**Aus der Klinik für Allgemein-, Viszeral-, Gefäß-**

**und Transplantationschirurgie**

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# Bedeutung des Ischämie-Reperfusionsschadens und therapeutische Strategien für die Organfunktion bei Lebertransplantation

**Habilitationsschrift nach neuem Recht**


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## 1. Einleitung:

Der gegenwärtige Mangel an Spenderorganen führt zum Rückgang der Transplantationszahlen. Die Rate an postmortalen Organspenden sank in Deutschland dabei von 15,8 im Jahr 2010 auf 10,7 pro eine Million Einwohner im Jahr 2014. In Österreich lag dieser Wert zuletzt bei 25,5 und in Spanien bei 35,9 postmortalen Organspenden pro eine Million Einwohner (s. Abb.1). Die absolute Zahl der postmortal gespendeten Organe in Deutschland sank von 3200 (2010) auf rund 1900 Organe (2015), entsprechend einem Rückgang von über 40 %.

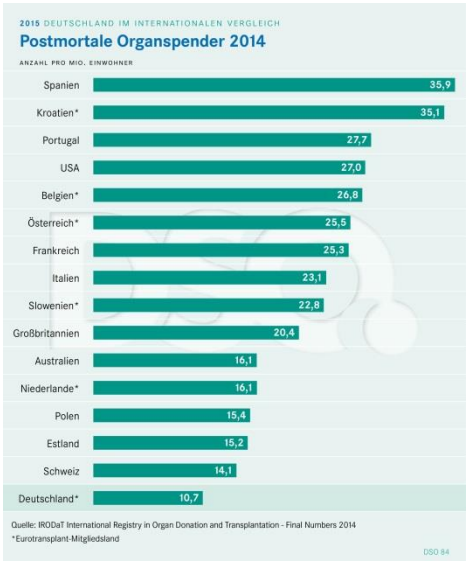



**Abb.1: Postmortale Organspende in Deutschland im internationalen Vergleich, Quelle: DSO**

Im Jahr 2015 wurden in Deutschland 846 Lebern aus postmortalen Organspenden transplantiert. Demgegenüber standen rund 1300 Neuanmeldungen auf Wartelisten zur Lebertransplantation (12). Diese Diskrepanz wird durch Lebendspenden und Split-Lebertransplantationen nur unzureichend ausgeglichen.

Infolge des Organmangels kommt es zu verlängerten Wartezeiten für eine Organtransplantation und zu einer erhöhten Wartelistenmortalität: Eine statistische Anfrage bei Eurotransplant ergab für den Verlauf des Jahres 2010 eine Letalität von 10,9 % unter den Patienten auf Wartelisten zur Lebertransplantation an deutschen Transplantationszentren. Darüber ist über die vergangenen Jahre ein schlechterer Gesundheitszustand der Organempfänger zu beobachten: Der durchschnittliche MELD-Score (model for endstage liver disease), der aus den Größen Kreatinin, Bilirubin und INR berechnet wird, liegt in Deutschland inzwischen bei einem Wert von >30 Punkten bei Standard-Organallokationen an (36). Der MELD-Score dient als Maß für die zu erwartende Überlebensdauer von Patienten mit schweren Lebererkrankungen und dient als Grundlage der Organverteilung durch

Eurotransplant (17). Ein schlechterer Gesundheitszustand der Organempfänger muss daher als eine Folge des Organmangels gewertet werden.

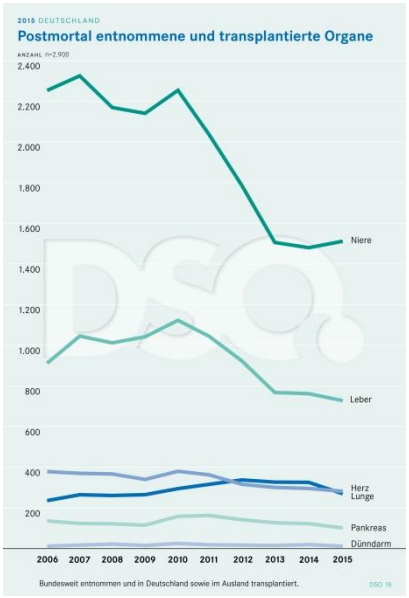
Um den Organmangel auszugleichen müssen Organe mit reduzierter Qualität (sog. „marginale Organe“, englisch: extended criteria donors ECD) zur Transplantation verwendet werden (z.B. verfettete Organe, Spender mit hohem Alter oder mit kardiovaskulären Vorerkrankungen). Diese Organe weisen eine erhöhte Rate an Organdysfunktion sowie ein reduziertes Organüberleben auf (1;10;21;28;37).

Als zentrale Ursache für schlechte Organfunktion und reduziertes Organüberleben gilt neben immunologischen Prozessen der Ischämie-Reperfusionsschaden (IRS), welcher bei Transplantation marginaler Organe verstärkt ausgeprägt ist (34;35).

Der IRS ist ein primär allogen-unabhängiger inflammatorischer Prozess, der auf molekularer, zellulärer und klinischer Ebene gut charakterisiert ist. Während der Reperfusion verstärken sich während der Ischämie beginnende Prozesse, die durch proinflammatorische Zytokine (z.B. TNFα, IL6, etc.) und eine verstärkte endotheliale Interaktion mit zirkulierenden Zellen (Leukoyzyten, Thrombozyten) und ggf. deren Transmigration in das Gewebe die Freisetzung verschiedener radikaler Sauerstoffspezies (ROS) sowie durch Mikrozirkulationsstörungen eine lokale und systemische Entzündungsreaktion hervorrufen (11;15;19). Diese Schädigungsmechanismen führen je nach Ausprägung zu Organdysfunktion und im schwersten Fall zum Organverlust (34;35). Letzteres belastet durch die Notwendigkeit einer Retransplantation den knappen Organpool zusätzlich.

Vor diesem Hintergrund müssen aktuelle Zahlen zum Organüberleben interpretiert werden, welche ein reduziertes 5-Jahres Organüberleben in Deutschland im Vergleich zum internationalen Mittel zeigen (s. Abb. 2; Quelle: DSO-Jahresbericht 2011 / CTS-Studie, Opelz G Transplantation 2013).

Die Ursachen des Organmangels können von Medizinern nur begrenzt beeinflusst werden. Daher müssen Strategien entwickelt werden, um die Funktion marginaler Organe zu verbessern (z.B. durch eine Reduktion des IRS) und durch Nutzbarmachung zusätzlicher Organe eventuell eine Vergrößerung des Spenderpools zu erreichen.



