) from the liver taken out of the ice to partial portal reperfusion - Presence of portal thrombosis, partial or complete - Vena cava preservation - Use of veno-venous bypass - Use of intraoperative porto-caval shunt - Use of arterial interposition-graft - Laboratory values before the reperfusion: serum Hemoglobin (Hb; g/L), serum Platelets count (PLTs; x 10^9/L), International Normalized Ratio (INR), Activated Partial Thromboplastin Time (APTT; seconds)

Variables related to intraoperative blood loss and transfusions
- Total intraoperative blood loss - Total Red Blood Cells (RBC) Units transfused, including Red Blood Cells Units from Blood Bank and Red Blood Cells Units from Cell-Saver - Total Plasma Units transfused, including Fresh Frozen Plasma (FFP) and Fresh Plasma Units - Total Platelets ´(PLTs) Units transfused - Use of Cell-saver (Intraoperative Cell Salvage Machine) - Use of Antifibrinolytics, Prohemostatic agents or Coagulation Factors

TRANSPLANT OUTCOME

Variables related to postoperative period
- Length of postoperative stay in Intensive Care Unit (ICU) (days) - Total post transplant hospitalization at transplant center (days) - Postoperative treatment with Continuous Renal Replacement Therapy (CRRT) and Hemodialysis treatment. - Sepsis - Gastrointestinal bleeding - Need for postoperative transfusions - New onset of post transplant diabetes mellitus (NODM) - Episodes of acute rejection - Post–transplant re-operation - Postoperative laboratory values: serum Haemoglobin (Hb; g/L), serum Platelets count (PLTs; x 10^9/L), International Normalized Ratio (INR), Activated Partial Thromboplastin Time (APTT; seconds), serum Creatinine (μmol/L), serum Urea (μmol/L), serum Bilirubin(μmol/L), serum Albumin (g/L)

Variables related to postoperative transfusions
- RBC Units Transfused during the first 24 h post transplant - RBC Units Transfused during the 2^{nd}-30^{th} days post transplant - Plasma Units Transfused during the first 24 h post transplant - Plasma Units Transfused during the 2^{nd}-30^{th} days post transplant - Platelets Units Transfused during the first 24 h post transplant - Platelets Units Transfused during the 2^{nd}-30^{th} days post transplant

Survival
- Graft survival at 1 month, 1 year, 3 years - Patient survival at 1 month, 1 year, 3 years

Anaesthesiology management and transfusion protocol

A specially trained team consisting of 6-12 anaesthesiologists was involved in the pre- and intraoperative care of all the liver transplanted patients. The anaesthesiology management was performed according to the clinical guidelines, worked out at the Department of Anaesthesiology and Intensive Care, Karolinska, Huddinge.

Anaesthesia induction was performed with pentothal (Pentothal®), or more often recently with propofol (Diprivan®), fentanyl (Leptanal®) and atracurium (Tracrium®) as the muscle relaxant. Succinylcholine was used as an alternative in patients with a high risk of aspiration. Anaesthesia was maintained with sevoflurane (Sevorane®) in oxygen, fentanyl and atracurium. All patients were monitored with 5-lead electrocardiography and pulse oximetry. Invasive hemodynamic monitoring was assured by bilateral radial arterial catheters (one line used exclusively for blood sampling), a large central venous catheter (recently Mahurkar®), and a pulmonary artery catheter used with the Vigilance monitor which enabled continuous cardiac output monitoring. Patients also received a nasogastric tube and urinary catheter. All patients were positioned carefully on the operation table to avoid compression or stretch injury. An air warming system (WarmTouch®) and intravenous fluid warmer systems were used during the operation to avoid hypothermia.

Intravenous fluid administration consisted of the infusion of crystalloids (Ringer's acetate) and colloids (5% albumin solution) were used to maintain mean arterial pressure greater than or equal to 60 mmHg. Volume replacement was combined with the use of a vassopressor (noradrenaline). Dopamine was routinely used at low dosage due to its potential diuretic effect. Intraoperative transfusion needs were guided mainly by the clinical needs and not by the results of the coagulation status. The aim was to maintain the Hb level around 90-100 g/L. However, plasma and/or platelets were given preoperatively to correct coagulation defects before performing invasive procedures like inserting the central venous catheter. This practice has changed during recent times and the policy of giving blood products became more restrictive. A rapid infusion system (previously RIS, currently FMS-2000 - Belmont Instrument Corporation, 780 Boston Road, Billerica, MA 01821, USA) was used to enable the rapid delivery of large volumes of warmed blood products in case of extensive bleeding. The Cell-Saver device was used in all cases, except for patients with cancer, HCV and/or HBV positive. The laboratory tests, including arterial blood gas analysis, were taken every hour during the operation, except for the anhepatic phase during

which the arterial blood gases were taken every 15 minutes. Electrolyte disturbances were corrected according to the laboratory results. Patients were extubated on the operation table or transferred to the Intensive Care Unit for delayed extubation with continuous postoperative monitoring of vital functions.

Surgical technique

Only orthotopic liver transplants performed with a whole organ were included in the study. All the liver transplantations were performed using the classic technique with the use of veno-venous bypass during the anhepatic phase or using the cava preservation technique for implantation of the liver graft (piggyback technique). All the donors were brain death donors or living donors with Familial Amyloid Polineuropathy (FAP) donating their liver to domino transplantation. All the liver transplants were performed with ABO identical or compatible correspondence between the donor and recipient blood group, including A_2 to O liver transplants. A liver biopsy was performed during every transplant after complete revascularization of the graft. Immunosuppression was mantained according to one of the two standard immunosuppressive triple regimen therapies:

1. Cyclosporine, Azathioprine and Prednisolone used in HCV patients;
2. Tacrolimus, Mycophenolate mofetil and Prednisolone used in all the other patients.

Statistical analysis

The study character is a retrospective quality control study.

General descriptive statistics were used for data presentation. Continuous variables are presented as medians with Standard Deviation (SD) and ranges. Categorical variables are presented as a number with percentages. For every variable the total number of observations (n) is specified. Differences in continuous parameters over time were tested using the Kruskal-Wallis test for comparison of the medians. The effects of intraoperative bleeding and blood transfusions on survival were tested in a log-linear regression analysis. Patient survival was tested with the Cox-hazard test and presented using the Kaplan-Meier survival curve. The variables tested in the univariate analysis with a $p<0,05$ were included in the multivariate analysis. The statistical analysis was performed using STATISTICA 10 (StatSoft Inc., 2011).

RESULTS

The study analyzed data concerning 227 liver transplants of the 275 liver transplants performed at Karolinska University Hospital during the period 2005-2009.

A total of 48 transplants were excluded: 2 reduced liver transplants, 29 transplants performed with a split liver on both adults and children, 8 simultaneous combined solid organ transplants (kidney-liver; lung-liver), and 9 whole organ transplants performed in the paediatric population.

Among the 227 cases included in the study, 17 re-transplantations, 21 domino liver transplantations and 30 liver transplantations for Familial Amyloid Polyneuropathy were included. 4 of the re-transplantations included in the study were performed on patients previously transplanted during the same observation period (2005-2009).

Table 7. Liver transplants included and excluded from the study

	2005	**2006**	**2007**	**2008**	**2009**	**Total**
Total liver transplants	60	59	52	58	46	**275**
Liver transplants included in the study	46	49	44	49	39	**227**
Liver transplants excluded from the study	14	10	8	9	7	**48**
- **Reduced liver transplants**	0	0	0	1	1	**2**
- **Split liver transplants**	10	6	6	3	4	**29**
- **Combined transplants**	1	2	0	4	1	**8**
- **Pediatric whole organ transplants**	3	2	2	1	1	**9**

Not all the variables considered show a complete number of observations. The number of effective observations (n) is specified for every variable alongside the median and ranges or percentages. Results of the observations are listed below.

Donor data

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
Age	57 ± 12 (20-75) n=45	52 ± 16 (14-81) n=49	5 ± 15 (11-77) n=44	8 ± 16 (12-77) n=49	54 ± 15 (10-78) n=39	**56 ± 15 (10-81) n=226**
Male	20 (44%) n=45	28 (57%) n=49	26 (59%) n=44	27 (55%) n=49	18 (46%) n=39	**119 (53%) n=226**
Female	25 (56%) n=45	21 (43%) n=49	18 (41%) n=44	22 (45%) n=49	21 (54%) n=39	**107 (47%) n=226**
BMI	25,2 ± 3,4 (18,8-34,6) n=42	24,3 ± 4,2 (18,3-37) n=48	25,6 ± 4,5 (15,6-40,4) n=44	24,6 ± 4 (19,4-36,6) n=49	24,3 ± 3,8 (16,1-33) n=39	**24,8 ± 3,9 (15,6-40) n=222**
ICU stay (days)	2 ± 3 (0-12) n=36	2 ± 3 (0-16) n=45	2 ± 3 (0-15) n=43	1 ± 4 (0-21) n=49	1 ± 1 (0-6) n=39	**2 ± 3 (0-21) n=212**
Use of inotropic agents	27 (82%) n=33	37 (84%) n=44	38 (86%) n=44	45 (92%) n=49	35 (90%) n=39	**182 (87%) n=208**
Cardiac arrest	7 (22%) n=32	12 (27%) n=44	8 (18%) n=44	12 (24%) n=49	5 (13%) n=38	**44 (21%) n=207**
S-Natrium	145 ± 8,7 (132-166) n=27	145 ± 10 (131-176) n=39	150 ± 8 (133-169) n=35	146 ± 7 (134-162) n=47	145 ± 7 (130-165) n=38	**146 ± 8,2 (130-176) n=186**

The overall median donor age was 56 ± 15 years; 53% were male and 47% females of a total of 226 observations. In one case gender and age of the donor, like other data, were not available. No differences among data were observed over the years. Donors were largely brain death donors (91%), and in a small percentage living donors (9%), such as patients affected by Familial Amyloid Polineuropathy in the case of Domino Transplants. Living donors for split liver transplants are not included in our analysis. In 4 cases the data on the cause of death in cadaveric donors were not available.