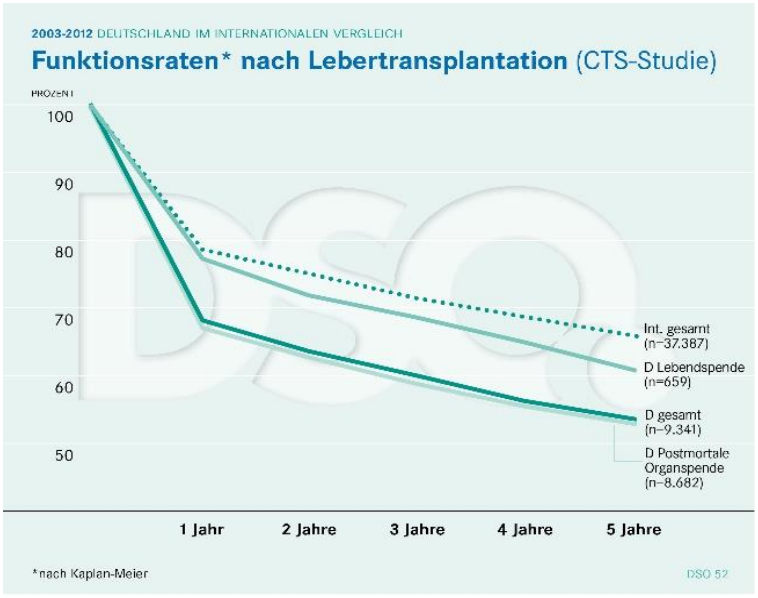




**Abb. 2:**
**A) Postmortal entnommene Organe in Deutschland**
**B) Funktionsraten nach Lebertransplantation: Deutschland im internationalen Vergleich**
**(Quelle: DSO - Deutsche Stiftung Organtransplantation)**

## 2. Ziel der Arbeit:

Diese Arbeit soll Risikofaktoren bei Lebertransplantation und zugrundeliegende Pathomechanismen in experimentellen und klinischen Studien identifizieren. Auf dem Boden dieser Erkenntnisse werden organprotektive Verfahren entwickelt, die insbesondere in marginalen Organen den IRS verbessern sollen. Dadurch könnte auch ggf. der limitierte Spenderpool erweitert werden.

## 3. Forschungsarbeiten

### 3.1 Klinische Risikofaktoren für den Ischämie-Reperfusionsschaden (IRS)

*Die Transplantation marginaler Lebern: Ein Ausweg aus dem Organmangeldilemma? Pratschke S, Loehe F, Graeb C, Jauch KW, Angele MK. Zentralbl Chir 2009 Apr;134(2):107-12.* (28)

Beim Organspender werden in der Literatur verschiedene Einflussgrößen beschrieben, mit denen eine potentiell verschlechterte Organfunktion nach Lebertransplantation assoziiert ist (Marginalitätskriterien, englisch: EDC – extended donor criteria). Aus klinischer und wissenschaftlicher Sicht ist bislang keine eindeutige Definition von Marginalität für Transplantatlebern verfügbar. Je nach Publikation und Transplantationszentrum werden bis zu 15 Kriterien (Extended Donor Criteria - EDC) beschrieben (28). Die Daten aus der Literatur sind dabei zum Teil widersprüchlich, da die Ergebnisse der meistens Studien retrospektiv erhoben wurden und

schwierig vergleichbar sind auf Grund der unterschiedlichen Definitionen für marginale Organe. Zusätzlich erscheint der zum Teil sehr unterschiedliche Erhebungszeitraum für die Beurteilung der Ergebnisse problematisch. Die unklare Datenlage stellt auch ein ethisches Problem bei der Akzeptanz von marginalen Organen zur Transplantation dar, da die zu erwartende Sterblichkeit für einen individuellen Empfänger nicht ermittelt werden kann.

Von der Bundesärztekammer wurde eine Definition marginaler Organe für Spenderlebern mit folgenden Kriterien erstellt (Bundesärztekammer: Richtlinien zur Organtransplantation gem. § 16 TPG; Richtlinie gemäß § 16 Abs. 1 S. 1 Nrn. 2 u. 5 TPG für die Wartelistenführung und Organvermittlung zur Lebertransplantation) (9): Positive Serologie für HBV oder HCV, Sepsis mit positiver Blutkultur, Meningitis, extrahepatische Tumorerkrankungen, Drogenabusus, Spenderalter > 65 Jahre, Fettleber > 40% (histologisch gesichert), Na >165 mmol/l, Intensivstation einschließlich Beatmung des Spenders > 7 Tage, Adipositas des Spenders mit BMI > 30, Kaltischämiezeit > 13 Stunden, GOT od. GPT > 3x normal, Bilirubin > 3 mg/dl. Auch von Eurotransplant wurde eine ähnliche Definition von erweiterten Spenderkriterien für Lebern erstellt (13).

Unter Berücksichtigung dieser Definition betrug der Anteil von Organen mit mindestens einem Marginalitätskriterium in Deutschland zuletzt 70% (Quelle: Statistische Anfrage Eurotransplant). Die im klinischen Alltag häufigsten Marginalitätskriterien bei Spenderorganen sind eine ausgeprägte Leberverfettung, ein hohes Spenderalter sowie eine lange Kaltischämiezeit beim Spender. In einer Auswertung der aktuellen Literatur und in Daten aus unserer Klinik konnte dabei am Beispiel der Leberverfettung gezeigt werden, dass das Vorhandensein von einem Marginalitätskriterium einen Risikofaktor für Organdysfunktion darstellt, daraus jedoch nicht zwingend ein verkürztes Organüberleben resultiert (1;28). Vielmehr scheint eine Kombination aus mehreren Risikofaktoren das Organüberleben negativ zu beeinflussen (21;34;37).

Daher wurde in einer Übersichtsarbeit die Bedeutung verschiedener erweiterter Spenderkriterien anhand aktueller Literatur dargestellt (28). Die Transplantation marginaler Organe in Spender mit hohem MELD-Score erscheint mit einer höheren Sterblichkeit verbunden zu sein. Insbesondere für die Kombination aus hohem Spenderalter und langer Ischämiezeit oder aus fortgeschrittenem Spenderalter und Hepatitis-C-Infektion des Empfängers konnte eine signifikante Verschlechterung des Organüberlebens nachgewiesen werden. Angesichts des herrschenden Organmangels sollten Anstrengungen unternommen werden, durch Verkürzung der Kaltischämiezeit und die Entwicklung von therapeutischen Strategien die Funktion und Nutzbarmachung marginaler Organe zu erhöhen und dadurch den Spenderpool zu erweitern. Die Ablehnung marginaler Organe auf dem Boden von einzelnen EDC ohne Berücksichtigung des Empfängerstatus erscheint nach derzeitigem Kenntnisstand nicht adäquat.