Details on donor characteristics are listed below.

Table 8. Type of donors

	n	%
Brain Death Donors - Cause of Death		
Intracranial hemorrhage/Stroke	154	68%
Asphyxiation	13	6%
Cardiovascular	8	4%
Blunt injury	12	5%
Hydrocephalus	5	2%
Cerebral abscess	3	1%
Meningitis	3	1%
Gunshot wound	2	1%
Stab	1	0,5%
Drug intoxication	1	0,5%
Missing data	4	2%
Living Donors		
FAP Patients as Donors for Domino Transplant	21	9%

182 (87%; n=208) of the donors were supported by vaso-active agents, while 44 (21%; n=207) of them experienced at least one episode of cardio-circulatory arrest. Only 3 donors in a total of 209 observations were HCV positive, while 7 had anti-HBc antibodies .

No significant differences over the years were found regarding the characteristics of the donors such as age, BMI, ICU stay, use of vaso-active agents and episodes of cardio-circulatory arrest. All of the liver transplants were performed with ABO identical or compatible blood groups between the donor and recipient, including liver transplants performed between a donor with blood group A_2 and a recipient with blood group 0.

Recipient Demographic Data at the Time of Transplant

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
Age	55 ± 10 (29-73) n=46	53 ± 10 (25-69) n=49	55 ± 13 (19-68) n=44	57 ± 9 (27-71) n=49	54 ± 13 (20-66) n=39	**55 ± 11 (19-73) n=227**
Male	26 (57%) n=46	31 (63%) n=49	37 (84%) n=44	33 (67%) n=49	21 (54%) n=39	**148 (65%) n=227**
Female	20 (43%) n=46	18 (37%) n=49	7 (16%) n=44	16 (33%) n=49	18 (46%) n=39	**79 (35%) n=227**
BMI	25 ± 4,6 (17,9-37,1) n=45	24,8 ± 3,3 (19,8-37,7) n=49	26,7 ± 4,8 (16,5-41) n=44	24,2 ± 4,5 (19,3-35,6) n=48	24,5 ± 5,4 (17,1-40) n=39	**25 ± 4,5 (16,5-41) n=225**

The recipient population showed similar characteristics over the years and no statistically significant differences were found. Overall median age was 55 years. Regarding gender, 148 cases (65%) were males and 79 cases (35%) females. The median BMI was 25.

Waiting List Time

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
Waiting List Time (days)	44 ± 85 (0-398) n=46	64 ± 61 (0-304) n=49	85 ± 61 (3-310) n=44	90 ± 109 (2-506) n=49	103±90 (9-281) n=39	**71 ±86 (0-506) n = 227**

Despite wide variability among the years, the overall median waiting list time was 71 days (range 0-506; n=227). Differences among the years were not significant.

Liver disease diagnosis

Table 9. Liver disease diagnosis

	n	%
Cirrhosis		
Viral	20	9
Alcoholic	16	7
Viral + Alcoholic	17	7
Autoimmune (AIH)	9	4
Primary Biliary Cirrhosis (PBC)	8	3
Primary Biliary Cirrhosis (PBC) with overlapping Autoimmune hepatitis	4	2
Cryptogenic	8	3
Cholestatic diseases		
Primary Sclerosing Cholangitis	23	10
Primary Sclerosing Cholangitis with overlapping Autoimmune hepatitis	3	1
Other	9	4
Tumour		
Hepatocellular carcinoma (HCC) on viral cirrhosis	43	19
Hepatocellular carcinoma (HCC) on non – viral cirrhosis	15	7
Cholangiocellular carcinoma	1	0,3
Other	2	1
Metabolic Disease		
Familial Amyloid Polineuropathy	30	13
α-1-antitrypsin-deficiency	2	1
Acute Liver Failure	10	4
Miscellaneous		
Budd-Chiari Syndrome	3	1
Alagille Syndrome	2	1
Caroli Disease	1	0,3
Small for size	1	0,3

A major indication for liver transplantation was cirrhosis (35%) caused by any disease, followed by tumuors (27,3%) and cholestatic diseases (15%). Transplants for Familial Amyloid Polineuropathy were performed in 13% of cases.

Among liver cirrhosis, viral cirrhosis due to chronic hepatitis C, chronic hepatitis B and the association of both was the leading diagnosis (9%), followed by alcoholic liver cirrhosis (7%) and the association of viral and alcoholic cirrhosis (7%). Among tumours, hepatocellular carcinoma on cirrhosis was the main diagnosis (26% of total): it developed in 19% of cases of viral cirrhosis and in 7% of non-viral cirrhosis cases (alcoholic cirrhosis, primary biliary cirrhosis, cryptogenic cirrhosis). Primary sclerosing cholangitis (10%) was the leading cause of cholestatic diseases. Liver transplant for acute liver failure was only performed in 10 cases (4%).

Other diseases (Alagille syndrome, Caroli disease, Budd-Chiari syndrome, α-1-antitripsin deficiency, small for size) represented rare indications for liver transplant.

Liver disease and related complications

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
Presence of cirrhosis	37 (80%) n=46	42 (86%) n=49	32 (73%) n=44	32 (65%) n=49	29 (74%) n=39	**172 (76%) n=227**
Anamnesis for varices and/or portal hypertensive gastropathy	33 (72%) n=46	38 (77%) n=49	26 (60%) n=43	30 (65%) n=46	25 (64%) n=39	**152 (67%) n=223**
Presence of splenomegaly	33 (72%) n=46	38 (77%) n=49	23 (52%) n=44	27 (57%) n=47	27 (69%) n=39	**148 (66%) n=225**
Previous gastrointestinal bleeding	10 (22%) n=46	22 (45%) n=49	13 (29%) n=44	14 (30%) n=47	16 (41%) n=39	**75 (33%) n=225**
Anamnesis for portal thrombosis	7 (15%) n=45	5 (10%) n=49	5 (11%) n=44	2 (4%) n=46	5 (13%) n=39	**24 (11%) n=223**
Presence of TIPS	1 (2%) n=46	3 (6%) n=49	0 (0%) n=44	2 (4%) n=48	0 (0%) n=39	**6 (3%) n=226**
Hepatorenal Syndrome	3 (6%) n=46	8 (16%) n=49	2 (4%) n=44	9 (19%) n=48	10 (26%) n=39	**32 (14%) n=226**
Hepatopulmonary Syndrome	0 (0%) n=46	0 (0%) n=49	2 (4%) n=44	1 (2%) n=48	2 (5%) n=39	**5 (2%) n=226**

A large number of the recipients were affected by liver cirrhosis (76%). A large part of the patients (67%) had a positive anamnesis for oesophageal varices and/or hypertensive gastropathy and splenomegaly (66%). Nevertheless, only a third of the observed patients (33%) experienced at least one episode of gastrointestinal bleeding due to varices and only 6 patients (3%) underwent a procedure of Transjugular Intrahepatic Portosystemic Shunt positioning. Portal thrombosis was present in 24 patients (11%). Hepatorenal syndrome was experienced in 32 cases during the study period, whilst hepatopulmonary syndrome was a rare event in the population in analysis, affecting only 5 patients.

Patient medical history and clinical conditions at Waiting List time

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	Total n=227
Previous abdominal surgery	20 (43%) n=46	21 (43%) n=49	26 (59%) n=44	26 (53%) n=49	25 (64%) n=39	**118 (52%) n=227**
Previous upper abdomen surgery (included in abdominal surgery)	9 (19%) n=46	10 (20%) n=49	12 (27%) n=44	16 (33%) n=49	14 (36%) n=39	**61 (27%) n=227**
Previous transfusions	18 (47%) n=38	21 (47%) n=45	20 (50%) n=44	25 (57%) n=44	20 (53%) n=38	**104 (51%) n=205**
Diabetes Mellitus II insulin treated	8 (17%) n=46	8 (16%) n=49	5 (11%) n=44	9 (20%) n=45	10 (26%) n=39	**40 (18%) n=223**

Regarding previous surgical operations, 118 (52%) of the 227 cases underwent a surgical abdominal procedure. These observations also include the 61 cases (27%) that underwent at least one upper abdominal surgical procedure. Previous liver surgery, including previous liver transplantation, is included in this group: 35 of the patients underwent liver surgery of any type (cholecystectomy, hepatico-jejunostomy, liver resections, liver transplantation). A small percentage of the patients had a pretransplant diagnosis of Diabetes Mellitus type II treated with insulin.

No statistical difference over time was shown for any of the variables considered.

Recipient clinical status at transplant time

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
MELD	14,1 ± 6,4 (6,4-34,9) n=46	14,1 ± 9,7 (6,8-52,7) n=49	11,2 ± 6 (6,4-38,4) n=44	14,3 ± 9,1 (6,4-41,7) n=49	13,5 ± 8 (6,4-33,5) n=39	**13,5 ± 8,1 (6,4-52,7) n=227**
Child –Pugh Class	A = 13 B = 22 C = 11 n=46	A = 10 B = 19 C = 20 n=49	A = 17 B = 16 C = 11 n=44	A = 10 B = 23 C = 16 n=49	A = 12 B = 11 C = 16 n=439	**A = 62 B = 91 C = 74 n=227**
Child-Pugh Score	8 ± 2 (5-15) n=46	9 ± 3 (5-15) n=49	8 ± 2 (5-13) n=44	9 ± 2 (5-13) n=49	9 (5-13) n=39	**8 ± 2 (5-15) n=227**
Presence of ascites	18 (39%) n=46	21 (43%) n=49	14 (32%) n=44	19 (39%) n=49	19 (49%) n=39	**91 (40%) n=227**
Presence of encephalopathy	3 (6%) n=46	8 (16%) n=49	7 (16%) n=44	9 (18%) n=49	9 (23%) n=39	**36 (16%) n=227**
Pre-transplant hemodialysis (CVVH)	2 (4%) n=46	2 (4%) n=49	0 (0%) n=44	4 (8%) n=49	1 (3%) n=39	**9 (4%) n=227**
Pre-transplant liver dialysis (MARS®)	0 (0%) n=46	3 (6%) n=49	1 (2%) n=44	5 (10%) n=49	0 (0%) n=39	**9 (4%) n=227**
Pre-transplant hospitalization	8 (17%) n=46	10 (20%) n=49	3 (7%) n=44	11 (22%) n=49	7 (18%) n=39	**39 (17%) n=227**

The overall median MELD at the time of transplant was 13,5 and was roughly constant among the years. The MELD was calculated using the original formula[76]. It was not corrected for FAP or tumours. No statistical difference over time was found. Also, the more represented Child-Pugh class was class B (40%) with a median of 8 points (range 5-15). At the time of transplant, 40% of the cases presented with ascites of any amount, while only 16% presented with encephalopathy of any grade. Only a few patients required pre-transplant treatment with hemodialysis (4%) using CVVH, or liver dialysis (4%) with MARS® (Molecular Adsorbent Recirculating System).