Vor diesem Hintergrund müssen klinisch relevante und einfach zu bestimmende Marker zur Identifikation von Hochrisikoorganen entwickelt werden, um das perioperative Management bei Transplantation dieser Organe zu optimieren.

**Auswertung der chirurgischen Lebertransplantationsdatenbank am Klinikum der Universität München**

Um eine exaktere Identifikation von Risikofaktoren bei Organspendern und –empfängern zu erreichen, wurde die Lebertransplantationsdatenbank des Klinikums Grosshadern (n=448 Lebertransplantationen) ausgewertet.

In einer Univariatanalyse konnten u.a. folgende Parameter als statistisch signifikante Prognosefaktoren für das Organüberleben gezeigt werden ($p < 0,05$): Eine Retransplantation, ein LabMELD-Score ≥ 35, ein Bilirubinanstieg im postoperativen Verlauf um mehr als 20 % und ein DRI ≥ 0,125 (29). Dabei wurde auch die positive Rolle eines intraoperativen portosystemischen Shunts sowie die prädiktive Bedeutung der intraoperativ gemessenen Blutflüsse in Leberarterie und Pfortader gezeigt (29;30) (s. 3.2 Beeinflussbare Prognosefaktoren bei Lebertransplantationon). Außerdem weisen Organe, die bei akutem Leberversagen und bei primär biliärer Zirrhose transplantiert werden, ein signifikant kürzeres Überleben auf als bei Transplantation bei malignen Grunderkrankungen, nutritiv-toxischer oder viral bedingter Leberzirrhose. In einer Multivariatanalyse konnte dabei die Bedeutung der Organqualität für das Ergebnis nach Lebertransplantation gezeigt werden: Es wurden unter anderem ein Donor Risk Index (DRI) (HRR [CI]: 3,2 [1,7–5,9], P<0,001), ein intraoperativer arterieller Blutfluss (A. hepatica) <100 ml/min (2.1 [1.3–3.2], P=0.001) als unabhängige Variablen identifiziert (29).

### 3.2 Beeinflussbare Risikofaktoren bei Lebertransplantation

Die Organperfusion spielt eine zentrale Rolle für die Transplantatfunktion nach Lebertransplantation. Während der Reperfusion auftretenden Mikrozirkulationsstörungen kommt eine Schlüsselrolle in der Pathogenese des Ischämie-Reperfusionsschadens zu (11;39). Puhl et al. konnten eine Korrelation zwischen der initialen Mikrozirkulation und der frühen Organfunktion nach Lebertransplantation zeigen (32). Gleichzeitig belegen Studien den Zusammenhang zwischen Mikrozirkulation und dem makrovaskulären Blutfluss der zuführenden Lebergefäße (41). Außerdem beeinflusst die systemische Hämodynamik die Leberdurchblutung, zum Beispiel der mittlere arterielle Druck oder der venöse Abfluss infolge technischer Probleme oder durch ein mögliches Rechtsherzversagen (8).

**Einfluss der intraoperativen Makroperfusion auf Organfunktion und -überleben**

*Arterial blood flow predicts graft survival in liver transplant patients. Pratschke S, Meimarakis G, Mayr S, Graeb C, Rentsch M, Zachoval R, et al. Liver Transpl 2011 Apr;17(4):436-45.* (30)

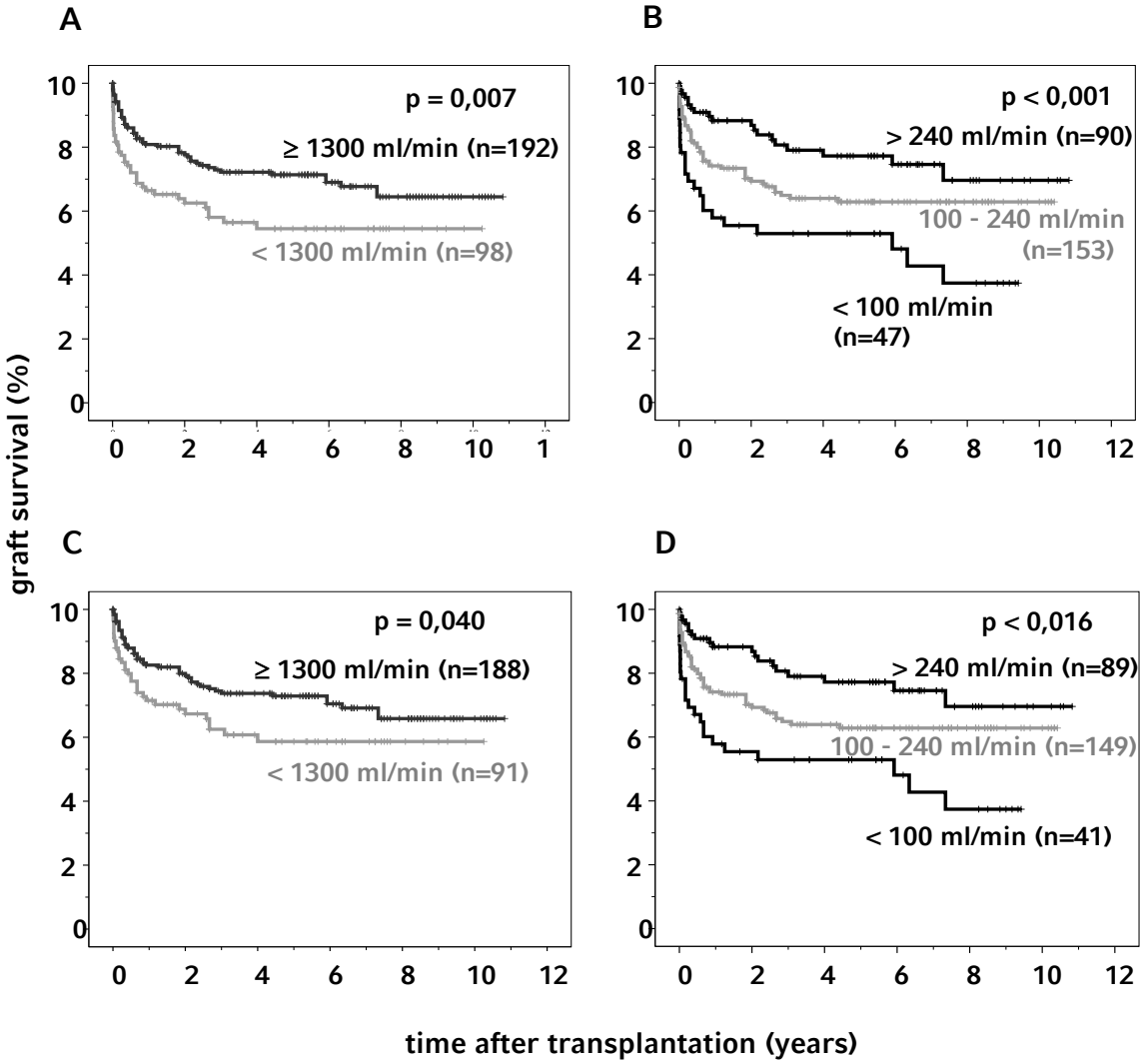
In einer klinischen Arbeit konnte als klinisch einfach durchführbare Methode zur Identifikation von Hochrisikoorganen die intraoperative Messung des Blutflusses von Pfortader und Leberarterie bei Lebertransplantation identifiziert werden (30). Bei 290 Lebertransplantationen am Klinikum Grosshadern wurden hierzu intraoperativ nach Reperfusion die portalvenösen und arteriellen Blutflüsse gemessen und mit der Organfunktion und dem Organüberleben korreliert. Als cut-off Punkte wurden mittels CRT-Analyse für den arteriellen Blutfluss 100 ml/min und 240 ml/min (ART I: <100 ml/min, ART II: 100-240 ml/min, ART III: ≥240 ml/min) bzw. 1300 ml/min (PV I: <1300 ml/min, PV II: ≥1300 ml/min für den portalvenösen Fluss identifiziert.