Preoperative laboratory values

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
S-Hemoglobin (g/L)	115 ± 18 (77-158) n=46	119 ± 19 (85-153) n=49	128 ± 20 (85-169) n=44	118 ± 21 (84-157) n=47	118 ± 22 (76-162) n=39	**119 ± 20 (76-169) n=225**
S-Platelets (x 10^9/L)	96 ± 108 (30-537) n=46	96 ± 74 (19-308) n=49	116 ± 115 (31-583) n=44	89 ± 114 (12-633) n=47	109 ± 90 (33-394) n=39	**101 ± 102 (12-633) n=225**
INR	1,4 ± 0,3 (0,9-2,6) n=46	1,4 ± 1 (1-8) n=49	1,3 ± 0,5 (1-4,4) n=44	1,3 ± 1,1 (0,9-8) n=46	1,3 ± 0,7 (0,9-3,8) n=39	**1,4 ± 0,8 (0,9-8) n=224**
APTT (seconds)	39 ± 10 (27-68) n=45	41 ± 22 (30-181) n=47	38 ± 6 (29-57) n=43	40 ± 18 (26-135) n=44	40 ± 30 (25-218) n=38	**40 ± 19 (25-218) n=217**
S-Bilirubin (µmol/L)	36 ± 100 (3-550) n=46	42 ± 207 (6-933) n=48	26 ± 92 (4-426) n=44	34 ± 151 (5-672) n=46	40 ± 112 (3-560) n=39	**36 ± 142 (3-933) n=223**
S-Albumin (g/L)	30 ± 7 (15-46) n=44	29 ± 6 (16-44) n=48	32 ± 7 (14-48) n=43	28 ± 7 (19-47) n=45	31 ± 8 (18-48) n=39	**30 ± 7 (14-48) n=219**
S- Urea (µmol/L)	6 ± 12 (2,9-77) n=46	5,4 ± 13,7 (2,1-97) n=49	5,5 ± 2,8 (2-17,5) n=42	6 ± 5,6 (0,8-26,6) n=45	6 ± 13,4 (2,9-64) n=39	**5,9 ±10,5 (0,8-97) n=221**
S- Creatinine (µmol/L)	71 ± 31 (31-166) n=46	71 ± 71 (20-436) n=49	70 ± 17 (39-136) n=44	79 ± 43 (40-254) n=47	82 ± 53 (36-332) n=39	**72 ± 47 (20-436) n=225**

The overall median preoperative haemoglobin value was 119 g/L, median preoperative platelets count 101 x 10^9/L. Regarding coagulation tests, the overall median INR was 1,4 and the median Activated Partial Thromboplastin Time (APTT) 40 seconds. Preoperative median bilirubin was 36 µmol/L and albumin 30 g/L. Regarding renal function at the time of transplant, overall median uremia was 5,9 µmol/L while creatinine was 72 µmol/L.

Intraoperative variables

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	Total n=227
Operation Time (minutes)	530 ± 118 (285-840) n=46	540 ± 132 (270-870) n=49	570 ± 128 (380-1010) n=43	520 ± 113 (247-785) n=47	498 ± 126 (235-896) n=39	**530 ± 124 (235-1010) n=224**
Cold Ischemia Time (minutes)	521 ± 158 (215-857) n=38	578 ± 170 (69-890) n=48	546 ± 148 (158-903) n=36	491 ± 131 (218-755) n=43	479 ± 148 (146-756) n=33	**527 ± 154 (69-903) n=198**
Warm Ischemia Time (minutes)	61 ± 15 (23-109) n=38	59 ± 80 (25-605) n=48	59 ± 35 (30-235) n=36	49 ± 13 (25-86) n=44	49 ± 21 (20-140) n=34	***55 ± 44 (20-605) n=200^***
Cava preservation (piggyback technique)	8 (17%) n=46	19 (39%) n=49	23 (52%) n=44	29 (59%) n=49	24 (61%) n=39	***103 (45%) n=227^***
Use of intraoperative veno-venous bypass	39 (85%) n=46	32 (65%) n=49	21 (48%) n=44	19 (4%) n=49	13 (33%) n=39	***124 (55%) n=227^***
Use of porto-caval shunt	0 (0%) n=46	2 (4%) n=49	0 (0%) n=44	0 (0%) n=48	1 (3%) n=39	**3 (1%) n=226**
Use of arterial interpositiongraft	1 (2%) n=46	0 (0%) n=49	2 (4%) n=44	0 (0%) n=48	1 (3%) n=39	**4 (2%) n=226**
Intraoperative evidence of portal thrombosis	2 (4%) n=46	5 (10%) n=49	4 (9%) n=44	2 (4%) n=48	4 (10%) n=39	**17 (7%) n=227**

In red significant different results among years (p<0,05).

^p=0,001

The percentage of liver transplants performed with cava preservation increased over the years (17% in 2005 vs 61% in 2009) and the difference over time is statistically significant. At the same time, the use of intraoperative veno-venous bypass has been progressively reduced over the years (85% in 2005 vs 33% in 2009) in a significant way.

Operation times did not vary statistically over the years: the overall median is 530 ± 124 minutes. Cold Ischemia Time had an overall median of 527 ± 154 minutes with no statistical difference over the years. Contrary to this, Warm Ischemia Time (overall median 55 ± 44 minutes) showed a significant decrease over time.

The use of an intraoperative temporary port-caval shunt only occurred in 3 cases.

The necessity to perform an arterial re-vascularization of the liver graft using an interposition-graft was only present in 4 cases. Intraoperative portal vein thrombosis was evident in 17 cases. Differences over time regarding these data are not significant.

Laboratory values before perfusions

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
S-Hemoglobin (g/L)	107 ± 11 (82-130) n=42	103 ± 14 (79-146) n=47	106 ± 19 (11-129) n=41	107 ± 11 (84-127) n=45	101 ± 14 (68-124) n=36	**106 ±14 (11-146) n=211**
S-Platelets (x 10^9/L)	75 ± 106 (21-528) n=42	86 ± 63 (19-275) n=47	94± 96 (22-489) n=41	78 ± 74 (13-383) n=46	84 ± 68 (27-277) n=36	**212 ± 83 (15-528) n=212**
INR	1,4 ± 0,2 (1,1-1,9) n=42	1,5 ± 0,3 (1,1-2,5) n=48	1,5 ± 0,3 (1-2,2) n=41	1,5 ±0,2 (0,9-2,1) n=46	1,4 ± 0,4 (1-2,9) n=35	**1,5 ± 0,3 (0,9-2,9) n=212**
APTT (seconds)	44 ± 41 (31-300) n=42	54 ± 26 (33-173) n=47	44 ± 32 (30-226) n=41	46 ± 15 (30-103) n=46	48 ±87 (29-556) n=35	**47 ± 44 (29-556) n=211**

Regarding intraoperative laboratory values, the ones available just before the portal reperfusion were collected. However, the intraoperative blood samples are taken by time, every hour during the liver transplant procedure and are not in relation to specific intraoperative phase, so there is certain variability in respect to the interval time between blood sampling and reperfusion.

The overall median haemoglobin value was 106 g/L and the median platelets count was 212 x 10^9/L. With regards to coagulation tests, the overall median INR was 1,5 and the median Activated Partial Thromboplastin Time (APTT) was 47 s.

Intraoperative blood loss and transfusions

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
Total intra-operative blood loss (l)	5,5 ± 5,1 (0,5-23) n=45	5,5 ± 9,5 (0,7-46) n=49	4,7 ± 14,3 (0,6-85) n=44	5,3 ± 14,6 (0,8-95) n=48	7 ± 7,2 (0,8-27) n=39	**5,5 ± 10,9 (0,5-95) n=225**
RBC – Total Units	10± 9 (0-36) n=46	12 ± 17 (0-77) n=49	7 ± 28 (0-170) n=44	9 ± 29 (1-183) n=46	11 ± 13 (1-54) n=39	**10 ± 21 (0-183) n=224**
RBC – Blood bank Units	8 ± 8 (0-36) n=46	9 ± 16 (0-77) n=49	6 ± 28 (0-170) n=44	8 ± 18 (0-95) n=46	10 ± 12 (0-49) n=39	**8 ± 17 (0-170) n=224**
RBC – Cell saver Units	0 ± 4 (0-12) n=46	0 ± 4 (0-19) n=49	0 ± 6 (0-34) n=44	0 ± 13 (0-88) n=46	0 ± 5 (0-20) n=39	**0 ± 7 (0-88) n=224**
Plasma- Total Units	13 ± 10 (0-45) n=44	13 ± 18 (1-80) n=49	8 ± 28 (0-170) n=44	10 ± 19 (1-98) n=46	12 ±13 (0-50) n=39	**12 ± 19 (0-170) n=222**
Plasma- FFP Units	0 ± 0 (0-0) n=44	0 ± 1 (0-4) n=49	0 ± 0 (0-2) n=44	0 ± 0 (0-0) n=46	0 ± 1 (0-7) n=38	**0 ± 1 (0-7) n=221**
Plasma- Fresh Plasma Units	13 ± 10 (0-45) n=44	13 ± 18 (0-80) n=49	8 ± 28 (0-170) n=44	10 ± 19 (1-98) n=46	12 ±13 (0-50) n=39	**12 ± 19 (0-170) n=222**
Platelets Units	0 ± 1 (0-4) n=44	0 ± 1 (0-4) n=49	0 ± 2 (0-6) n=44	0 ± 2 (0-14) n=46	0 ± 1 (0-6) n=39	**0 ± 2 (0-14) n=222**

The median intraoperative blood loss was 5,5 litres.