Die Höhe des arteriellen und portalvenösen Blutflusses hatte keine Auswirkungen auf die akute postoperative Organfunktion und –schädigung (GOT, GPT als klinisches Maß für den IRS; Bilirubin, Quick) während der ersten sieben Tage nach Transplantation ($P > 0,05$). Dagegen führte insbesondere ein reduzierter arterieller Blutfluss zu einem signifikant schlechteren Organüberleben (s. Abb.3).

Gleichzeitig waren niedrige intraoperative Flüsse assoziiert mit einem erhöhten Risiko für eine primäre Transplantat Nonfunktion (PNF), sowohl bei Pfortader (PV I: 7,1% vs. PV II: 2,1%; P=0.0487) als auch Leberarterie (ART I: 12,8%, ART II: 2,6%, ART III: 1,1%). Auch in der multivariaten Analyse zeigte sich ein signifikant erhöhtes Risiko für eine primäre Transplantat Nonfunktion (bei Patienten mit einem arteriellen Blutfluss < 100 ml/ min (HRR 7,4; CI [2,1-26,3]; P=0,002) und einem Gesamt-Fluss < 1400 ml/ Minute (HRR 4,8; CI [1,3-17,6]; P=0,017).

Die Retransplantationsrate korrelierte ebenfalls mit dem arteriellen Blutfluss (ART I: 21,3%, ART II: 8,5%, ART III: 5,6%; P=0.010). Auch in der multivariaten Analyse war ein arterieller Fluss < 100 ml/ min ein Risikofaktor für Retransplantation (HRR 3,4; CI [1,4-7,9]; $P < 0,005$).

In der Multivariatanalyse erhöhte ein arterieller Blutfluss < 100 ml/min als unabhängige Variable das Risiko für Organversagen nach Transplantation um mehr als das doppelte mit einer hazard rate ratio




von 2,5; CI [1,6-4,1]; P<0,001.

**Abb. 3: Kumulatives Transplantatüberleben 10 Jahre nach Lebertransplantation bezogen auf (A) Pfortaderfluss (B) arterieller Blutfluss (C) Pfortaderfluss (Patienten mit PNF ausgeschlossen) (D) arterielle Durchblutung (Patienten mit PNF ausgeschlossen)**

Eine intraoperative Blutflussmessung ist daher eine prädiktive und leicht durchführbare Methode zur Identifikation von Hochrisikoorganen bei Lebertransplantation (30). Gleichzeitig müssen Therapien zur Verbesserung der Organperfusion entwickelt werden (s. 3.4 Prospektive klinische Studien).

**Bedeutung eines intraoperativen portosystemischen Shunts für Organfunktion und -überleben**

- *Temporary intraoperative porto-caval shunt: useless or beneficial in piggy back liver transplantation? Pratschke S, Meimarakis G, Bruns CJ, Kaspar M, Prix N, Zachoval R, et al. Transpl Int 2013 Jan;26(1):90-8.* (29)
- *Temporary intraoperative porto-caval shunts in piggy-back liver transplantation reduce intraoperative blood loss and improve postoperative transaminases and renal function - a meta-analysis. Pratschke S., Rauch A., Albertsmeier M., Rentsch M., Kirschneck M., Andrassy J., et al. World J Surg. 2016 Dec;40(12):2988-2998.* (31)

Neben Messgrößen zur Identifikation von Risikoorganen wurde in bisherigen Arbeiten nach beeinflussbaren Faktoren gesucht, die mit einfach durchführbaren Maßnahmen zur Verbesserung der Funktion marginaler Organe beitragen können.