Red blood cells units and plasma units transfusions are tightly correlated to intraoperative blood loss and follow its trend truthfully. The volume of one red blood cells unit is assumed to be 250 ml; the same for one plasma unit. Intraoperative blood transfusions are usually made with a ratio of 1:1 (RBC: Plasma unit). The overall median RBC units transfused were 10, with a median of 8 units from the Blood Bank (allogenic blood) and a median of 0 Cell-saver units (autologous blood). Intraoperative cell-savage with use of a Cell-saver is routinely used except in cases of cancer or recipient HCV and/or HBV positive. In the present series, the Cell-saver was used in 87 of the 224 observed cases (39%).

The overall median for plasma units transfused was 12 with a median of 0 fresh frozen plasma units and a median of 12 fresh plasma units. At Karolinska University Hospital fresh plasma is routinely transfused instead of fresh frozen plasma. Fresh plasma is a Swedish variant of plasma used for transfusions. It is not frozen, but is stored for up to 14 days from the moment of collection in the refrigerator (4-6 °C). The overall median for platelets units transfused was 0. No difference was found over the years regarding intraoperative blood loss and transfusion requirements.

Laboratory post-operative values

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total** **n=227**
S-Hemoglobin (g/L)	104 ± 13 (78-128) n=44	107 ± 12 (89-143) n=48	102 ± 14 (67-142) n=43	100 ± 12 (82-140) n=46	100 ± 14 (72-137) n=36	**103 ± 13** **(67-143)** **n=217**
S-Platelets (x 10^9/L)	54 ± 66 (17-365) n=44	47 ± 46 (18-185) n=48	68 ± 76 (26-421) n=43	61 ± 49 (19-263) n=46	53 ± 49 (21-240) n=37	**57 ± 59** **(17-421)** **n=218**
INR	1,7 ± 0,3 (1,2-2,7) n=44	1,7 ± 0,3 (1,3-2,4) n=47	1,8 ± 0,3 (1,1-2,6) n=41	1,8 ± 0,4 (0,9-2,7) n=46	1,7 ± 0,4 (1,2-2,9) n=37	**1,7 ± 0,4** **(0,9-2,9)** **n=215**
APTT (seconds)	48 ± 11 (34-92) n=43	53 ± 21 (39-142) n=47	46 ± 20 (33-146) n=42	46 ± 18 (36-143) n=46	46 ± 10 (37-75) n=36	**48 ± 17** **(33-146)** **n=214**
S-Bilirubin (µmol/L)	55 ± 53 (10-246) n=44	57 ± 73 (22-382) n=47	53 ± 33 (14-178) n=42	56 ± 60 (10-265) n=45	57 ± 36 (18-179) n=34	**55 ± 54** **(10-382)** **n=212**
S-Albumin (g/L)	26 ± 5 (16-39) n=44	26 ± 5,5 (17-44) n=45	31 ± 8 (19-53) n=38	28 ± 6 (16-43) n=39	32 ± 6 (21-45) n=26	**28 ± 6** **(16-53)** **n=192**
S- Urea (µmol/L)	7,6 ± 4,2 (3-22) n=43	6,7 ± 4,2 (3,7-25,6) n=45	6,7 ± 9,8 (3,2-66) n=40	9 ± 4 (3,2-28,7) n=41	6,5 ± 4,9 (2,5-26,5) n=33	**7,2 ± 5,8** **(2,5-66)** **n=202**
S- Creatinine (µmol/L)	79 ± 28 (41-144) n=43	67 ± 45 (43-262) n=45	78 ± 25 (38-160) n=41	91 ± 40 (40-257) n=43	80 ± 36 (34-192) n=34	**79 ± 36** **(34-262)** **n=206**

A complete postoperative laboratory panel test is usually taken at a variable time after the end of the operation. The overall median postoperative haemoglobin value was 103 g/L and the median postoperative platelets count was 57 x 10^9/L. Concerning the coagulation tests, the overall median INR was 1,7 and the median APTT 48 seconds. Postoperative median bilirubin was 55 µmol/L and albumin was 28 g/L. Regarding renal function tests, the overall median uremia was 7,2 µmol/L while creatinine was 79 µmol/L.

Post-transplant hospitalization

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total** **n=227**
ICU stay **(days)**	1 ± 9 (0-52) n=46	1 ± 4 (0-18) n=49	1 ± 9 (0-41) n=44	1 ± 4 (0-22) n=49	1 ± 6 (0-29) n=39	**1 ± 7** **(0-52)** **n=227**
Total hospitalization **at Transplant Centre** **(ICU stay included)**	20 ± 23 (0-145) n=46	21 ± 14 (0-82) n=49	16 ± 19 (0-107) n=44	18 ± 20 (0-127) n=49	18 ± 12 (9-71) n=39	**19 ± 18** **(0-145)** **n=227**

The overall median total post-transplant hospitalization at the transplant centre was 19 days, with a median of 1 day of ICU stay. No difference was observed over time.

Post-transplant complications

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total n=227**
CVVH Treatment	2 (4%) n=45	6 (13%) n=47	3 (7%) n=44	11 (23%) n=48	2 (5%) n=39	**24 (11%) n=223**
Hemodialysis treatment	1 (2%) n=45	3 (6%) n=47	3 (7%) n=44	3 (6%) n=49	0 (0%) n=39	**10 (4%) n= 224**
Postoperative transfusions	32 (76%) n=42	35 (71%) n=49	26 (62%) n=42	30 (61%) n=49	29 (76%) n=38	**152 (69%) n= 220**
Gastrointestinal bleeding	3 (7%) n=41	4 (10%) n=41	2 (5%) n=43	4 (8%) n=48	3 (8%) n=39	**16 (7%) n= 212**
Re-operation	6 (13%) n=46	9 (18%) n=49	8 (18%) n=44	13 (26%) n=49	5 (13%) n=39	**41 (18%) n= 227**
Sepsis	2 (4%) n=44	7 (14%) n=48	5 (11%) n=44	9 (19%) n=48	5 (14%) n=36	**28 (13%) n= 220**
NODM	11 (24%) n=45	24 (50%) n=48	23 (52%) n=44	20 (42%) n=48	5 (13%) n=39	***83 (37%) n= 224****
Rejection episodes	18 (39%) n=46	28 (57%) n=49	13 (29%) n=44	20 (42%) n=48	19 (49%) n=38	**98 (43%) n= 226**

In red significant different results among years (p<0,05).

*** p<0,0005**

Regarding post-transplant complications in the early post-operative period (first 30 days post transplant and in any case until the end of hospitalization period), Continuous Veno-Venous Hemofiltration (CVVH) was performed in 24 cases and hemodialysis treatment was only necessary in 10 cases.

Postoperative gastrointestinal bleeding occurred in 16 cases.

Re-operation was performed in 41 cases and 19 of them were due to intra-abdominal bleeding. Blood transfusions during the post-operative period were necessary in 152 cases (69%). In 28 cases at least an episode of sepsis, defined as a bacterial positive blood culture, developed.

Diabetes mellitus, defined as the need for insulin treatment persisting for more than 10 days post-transplant, was newly diagnosed in 83 cases post-transplant. A significant trend in the reduction of diabetes mellitus post-transplant has been shown when comparing data from 2006 and 2007 to 2009.

In 98 cases (43%) at least one episode of acute rejection developed after transplant. Rejection episodes include both biopsy-proven rejections and clinical rejections. In both cases the treatment of choice was the increase of baseline immunosuppression and the administration of Methylprednisolone 500 mg in a single dose every day for three consecutive days, followed by a step-reducing steroid treatment.

Post-operative transfusions during the first 24 hours post-transplant

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total** **n=227**
RBC Units Transfusions	1 ± 2 (0-5) n=21	0 ±1 (0-7) n=48	0 ± 1 (0-5) n=43	0 ± 1 (0-5) n=49	0 ± 2 (0-10) n=39	***0 ± 1*** ***(0-10)*** ***n=200^***
Plasma Units Transfusions	0 ± 1 (0-2) n=21	0 ± 1 (0-5) n=48	0 ± 2 (0-10) n=43	0 ± 0 (0-2) n=49	0 ± 0 (0-1) n=39	**0 ± 1** **(0-10)** **n=200**
Platelets Units Transfusion	0 ± 1 (0-4) n=21	0 ± 1 (0-2) n=48	0 ± 1 (0-3) n=43	0 ± 1 (0-7) n=49	0 ± 1 (0-2) n=39	**0 ± 1** **(0-7)** **n=200**

In red significant different results among years (p<0,05).

^p=0,001

During the first 24 post-operative hours a median of 1 RBC unit, a median of 0 plasma unit and a median of 0 platelets unit were transfused. Comparing transfusion rates among the different years, it is clear that since 2006 post-operative RBC unit transfusions decreased significantly. Regarding plasma transfusions during the same post-operative period, a significant difference among the years was not shown, but the trend is toward a progressive reduction in the transfusion rate.

Post-operative transfusions during the 2nd-30th days post-transplant

	2005 n=46	2006 n=49	2007 n=44	2008 n=49	2009 n=39	**Total** **n=227**
RBC Units Transfusions	2 ± 3 (0-10) n=22	2 ± 8 (0-43) n=48	0 ± 6 (0-38) n=43	2 ± 15 (0-93) n=49	2 ± 8 (0-37) n=39	**2 ± 9** **(0-93)** **n=201**
Plasma Units Transfusions	0 ± 1 (0-6) n=21	0 ± 7 (0-49) n=48	0 ± 2 (0-7) n=43	0 ± 13 (0-92) n=49	0 ± 3 (016) n=39	**0 ± 8** **(0-92)** **n=200**
Platelets Units Transfusion	0 ± 1 (0-4) n=22	0 ± 3 (0-21) n=48	0 ± 2 (0-10) n=43	0 ± 4 (0-16) n=49	0 ± 1 (0-5) n=39	**0 ± 3** **(0-21)** **n=201**

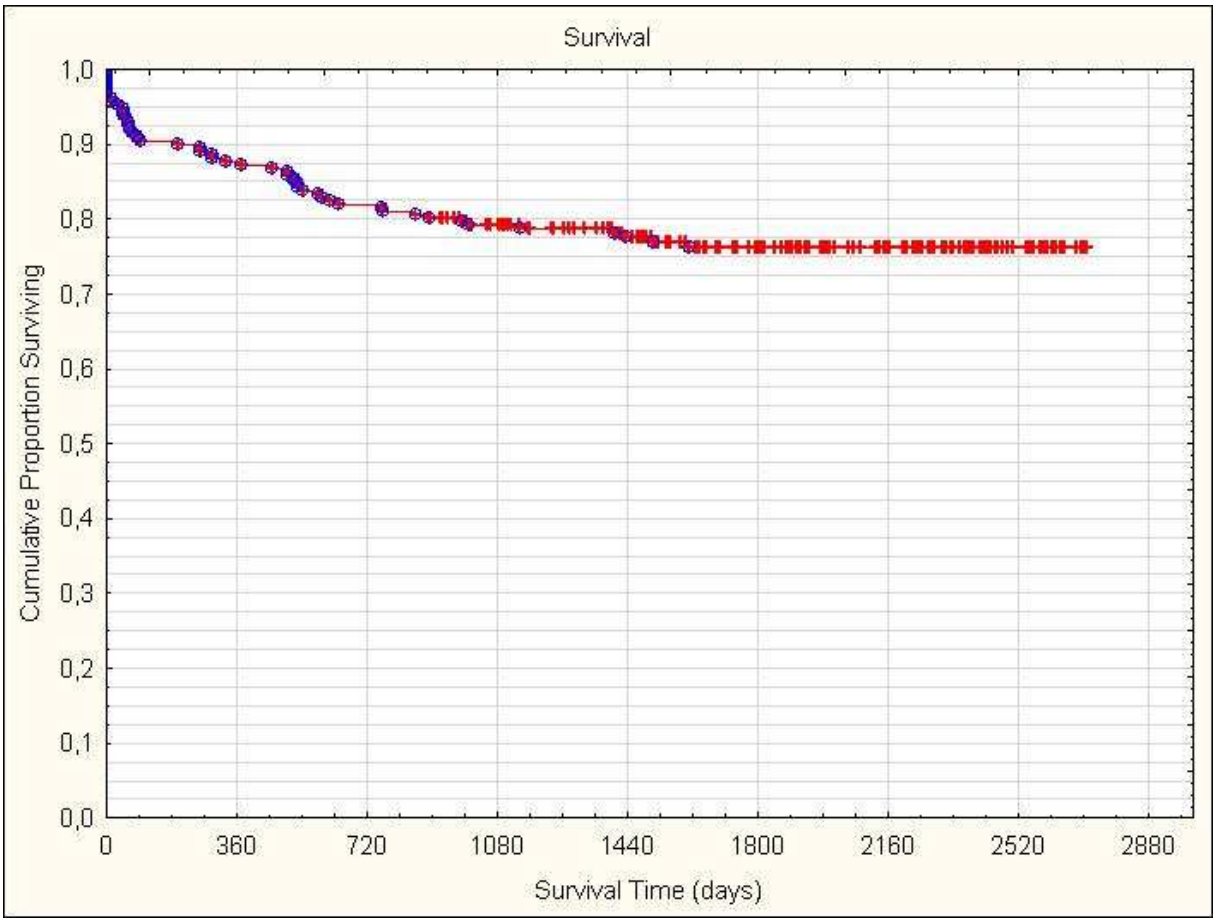
During the period from the 2nd to the 24th post-operative days there was a median of 2 RBC Units. A median of 0 plasma units and a median of 0 platelets units were transfused.
No statistical differences were found over time.

Patients survival

In our analysis 4 (2%; n=227) intraoperative deaths are included. 3 patients died because of massive intraoperative bleeding and 1 patient died because of cardiac arrest.

Overall, patients' survival is 90% at 6 months, 88% at 1 year and 79% at 3 years.

Graph 1. General survival of the patients included in the study

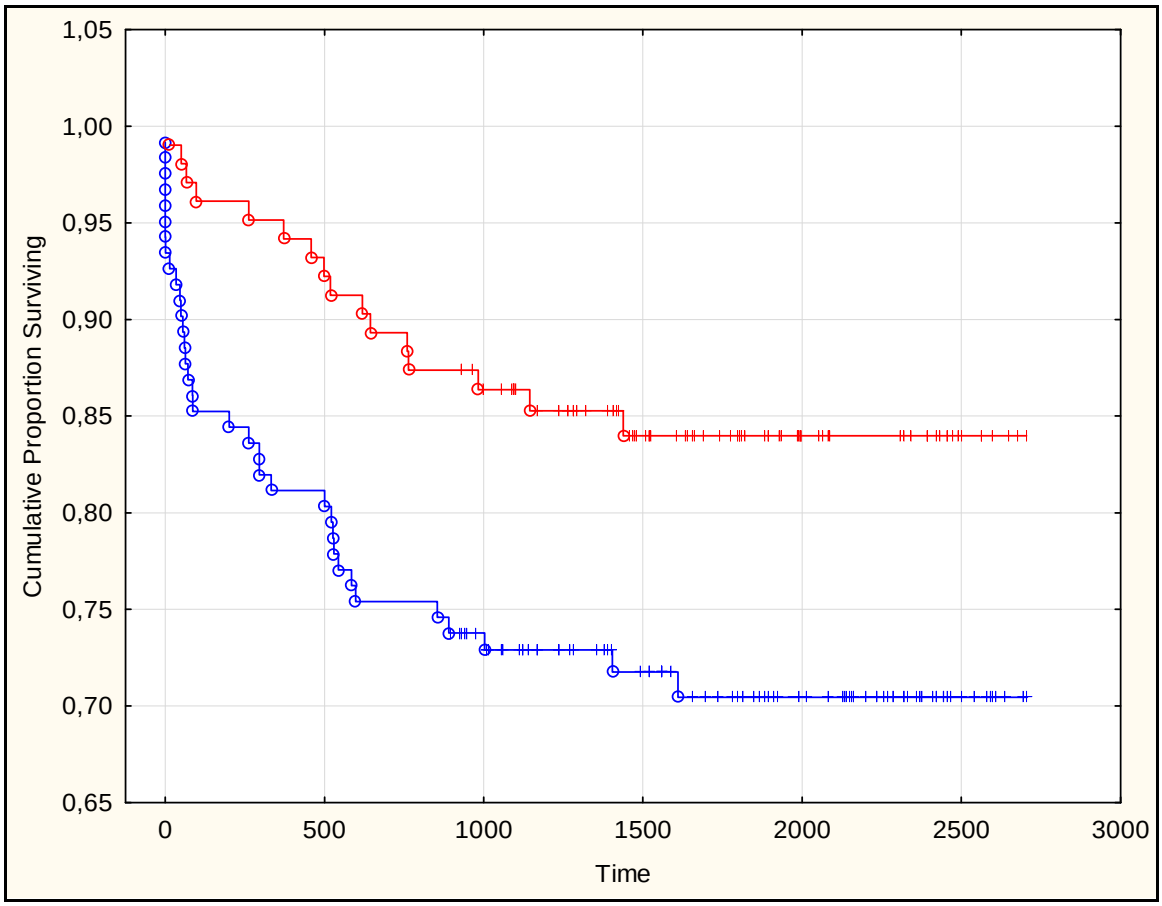


In our analysis only intraoperative bleeding has an impact on patient survival, and no other variable had an effect.

Transfusion requirements did not show any impact on survival ($p<0,05$), because they are strictly correlated to intraoperative bleeding.

A cut-off of intraoperative bleeding of 5 litres was shown to have an impact on survival. Patients with intraoperative bleeding of more than 5 litres have a survival rate of 70% at 7 years post-transplant, while patients receiving an amount of less than 12 red blood cells units have a survival rate of 84% ($p < 0,05$).