Eine wichtige Rolle könnte hierbei die Anlage eines temporären, intraoperativen porto-cavalen Shunts bei Lebertransplantation spielen. Während der anhepatischen Phase, nach dem Abklemmen der Pfortader, fördert die venöse Stase im Darm die Freisetzung von Endotoxin, das bei der Reperfusion zunächst über die Pfortader in die Leber und sekundär in die systemische Zirkulation gelangt (2). Endotoxin aktiviert intrahepatische Kupfferzellen, die eine zentrale Rolle bei der Initiierung des hepatischen Ischämie-Reperfusionsschadens durch Freisetzung proinflammatorischer Zytokine und von Sauerstoffradikalen spielen (2;22). Bei der orthotopen „en bloc“ Transplantation wird die Leber inklusive der Vena Cava entnommen und es kommt zur kompletten venösen Abstromunterbrechung aus der unteren Körperhäfte. Daher wurde als Weiterentwicklung der „en bloc“ Transplantation, bei der die Leber inklusive der, die sog. Cava-erhaltende Lebertransplantation (z.B. „Piggy Back“) entwickelt (5;45). Dennoch kann auch durch diese Technik die mesenteriale Stase während des Abklemmens der Pfortader nicht vollständig verhindert werden. Daher wurde durch Belghiti ein zusätzlicher intraoperativer portosystemischer Shunt bei Cava-erhaltender Lebertransplantation durch eine temporäre End-zu-Seit Anastomose zwischen Pfortader und Vena Cava beschrieben (4) (s. Abb.4). Am Klinikum Grosshadern wird dieser Shunt als Kunststoffkatheter zwischen Pfortader und Vena femoralis angelegt (s. Abb.4). Die Indikation zur Anlage eines Shunts wird im Klinikum Grosshadern in Abhängigkeit von der individuellen Risikoeinschätzung der Spender-Empfängerkonstellation und dem Vorhandensein adäquater portosystemischer Kollateralen durch den Operateur gestellt.

In einer Untersuchung der Lebertransplantationsdatenbank des Klinikums Grosshadern wurden 448 Lebertransplantationen (1/1997-6/2010) im Hinblick auf Organschädigung und -überleben mit oder

ohne Anlage eines porto-femoralen Shunts untersucht (Shunt n=274, kein Shunt n=174; mittlerer Nachbeobachtungszeitraum 50 Monate) (29).

Als Maß für den Ischämie-Reperfusionsschaden diente der GOT- und GPT-Anstieg. Unter Berücksichtigung potenzieller Confounder (Alter, Geschlecht, Retransplantion) wurde eine univariate und multivariate Analyse hinsichtlich des Organüberlebens durchgeführt.

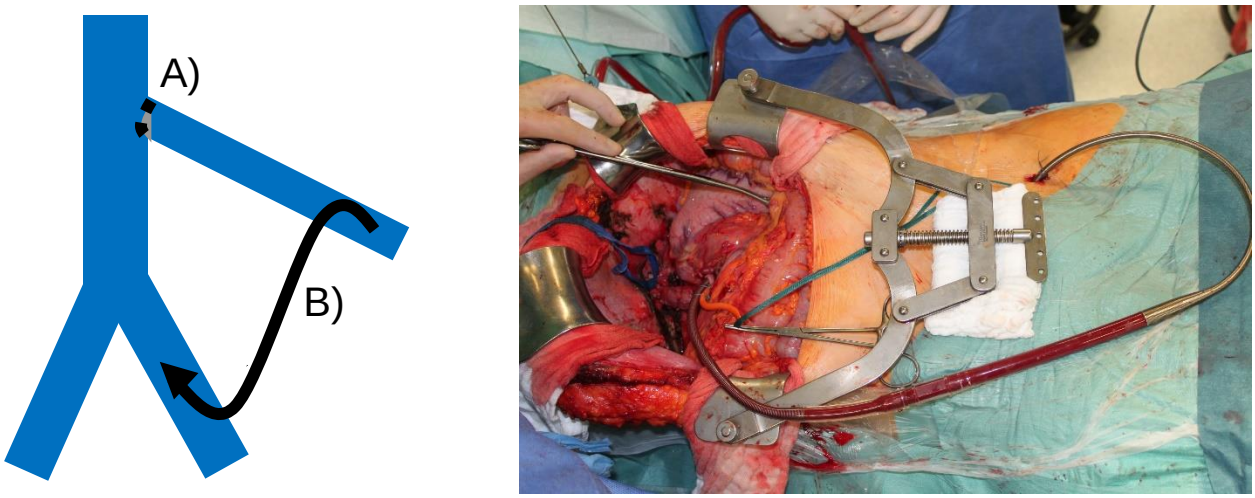



**Abb. 4: Temporärer intraoperativer Shunt. A: Portocavale End-zu-Seit Anastomose (Belghiti). B: Portofemoraler Katheter Shunt (Klinikum Grosshadern)**

Die Verwendung eines intraoperativen portofemoralen Shuntkatheters war mit einer signifikanten Verringerung der Transaminasen als klinischer Surrogatparameter des Ischämie-Reperfusionsschadens bis zum 7. postoperativen Tag verbunden (s. Abb.5).

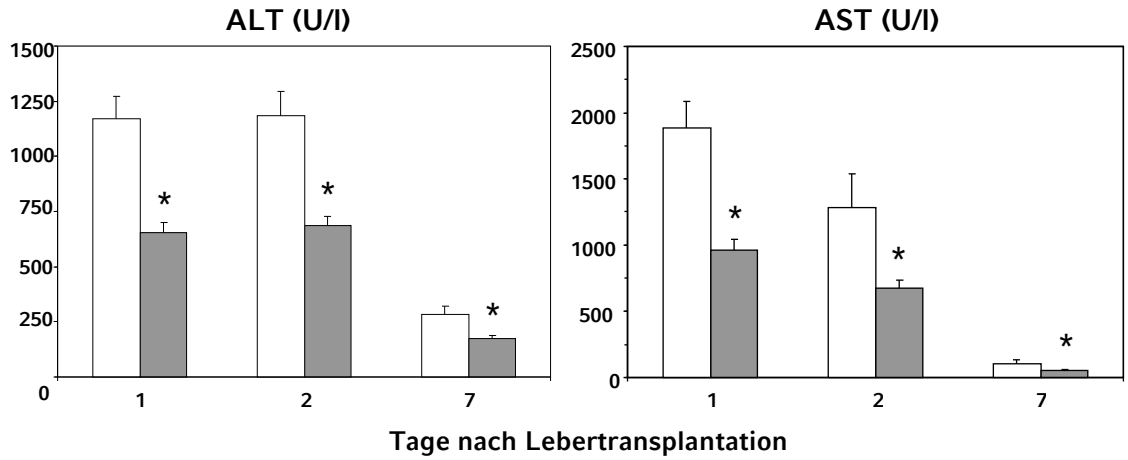



**Abb.5: Perioperative Organschädigung in Abhängigkeit von der Anlage eines portosystemischen Shunts; * P < 0,05 vs. kein Shunt (weiße Säulen: Kein Shunt, graue Säulen: Shunt)**

Darüber hinaus war das mittlere Organüberleben bei Anlage eines Shunts signifikant verlängert (MW in Monaten ± SD): Shunt 106,8 [98,0-115,7] vs. kein Shunt (86,5 [73,5-99,5]; p=0,001 (Abb. 6).