Graph 2. Cumulative survival according to intraoperative bleeding



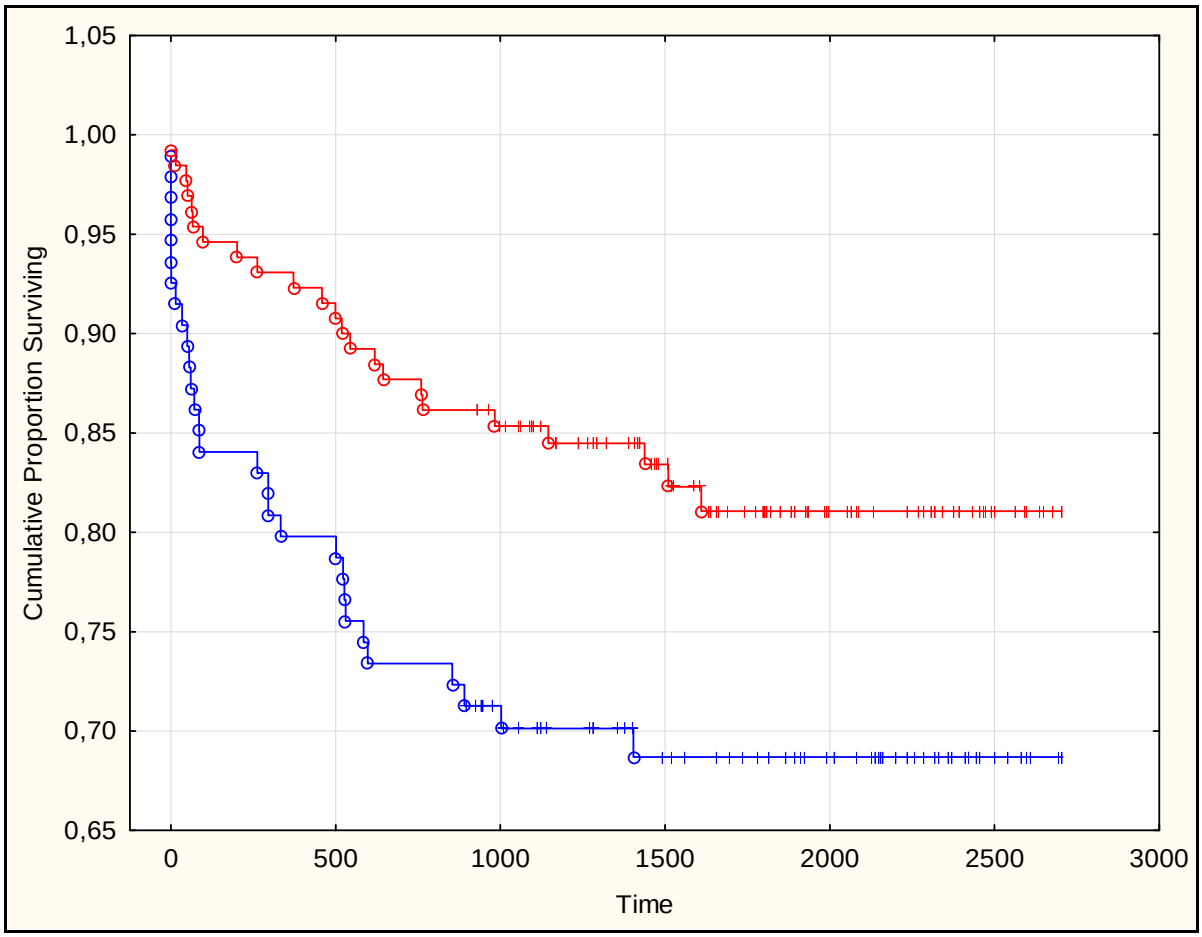
Group 1 = intra-operative bleeding > 5 litres

Group 2 = intra-operative bleeding < 5 litres

Similar results have been seen using a cut-off of 12 red blood cells units transfused: transfusion of more than 12 RBC units during the operation is correlated to a worse survival rate.

Patients transfused with more than 12 RBC units during the operation have a survival rate of 67% at 7 years post-transplant whilst patient receiving an amount of less than 12 RBC units have a survival rate of 81% ($p < 0.05$).

Graph 3. Cumulative survival according to intraoperative bleeding



Group 1 = > 12 RBC Units transfused

Group 2 = < 12 RBC Units transfused

Univariate analysis

In univariate analysis the waiting list time is inversely correlated to intraoperative bleeding and RBC and plasma transfusions. Factors that show a positive correlation to intraoperative bleeding and RBC transfusions are Child-Pugh score, MELD score, duration of surgery, Cold Ischemia Time, lower preoperative haemoglobin value, lower preoperative platelets count, higher preoperative INR value, higher preoperative bilirubin level, higher preoperative urea and creatinine value and lower preoperative albumin level.

Intraoperative bleeding and RBC/Plasma transfusions are associated to longer ICU stay and longer post-transplant hospitalization, the need of post-transplant transfusions and episodes of post-transplant gastrointestinal bleeding. Nevertheless, intraoperative blood loss and transfusions are correlated to platelets transfusions at 24 hours and during the first month post-transplant.

Multivariate analysis

In the multivariate analysis, only Cold Ischemia Time among the operation times was shown to be correlated to intraoperative blood loss and transfusion requirements ($p<0,001$). Low preoperative haemoglobin level is the strongest predictor of intraoperative blood loss and transfusions ($p<0,001$), but low preoperative platelets count ($p<0,001$) and higher preoperative INR value ($p<0,05$) are also good predictors of intraoperative bleeding and transfusions. Child-Pugh score and MELD are not predictive factors, but it is not the same for their components. In fact, preoperative creatinine ($p<0,05$), and albumin ($p<0,005$) like the INR mentioned above are correlated to intraoperative bleeding and transfusions. Anamnesis of previous bleeding ($p<0,05$) is a good predictor of higher intraoperative blood loss and the need for blood products transfusions along with pre-transplant hospitalization ($p<0,05$). No correlation was found between previous abdominal surgery and intraoperative bleeding. Among pre-transplant patients characteristics, only the presence of hepatorenal syndrome ($p<0,005$) is associated to intraoperative blood loss and transfusion requirements.

DISCUSSION

The aim of the present study was to identify the risk factors associated to blood loss and blood products transfusions and to evaluate the effect of transfusions on patient survival.

This is the first retrospective quality control study performed at Karolinska University Hospital, Huddinge, Stockholm, Sweden to assess the routine of blood products transfusions in liver transplanted patients. The critical review of the routine of intraoperative blood transfusion can lead us to a better understanding of liver transplant patients needs and improve our clinical practice. We reviewed adult liver transplantations performed during a period of five years to observe results in order to identify changes in patients perioperative treatment and to identify trends in recipient management.

Characteristics of donors and recipients were analyzed with regards to intraoperative bleeding and blood transfusion requirements. Results of the study have been reviewed to identify trends and differences in the centre over time.

The donor population showed similar characteristics over years and no statistic differences were found over time. None of the donor characteristics was correlated to intraoperative bleeding or intraoperative requirements for blood transfusions. These results confirm the observations shown by the literature[5].

The majority of the donors (91%) were brain death donors, while 9% of the donors were patients affected by Familial Amyloid Polineuropathy. These patients, who are recipients of a liver graft from a brain death donor, also act as living donors of a whole liver graft for other liver transplant recipients. This consequential liver transplantation process is defined as a "Domino Transplant"[77, 78].

Living donors for split liver transplants are not included in our analysis.

All the liver transplantations were carried out with ABO identical or compatible donors, including A_2 to O liver transplants. The most used perfusion solution for organ preservation was UW solution (Viaspan®) (57%), while HTK solution (Custodiol®) was used in 43% of cases.

Regarding liver graft quality, data on liver graft macrovesicular steatosis and ischemia grade were collected from pathology reports on Time-0-liver biopsies taken after complete reperfusion on recipients. These data are of course of paramount importance in order to

evaluate the quality of the liver graft, nonetheless we decided not to consider them in the statistical analysis because their analysis is beyond the purpose of the present study.

The overall median waiting list time was 71 days. Despite a wide variability in range, no significant statistical differences were found over the years. The fact that the median waiting list time is less than three months can reflect a dynamic policy in accepting patients onto the waiting list and transplanting them. Waiting list time depends on many factors such as the availability of donors and urgency of the liver transplant. In our series, patients transplanted for acute liver failure are included: these patients usually benefit from an urgent call for a liver, and therefore they are often transplanted after a very short waiting list time. Longer waiting list times could be related to less urgent cases or to the scarcity of donors. The timing of transplants is of paramount importance and affects the liver transplant outcome in a considerable manner. Transplants for patients in a relatively good condition lead to better results than transplants for patients with poorer clinical condition, heavy alterations in coagulation profile and severe malnutrition[8, 25, 28, 31, 33].

In the univariate analysis the waiting list time was inversely correlated to intraoperative bleeding and transfusion requirements. These results can be explained by the observations that patients with acute liver failure and poorer clinical conditions, usually requiring higher transfusions rates, are transplanted in a shorter time period. Nevertheless, this observation was not confirmed by the multivariate analysis.

The majority of the recipients were affected by liver cirrhosis (76%) caused by any disease. Cirrhosis (viral, alcoholic or a combination of these two) was the leading indication for liver transplantations during the study period. Other frequent indications were hepatocellular carcinoma on liver cirrhosis and cholestatic diseases. This distribution of diagnosis for liver transplants in the patients analyzed in the study recall the one present in Europe at present time[2].

It is clear from the literature[20, 61, 62] that portal hypertension and its complications play a pivotal role in intraoperative bleeding. The complexity of surgery in the presence of varices is one of the main causes of intraoperative blood loss[20, 61, 62]. In the absence of a direct hepato-venous pressure gradient measurement or portal flow velocimetry before liver transplantation in our patients, we entrusted the diagnosis of portal hypertension to indirect signs such as the presence of splenomegaly and anamnesis for oesophageal varices and/or hypertensive gastropathy. The majority of patients had a positive anamnesis for

oesophageal varices and/or hypertensive gastropathy (67%) and splenomegaly (66%). Nevertheless, only a third of the observed patients (33%) experienced at least one episode of gastrointestinal bleeding due to varices, and only 6 patients underwent a procedure of Transjugular Intrahepatic Portosystemic Shunt positioning. These observations could be the result of the good selection of patients and treatment and/or a good prophylactic treatment for oesophageal varices. Portal thrombosis was present in 24 patients (11%) at the waiting list time.

Portal thrombosis has not been shown to be directly correlated to intraoperative bleeding in the literature. Even if it is common to experience clinically higher intraoperative bleeding during liver transplantation due to portal hypertension and the presence of varices, results from our study did not show any correlation between the diagnosis of portal thrombosis and intraoperative blood loss.

The fact that the characteristics of patients listed for liver transplantation are quite similar over the years can reflect the use of constant criteria for the inclusion of patients in the liver transplant waiting list. Hepatorenal syndrome was experienced in 32 cases (14%) during the study period, while hepatopulmonary syndrome was a rare event in the population in analysis, affecting only 5 patients (2%). The presence of hepatorenal syndrome is one of the predictive factors for intraoperative bleeding and transfusion requirements in our analysis. An explanation of this result can be related to the fact that patients with hepatorenal syndrome are usually in poor clinical condition, and this can lead to a more complex surgery and medical support.

A large number of patients had a history of previous blood transfusions (51%); the percentages are roughly equal over time and no statistical differences were found among the years. The percentage of patients with previous blood transfusions is higher than the percentage of patients who experienced previous gastrointestinal bleeding, suggesting that the reasons for transfusions (ex. anaemia, surgical operations, trauma, delivery) are not necessarily correlated to liver disease. History of previous bleeding is correlated in the multivariate analysis to intraoperative blood loss and the need for transfusions. These results cannot be completely explained as a consequence of variceal and gastrointestinal bleeding because these other parameters do not show any correlation. Moreover, it is only anamnesis of previous bleeding and not previous transfusions that are correlated to intraoperative bleeding.

Regarding previous surgical operations, 118 (52%) of the cases observed underwent a surgical abdominal procedure. These observations also include the 61 cases (27%) that underwent at least one upper abdominal surgical procedure.

Some reports[8, 25] showed a correlation between previous abdominal surgery and intraoperative bleeding, but our analysis does not support this hypothesis. In our series no correlation was found between previous abdominal surgery and intraoperative blood loss during liver transplants, even if a strong tendency was shown. These results are probably due to the small number of patients included in the study, nevertheless they are also consistent with reports from the literature[8, 25].

A small percentage of patients had a diagnosis of diabetes mellitus type II treated with insulin before the liver transplant. There is no correlation, at least to our knowledge, between diabetes mellitus and bleeding. On the contrary, patients with diabetes mellitus can show a hypercoagulative profile with increased risk for arterial and venous thrombosis[76].

Renal function at the time of joining the waiting list was considered and data on urea, creatinine and GFR values were collected. Nevertheless, incompleteness of the data and the fact that some observations came from external laboratories (meaning variability of the results), discouraged us from using them in the statistical analysis.

Pre-transplant hospitalization is correlated to higher intraoperative bleeding and transfusion requirements. This result is fully comprehensible considering that pre-transplant hospitalization could be a surrogate marker of poorer clinical conditions.

A complete laboratory panel test is performed on every patient who undergoes a liver transplant. Among the laboratory values available, only the variables that have significance for the hematological (Hb, PLT) and coagulation status (INR, APTT) of the patients were selected for the analysis. Moreover, variables necessary to calculate MELD score (INR, Creatinine, Bilirubin) and Child-Pugh score (INR, Albumin, bilirubin) were included. Other variables such as C reactive protein were collected in the database, but the reference range for this value varied during time, making it difficult to perform a reliable analysis of the data.

At Karolinska University Hospital, MELD is one of the leading parameters for accepting patients for liver transplantation. Median MELD at the time of transplant was 13,5.

The median MELD was roughly constant among the years and no statistical difference was found over time. The MELD score was calculated on the basis of the original formula[76].

No corrections of MELD have been done for FAP or tumour diagnoses.

The most represented Child-Pugh class was class B with a median of 8 points. Child-Pugh score and MELD are not predictive factors of intraoperative bleeding, but the factors (INR, bilirubin, creatinine) that are the basis for their calculations are good predictors of intraoperative blood loss and blood transfusions instead. These data are consistent with previous studies[5, 8, 32, 35] even if there are controversial results in the literature[28, 30, 31, 32].

In our study, lower preoperative haemoglobin value was the strongest predictor of intraoperative blood loss and blood transfusion, as demonstrated by other studies[5]. Also, preoperative platelets count, INR, bilirubin, albumin and creatinine showed a positive correlation.

Regarding the surgical procedure, at Karolinska University Hospital, the classic liver transplant technique with the use of veno-venous bypass during the anhepatic phase has been the standard of care used since 1984, the year of the first liver transplant performed at the centre. The piggyback technique used for the hepatectomy with cava preservation has been increasingly used over time. Analysis showed that the percentage of liver transplants performed with cava preservation increased significantly over the years. Parallel to this, the use of intraoperative veno-venous bypass has been progressively reduced over the years.

These results reflect the general trend toward more frequent use of the piggyback technique.

There are no data demonstrating that the piggyback technique is superior to the classical technique, but it has been shown that the piggyback technique requires less blood products transfusions, probably due to shorter operation times, shorter warm ischemia time, and less fluid infusions[80, 81].

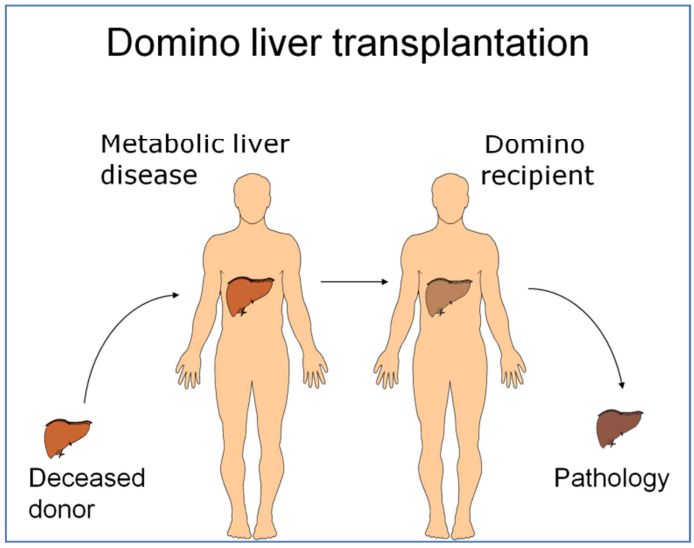
Our data did not show any correlation between the use or not of the piggyback technique and intraoperative bleeding. This could probably be due to the limited number of patients and to the surgeons' learning curve in the use of the technique. The actual trend at the centre is to use the piggyback technique as a standard technique during liver transplantation.

In our study, patients who underwent a liver transplant for Familial Amyloid Polyneuropathy (FAP) were included. These patients, according to their consent, donate

their livers to other patients, configuring the procedure known as the "Domino Liver Transplantation"[77, 78].

In this procedure, the patients affected by FAP are transplanted with a liver graft from a brain death donor. The liver removed from the FAP patient is used as a liver graft for another patient. In this complex sequential transplant procedure the classic technique with the use of intraoperative veno-venous bypass is often necessary during the hepatectomy phase in the FAP patient in order to provide a liver graft that can be used for the transplant and at the same preserve, as much as possible, the integrity of the donor-FAP patient.

Figure 1.Domino Liver transplantation (courtesy of Prof. Bo-Göran Ericzon)



The overall median operation time was 530 minutes and did not vary in a significant way over time. This can be related to the fact that at Karolinska University Hospital 6 transplant surgeons of different experience levels performed the 227 liver transplants included in the analysis, so the learning curve of some of them can explain the results. The duration of the surgical procedure was correlated to intraoperative blood loss and blood transfusions in the univariate analysis. These results were not confirmed in the multivariate analysis.

Median Cold Ischemia Time was 527 minutes and did not vary significantly over the years, even if a trend in the reduction of cold ischemia time is observed. CIT is correlated in the multivariate analysis to intraoperative bleeding and transfusions. This result, reported in other studies[28, 33] can be related to the quality of the graft: ischemic and steatotic grafts suffer a greater ischemia-reperfusion injury.

Median Warm Ischemia Time was 55 minutes and did vary significantly over the years (61 minutes in 2005 vs 49 minutes in 2009) with a significant reduction starting since 2008. These results can be explained with a progressive improvement in surgical technique. No significant differences regarding intraoperative bleeding and blood transfusions over time were observed. The use of blood products did not decrease over time and the differences over the years are not statistically significant.

Median intraoperative blood loss was 5,5 liters (range 0,5-95; n=225). Red blood cells units and plasma units transfusions are tightly correlated to intraoperative blood loss and follow its trend truthfully. In fact, the policy of the centre seems to be characterized by an intense rate of transfusions in order to mantain a haemoglobin level of 90-100 g/L, slightly higher than the level accepted in other centres[19, 26]. Intraoperative cell-savage with the use of Cell-saver is routinely used except in cases of cancer or recipient HCV and/or HBV positive. In the present series the Cell-saver was used in 39% of cases. No significant difference in the use of the device was observed during time. The use of the Cell-saver did not show any correlation with intraoperative blood loss: our results confirm the ones obtained from previous studies[34].

In our series, the use of an intraoperative porto-caval shunt is anecdotical because it was only used in 3 cases (1%) and was related to surgical needs more than being used as an instrument to reduce intraoperative blood loss[5, 82]. Also, the need for arterial interposition graft was confined to a few cases (2%). These few data did not allow us to use them for a significant statistical correlation analysis with intra-operative blood loss and transfusions.

Regarding the presence of portal thrombosis we observed that the effective presence of portal thrombosis during the surgical operation was inferior to the one expected from the patients anamnesis of portal thrombosis (7% vs 11%). These observations can be explained as an effective result of anticoagulant treatment with Low Molecular Weight Heparin (LMWH), but data regarding treated patients are lacking and we are not able to make any assumption on the effect of LMWH treatment on intraoperative blood loss.

The total intraoperative amount of cristalloids (Ringer's acetat, Natriumchloride, Glucose solutions) and colloids (Albumin) was taken into account in our data, as we wished to show the impact of fluid overload and eventual dilutional coagulopathy on intraoperative bleeding, however the paucity of data collected until now forced us not to use these data for the statistical analysis.

The use of intra-operative coagulation factors, pro-hemostatics and anti-fibrinolytics is essentially driven by clinical needs, and these agents are usually reserved to cases of massive intraoperative bleeding. The policy at the transplant centre has been to accept some intraoperative bleeding rather than overdue coagulation support by pharmacotherapy. Anti-fibrinolytics like Tranexamic acid (Cyklokapron®) and Aprotinin (Trasylol®) were used in 31 cases, while coagulation factors like factor I (Haemocomplettan®), factor II + factor VII + factor IX +factor X+ protein C + protein S (Ocplex®, Confidex®), recombinant factor VIIa (Novoseven®), antithrombin III, and von Willebrand-factor + factor VIII (Haemate®) were used, alone or in association to each other, in 11% of cases. Protaminsulfate was only used in 2 cases (<1%). The use of these pro-coagulation factors is always discussed before administration among surgeons and anhestesiologists involved in the transplants because of the risk of possible intravascular thrombosis. The policy at the centre is to mantain a high intravascular volume to avoid platelets aggregations that could promote arterial thrombosis. The counterpart of this type of management is sometimes to accept minimal residual bleeding. Vasoactive agents are routinely used in all transplants with variable frequency, and often in association with others. In this series, dopamine infusion was used in 94% of the liver transplants while noradrenalin infusion was used in 96% of the surgical procedures. Adrenalin was used in 46% of the liver transplants. Other inotropic agents such as Dobutamine, Terlipressin or Milniron were seldom used.