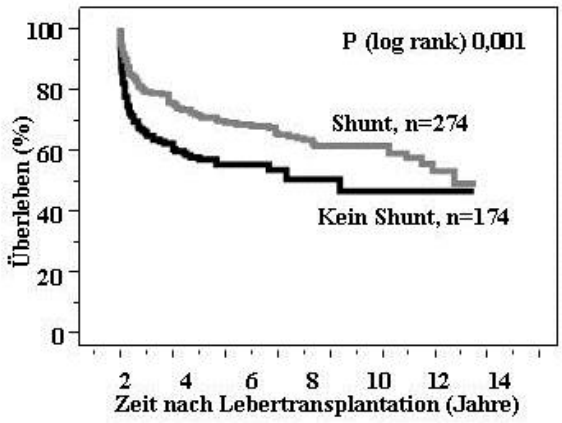



**Abb. 6: Transplantatüberleben in Abhängigkeit von der Anlage eines portosytemischen Shunts**

Interessanterweise war dieser Effekt besonders ausgeprägt bei Empfängern von Organen mit reduzierter Qualität mit einem Donor Risk Index ≥ 1.25 (s. Abb.7 B).

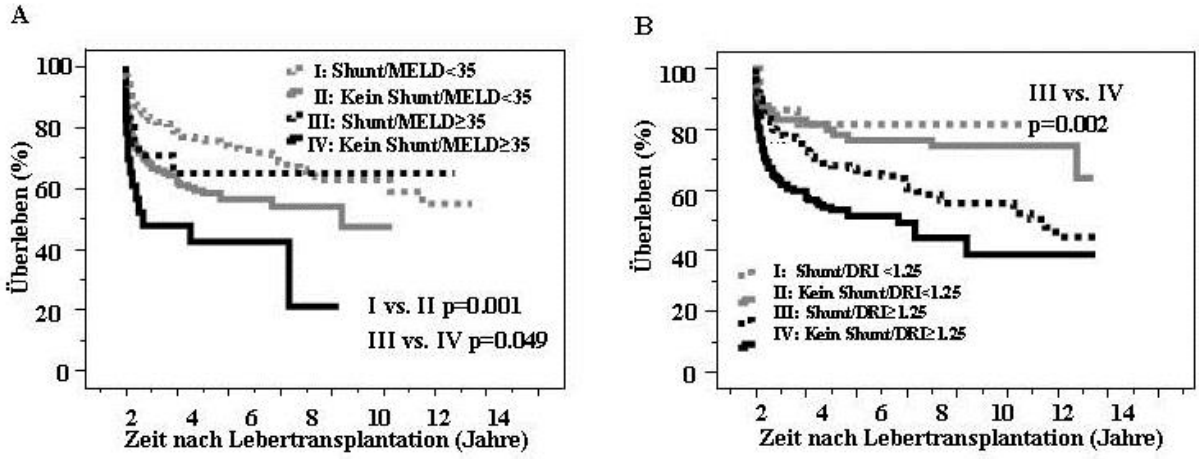



**Abb. 7: A: Transplantatüberleben in Abhängigkeit von der Anlage eines portosystemischen Shunts und dem MELD-Score. B: Transplantatüberleben in Abhängigkeit von der Anlage eines portosystemischen Shunts und dem Donor Risk Index.**

In einer Multivariatanalyse fanden sich als unabhängige Prognosefaktoren für ein schlechtes Ergebnis nach Lebertransplantation die Transplantation ohne intraoperativen portosystemischen Shunt mit einer hazard rate ratio von 2,1 ($p < 0,001$), ein intraoperativ gemessener Fluss in der Leberarterie unter 100 ml/min (hazard rate ratio 2,1; $p = 0,001$) sowie ein Donor Risk Index über 1,25 mit einer hazard rate ratio von 3,2 ($p < 0,001$) (29).

In einer Metaanalyse anhand aktueller Literatur zu portosystemischen Shunts konnte gezeigt werden, dass diese Technik zu signifikant verringerten AST-Werten, weniger Bluttransfusionen und verbesserter postoperativer Nierenfunktion führt ($P < 0,05$). Gleichzeitig fanden sich keine statistisch signifikanten Unterschiede bei der primären Transplantat Nonfunktion, der Länge des Krankenhausaufenthaltes sowie der OP-Dauer (31).

Die teilweise diskrepanten Ergebnisse zu diesem Thema verdeutlichen die Notwendigkeit prospektiver Daten zu diesem Thema. Eine randomisierte Studie zu portocavalen Shunts bei Lebertransplantation ist daher anzustreben.

Insbesondere bei Hochrisiko Konstellationen aus marginalen Organen (hoher Donor Risk Index) und kranken Empfängern (hoher MELD-Score) könnte durch die Verwendung eines intraoperativen Shunts eine Verbesserung der Organfunktion und des Organüberlebens erzielt werden.

### 3.3 Experimentelle Studien zu Pathomechanismen und Therapieformen des IRS

**Etablierung eines Schädigungsmodells bei experimenteller Lebertransplantation**

Zur Untersuchung von Schädigungsmechanismen marginaler Organe bei Ischämie und Reperfusion wurde ein orthotopes, arterialisiertes, syngenes Modell der Fettleber-Transplantation an Ratten etabliert. Als Spendertiere dienten hierbei Zuckerratten mit einem homozygoten Leptinrezeptor-Defekt (Phänotyp: Fat) bzw. mit heterozygotem Leptinrezeptor-Defekt (Phänotyp: Lean). Bei Spendertieren mit homozygotem Leptinrezeptordefekt weisen die Lebern im Alter von 10 Wochen einen Verfettungsgrad von 40% auf. Die kalte Ischämiezeit in hypothermer (4°C) UW-Lösung (University of Wisconsin) betrug bei schlanken Lebern 24 Stunden, bei verfetteten Lebern 4 Stunden. Empfänger waren ausschließlich schlanke Tiere.

Es konnte ein verstärkter Ischämie-Reperfusionsschaden auf dem Boden einer gesteigerten Produktion von Sauerstoffradikalen (ROS) im Vergleich zu Spenderlebern aus schlanken Zuckerratten mit heterozygotem Leptinrezeptor-Defekt (Phänotyp: Lean) anhand klinischer Parameter (ALT, AST, LDH) gezeigt werden. Dabei waren klinische Marker des IRS bei Transplantation dieser Organe gegenüber nicht verfetteten Lebern signifikant erhöht ($P < 0,05$) (24).

**Experimentelle Therapien des IRS**

**Untersuchung eines ROS Scavengers (Glutathion) bei Transplantation von Fettlebern**

*GSH attenuates organ injury and improves function after transplantation of fatty livers. Pratschke S, Angele MK, Grutzner U, Tufman A, Bilzer M, Loehe F, et al. Eur Surg Res 2010;45(1):13-9.* (24)

In nicht-verfetteten Organen ist Glutathion (GSH) ein effizienter Fänger von ROS, was zu einem reduzierten IRS nach GSH-Gabe führt (29). Reaktive Sauerstoffspezies (ROS) spielen dabei eine Schlüsselrolle in der Pathogenese des Ischämie-Reperfusionsschaden der Leber (7;33).

Ziel dieser Studie war es, zu untersuchen, ob GSH eine protektive Wirkung auf den Ischämie-Reperfusionsschaden in verfetteten Transplantaten entfaltet. Hierzu wurden die Empfängertiere von Fettlebern während der Reperfusion (120 min) systemisch intravenös mit GSH (200 mol/h/kg) oder mit Kochsalzlösung behandelt (jeweils n=5). Dabei wurden Parameter des hepatozellulären Schadens und der Gallefluss gemessen. Die Transplantation von verfetteten Lebern verstärkte den frühen Ischämie-Reperfusionsschaden im Vergleich zu nicht verfetteten Organen, was sich durch erhöhte AST-, ALT- und LDH-Spiegel äußerte (s. Abb.8A). Der Gallefluss als Maß für die exkretorische Funktion der Lebern war in steatotischen Transplantaten ebenfalls reduziert (s. Abb.8B; P < 0,05).

Die intravenöse Verabreichung von GSH reduzierte dabei signifikant Leberschäden und führte zu einer verbesserten Exkretionsleistung (Gallefluss) bei Transplantation von Fettlebern (P<0,05) (24).

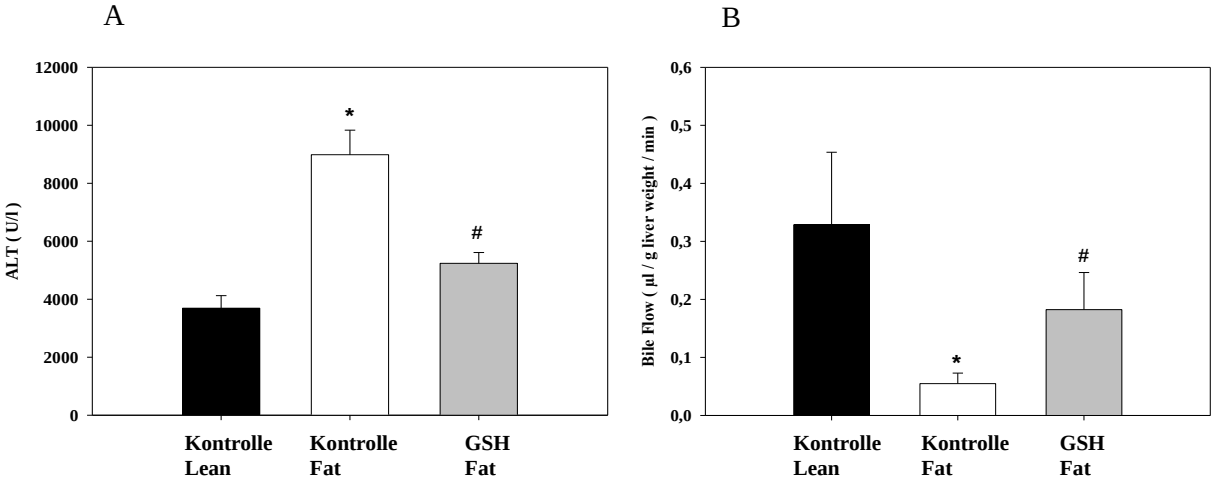



**Abb. 8** **A: MW ± SD, one-way ANOVA. * P<0,05 vs. Kontrolle Lean, # P<0,05 vs. Kontrolle fat**

**B: MW ± SD, one-way ANOVA. * P<0,05 vs. Kontrolle Lean, # P<0,05 vs. Kontrolle fat**

**Einfluss eines Calcineurin Inhibitors auf den IRS bei Lebertransplantation**

> *Tacrolimus Preconditioning of Rat Liver Allografts Impacts Glutathione Homeostasis and Early Reperfusion Injury. Pratschke S, Bilzer M, Grutzner U, Angele M, Tufman A, Jauch KW, et al. J Surg Res 2011 Aug 25.* (26)

Eine weitere experimentelle Studie untersuchte Effekte eines Immunsuppressivums auf den primär inflammatorischen Prozess des IRS. Die antiinflammatorische Wirkung von Calcineurininhibitoren (CNI, z.B. Tacrolimus, Cyclosporin, Sirolimus) auf ischämische Schädigungen verschiedener solider Organe ist dabei bekannt (3;16). In bisherigen Modellen wurden jedoch systemisch die Spender präkonditioniert, was die klinische Umsetzung solcher Therapien aufgrund der transnationalen Organallokation und einem daraus resultierenden unübersichtlichen Empfängerkreis behindert. Eine erfolgreiche Behandlung mit CNI zu einem späteren Zeitpunkt ist dagegen eine klinisch relevante Option zur Behandlung ischämischer Schädigungen.

In der vorliegenden Versuchsreihe wurden nicht verfettete Transplantatlebern aus männlichen Lewis Ratten in einem Akut-Modell des IRS einmalig ex vivo mit Tacrolimus präkonditioniert. Nach 24-stündiger kalter Ischämie (4°C) in University of Wisconsin (UW) Lösung wurden die Lebern am Ende der Kaltpräparation mit 20 ml verdünntem Tacrolimus perfundiert. Es wurden drei Gruppen (jeweils n=6) verglichen: Tacrolimus 50 ng/ml, Tacrolimus 10 ng/ml, NaCl 0,9% (Kontrolle). Die Reperfusionsdauer betrug 120 Minuten (26).