Intraoperative Continuous Venous-Venous Hemofiltration (CVVH) was only used in a few cases (<2%). The use of intraoperative CVVH is a valuable support to substaining the renal function necessary in the case of patients with liver failure and associated acute renal failure[83].

Our data do not consent for us to evaluate the intraoperative diuresis amount in the different phases of the transplant. At the same time, we only have data regarding intraoperative contraction of the diuresis (<30 ml/h) for a small number of the liver transplants.

During the operation, laboratory tests, mainly related to coagulation profile, (haemoglobin, platelets count, INR, APTT, fibrinogen, D-Dimer, ATIII) were taken every hour, except for the anhepatic phase during which the arterial blood gases were taken every 15 minutes. These data, including intraoperative highest INR, APTT and lactate values and lowest platelets, fibrinogen, and base excess values have been collected during the phase of data

collection. They are not included in the final analysis because the lack of many data would have minimized the power of the results. However, from a large part of the literature it is known that there is no direct correlation between coagulation parameters and intraoperative bleeding[26, 28, 31, 32, 33, 35]. The lowest intraoperative body temperature data (°Celsius) were also collected, but the absence of a clear source of the data, from oesophageal temperature probe or from bladder temperature probe, discouraged us from using it for the analysis as we do not have confounding results.

At the end of the operation, all patients were transferred to the Intensive Care Unit for post-operative care. A complete laboratory panel test is usually taken after the end of the operation. The overall median postoperative haemoglobin value was 103 g/L. This result is in line with the aim to maintain a haemoglobin level of around 80-100 g/L during the operation achieved by intense blood transfusion support during the surgical procedure.

The overall median total post-transplant hospitalization at the transplant centre was 19 days, of which there was a median of 1 day of ICU stay. No difference was observed over time.

In the univariate analysis, intraoperative bleeding and RBC/Plasma transfusions are associated to longer ICU stay and longer post-transplant hospitalization, the need for post-transplant transfusions and episodes of post-transplant gastrointestinal bleeding. These data observations were not confirmed by the multivariate analysis.

Regarding post-transplant complications in the early post-operative period (first 30 days post transplant and in any case until the end of hospitalization period), Continuous Veno-Venous Hemofiltration (CVVH) was performed in 24 cases: fluctuation in frequency of the use of the device was observed over the years, but these data do not reach a statistical difference. These observations could be interpreted as a low incidence of serious postoperative renal failure. There was no strict fluid restriction policy in the centre during the study period, moreover, the large use of intravenous bypass, avoiding clamping of the vena cava and mantaining a systemic circulation, offered a continuous support of the renal function during the surgical procedure.

Postoperative gastrointestinal bleeding occurred in 7% of cases, while re-operation was performed in 18% of cases, 46% of them due to intra-abdominal bleeding. The relatively high rate of re-operation for bleeding could be understood in regards to the policy of the

centre to actively promote anticoagulation immediately after the liver transplant procedure, with the use of Macrodex® and Fragmin® to minimize the risk for arterial thrombosis.

The counterpart is a higher rate of reoperation in those patients in which the bleeding cannot be controlled by transfusions and pharmacological agents. Nevertheless, in our series we observed less than 1% (0,8%) Hepatic Arterial Thrombosis and 1,3 % Portal Vein Thrombosis in the first 30 post-transplant days.

In 13% of cases, at least an episode of sepsis, defined as a bacterial positive blood culture, occurred. New onset post-transplant diabetes mellitus, defined as the need for insulin treatment persisting for more than 10 days post-transplant, was newly diagnosed in 37% of the observations and a significant trend in the reduction of diagnosis when comparing 2006-2007 to 2009, was observed over the years. This observation could be due to a progressive reduction in using high doses of steroids as an immunosuppressive regimen post transplant. In 43% of the observations at least one episode of acute rejection developed, usually treated with increasing basal immunosuppression and/or steroid treatment. Our intention was to insert data on the post-operative graft function to assess any impact of intraoperative bleeding on that, but we could not use any of the classification score present in the literature due to missing data or because they required parameters not usually collected at the centre[84, 85, 86].

Blood transfusions during the post-operative period were necessary in 69% of the cases. During the first 24 post-operative hours a median of 1 RBC unit, 0 Plasma units and 0 platelets units were transfused. Comparing transfusions rate among different years, it is clear that since 2006 post-operative RBC units transfusions decreased significantly. Regarding plasma transfusions during the same post-operative period, a significant difference was not shown among years, but the trend is toward a progressive reduction in transfusion rate. These results show a progressive reduction in post-operative transfusions due to a constant effort to improve intra and post-operative management.

During the post-operative period from the 2nd to the 24th post-operative days, a median of 2 RBC units, 0 plasma units and 0 platelets units were transfused, but there was no statistical difference over time. Intraoperative blood loss and transfusions showed a correlation to platelets transfusions at 24 hours and during the first month post-transplant in the univariate analysis, but the correlation was not confirmed in the multivariate analysis.

Our study showed that cold ischemia time is correlated to intraoperative blood loss and transfusion requirements, as demonstrated in other studies[28, 33]. The explanation could be related to an impact on the quality of liver graft, influenced by CIT, on the intraoperative phase after liver graft implantation.

Low preoperative haemoglobin level is the strongest predictor of intraoperative blood loss and transfusions. This result confirms observations from other studies[5, 8, 25, 32].

Low preoperative platelet counts and higher preoperative INR values are good predictors of intraoperative bleeding and transfusions. Our results are in line with observations made by Mangus[25] but contrast with results of the study performed by Massicotte[26], who showed no correlation between preoperative coagulation factors and intraoperative bleeding.

Child-Pugh score and MELD score are not predictive factors for intraoperative bleeding and transfusions, confirming results from other studies[32, 35].

Interestingly though the components of the formulas (MELD and Child-Pugh) are significant.

In fact, preoperative creatinine, albumin, and the INR mentioned above are correlated to intraoperative bleeding and transfusions. Anamnesis of previous bleeding is a good predictor of higher intraoperative blood loss and the need for blood products transfusions, as is pre-transplant hospitalization. No correlation was found between previous abdominal surgery and intraoperative bleeding. Among pre-transplant patient characteristics, only the presence of hepatorenal syndrome is associated with intraoperative blood loss and transfusions requirements. This result, to the best of our knowledge, has not been shown in other studies. Hovewer, patients with hepatorenal syndrome are in poor conditions and the result could be interpreted as a surrogate marker of poor clinical conditions and more complex surgery.

The results of the study will be used to write local guidelines to optimize the transfusion management in patients undergoing liver transplantation.

CONCLUSIONS

The retrospective study we performed has some limitations. Data are not complete and this limits the power of statistical calculations. Not all the donor and patients characteristics initially planned at the beginning of the study could be addressed in the statistical analysis due to a lack of data. However, despite these limitations we think that the results of this study can be useful to other centres.

In our analysis we demonstrated that cold ischemia time, lower preoperative haemoglobin level, lower preoperative platelets count, higher preoperative INR value, and preoperative bilirubin, creatinin and albumin are good predictors of intraoperative bleeding and the need for intraoperative transfusions.

Anamnesis of previous bleeding is a good predictor of higher intraoperative blood loss and the need for blood products transfusions, as is pre-transplant hospitalization.

No correlation was found between previous abdominal surgery and intraoperative bleeding.

Among pre-transplant patient characteristics, only the presence of hepatorenal syndrome is associated with intraoperative blood loss and transfusion requirements.

Moreover, in our analysis, only intraoperative bleeding has an impact on patient survival rates, and a cut-off of intraoperative bleeding of 5 litres and 12 RBC units transfusions showed to have a significant impact on survival rates.

FUTURE PERSPECTIVES

Our aim is to use the results of this study as a guidance to write local guidelines to optimize the transfusion management in patients undergoing liver transplantation.

On the basis of the results obtained from this study, new intraoperative transfusion protocols in liver transplantation will be discussed at Karolinska University Hospital.

New perioperative approaches will be discussed and evaluated by joint meetings between transplant surgeons and anaesthesiologists.

The increasing use of the piggyback technique has had an impact on anhestesia management because this hepatectomy technique would benefit from a lower CVP.

Mantainance of a low intraoperative CVP will be discussed, while also keeping in mind the risks that a low intravascular filling could have on the postoperative renal function.

A restrictive transfusion policy with the acceptance of a lower intraoperative haemoglobin level will be discussed, as well as the administration of erythropoietin to selected groups of the liver transplant recipients during waiting list time will be considered.

A limitation in fluid infusions and blood transfusions would reduce the systemic vascular overload and congestion in the splanchninc area. A reduction in portal pressure and abdominal varices would be helpful during the surgical prodecure, mainly during the hepatectomy phase, by minimizing the role of portal hypertension as a cause of intraoperative bleeding. At the same time, a reduction in vascular overload will reduce the risk of diluitional thrombocytopenia.

We think that these modifications in perioperative management, united to the use of intraoperative thromboelastometry, will reduce our rate of intraoperative transfusions. Intraoperative thromboelastometry by ROTEM®, which is an enhancement of the classic Thromboelstography, has been used for the last few months at the centre during liver transplantation and liver resection operations. This system allows us to guide transfusions of blood products and coagulation factors according to the real needs, thereby minimizing the risk of useless transfusions.

Intraoperative bleeding in liver transplantations and the need for intraoperative transfusions are a complex problem that requires a multidisciplinary and integrated approach.

We are convinced that we can only improve results through close and dynamic collaboration among professionals involved in the transplant.

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