Eine einmalige ex vivo Rinse der Transplantate unmittelbar vor Implantation mit Tacrolimus (Konzentration 10 bzw. 50 ng/ml) bewirkte eine relevante Organprotektion (ALT, AST, LDH, Bilirubin) 120 Minuten nach Reperfusion einhergehend mit Veränderungen der Glutathion-Homöostase: Bei Verabreichung von Tacrolimus (10 ng/ml) verringerte sich der ALT-Spiegel im Serum auf 1740 ± 1169 U/l vs. 3691 ± 1144 U/l (Kontrolle), $P<0,05$). Während die intrazelluläre GSH-Konzentrationen im Lebergewebe unverändert blieb, war GSSG, das Oxidationsprodukt von GSH, deutlich verringert in präkonditionierten Lebern (47,0 ± 10,4 nm/g vs. 71,8 ± 30,6 nm/g; $P<0,05$). Die Ursache hierfür könnte eine vermehrte Ausscheidung von GSSG in die Galle (0,19 ± 0,04 mm vs. 0,13 ± 0,04 mm (Kontrolle); $P<0,05$) sowie ins Plasma (2,4 ± 0,3 mM vs. 1,4 ± 0,2 mM (Kontrolle); $P<0,05$) sein (26). Diese Veränderungen sind relevant, da intrazelluläres GSSG in hohen Dosen zytotoxische Eigenschaften aufweist und somit u.a. für die beobachteten Effekte einer Tacrolimus Rinse verantwortlich sein könnte (14;26). Überraschenderweise waren die Effekte der Tacrolimus Rinse nicht dosisabhängig sondern stärker ausgeprägt in der niedrigeren Konzentration von 10 ng/ml gegenüber 50 ng/ml (Abb. 9).

**Hepatocellular Injury and Liver Function Following Transplantation**

| | Sham [$n = 5$] | Control [$n = 6$] | Tac 50 [$n = 6$] | Tac 10 [$n = 6$] | $P$ value [*$versus$ control] |
|---|---|---|---|---|---|
| ALT [U/l] | 108 ± 12 | 3691 ± 1444 | 2213 ± 816* | 1740 ± 736* | <0.05 |
| AST [U/l] | 72 ± 9 | 2854 ± 676 | 2312 ± 412* | 1470 ± 812* | <0.05 |
| BF baseline [$\mu$L/min/g] | 1.03 ± 0.6 | 1.1 ± 0.4 | 0.9 ± 0.3 | 1.1 ± 0.5 | - |
| BF maximum [$\mu$L/min/g] | 1.05 ± 0.2 | 0.43 ± 0.1 | 0.52 ± 0.08 | 0.68 ± 0.12* | <0.05 |
| BF cumulative [$\mu$L/2 h/g] | 111.5 ± 4.8 | 34.7 ± 5.9 | 41.2 ± 4.2 | 53.4 ± 6.1* | <0.05 |

Sham operated animals underwent only laparotomy, but had open abdomen for the same time period as necessary for transplantation and reperfusion time. Tac 50 = 50 ng/mL and Tac 10 = 10 ng/mL tacrolimus added to rinse solution. ALT and AST serum levels were analyzed at the end of the 2 h reperfusion period. BF = bile flow; baseline values represent bile flow as measured in donors.
*$P < 0.05$ versus controls.

**Abb. 9: Transaminasen, Gallefluss 120 Minuten postoperativ: Sham, Kontrolle, Tacrolimus 50 / 10 ng/ml. *P<0,05 vs. Kontrolle**

### 3.4 Prospektive klinische Studien

**Organkonditionierung mit Nitroglycerin**

Die retrospektiv erhobenen Daten zur Bedeutung der Leberperfusion für die Transplantatfunktion und das Überleben (30) waren Anlass für eine Untersuchung mit einer vasoaktiven Substanz bei Lebertransplantation. Es wurde eine klinische Studie zur intraoperativen Gabe des NO-Donors Nitroglycerin mit dem Ziel einer verbesserten Organdurchblutung bei Lebertransplantation durchgeführt (nicht veröffentlichte Daten). Bei 44 konsekutiven Lebertransplantation wurden Organe mit einem Donor Risk Index (DRI) ≥ 1.9 mit 5 mg Nitroglyzerin (Injektion in Arteria hepatica) unmittelbar vor Implantation präkonditioniert. Diese Transplantationen wurden verglichen mit 44 Lebertransplantationen ohne Gabe von Nitroglyzerin mittels matched pairs Analyse anhand der Lebertransplantationsdatenbank unserer Klinik. In die Untersuchung gingen ein der Lab-MELD-Score, der Donor Risk Index (DRI), die postoperative Leberschädigung (ALT, AST), Transplantatfunktion (Bilirubin, Quick), die intraoperativ gemessenen Blutflüsse in Leberarterie und Pfortader und das Organüberleben. Bei keinem der Parameter konnte ein Unterschied zwischen den Gruppen gefunden werden (P > 0,05). Trotz dieser Negativdaten sind weitere Untersuchungen mit vasoaktiven Substanzen erforderlich, um deren Interaktion mit dem Ischämie-Reperfusionsschaden zu untersuchen.

**TOP-Studie (Tacrolimus Organ Perfusion)**

- *Protocol TOP-Study (tacrolimus organ perfusion): a prospective randomized multicenter trial to reduce ischemia reperfusion injury in transplantation of marginal liver grafts with an ex vivo tacrolimus perfusion. Pratschke S, Eder M, Heise M, Nadalin S, Pascher A, Schemmer P,*

*Scherer MN, Ulrich F, Wolters H, Jauch KW, Wöhling D, Angele MK. Transplant Res. 2013 Mar 4;2(1):3.* (27)

- *Results of the TOP Study: Prospectively randomized multicenter trial of an ex vivo Tacrolimus rinse prior to Transplantation in EDC Livers. Pratschke S, Arnold H, Zollner A, Heise M, Pascher A, Schemmer P, et al. Transplantation direct 2016 May 4;2(6):e76.* (25)
- *The Authors' Reply. Pratschke S, Angele MK. Transplant Direct. 2017 Mar 28;3(4):e148. 2016***.**

Auf dem Boden der experimentellen Vorarbeiten zu einer Tacrolimus Rinse bei Lebertransplantation an der Ratte (26) wurde eine IIT (investigator initiated trial) bei Lebertransplantation durchgeführt (27). Für die als Immunsuppressivum zugelassene Prüfsubstanz Tacrolimus wurden in experimentellen Arbeiten und in zwei klinischen Studien protektive Wirkungen auf den hepatischen IRS beschrieben (20;23;26). Jedoch fehlten bislang Aussagen zur Wirksamkeit einer solchen Therapie bei marginalen Organen, wo der Wirksamkeit von Tacrolimus besondere Bedeutung zukommt.

Ziel der TOP-Studie (Tacrolimus Organ Perfusion) war, angesichts des herrschenden Organmangels und der daraus resultierenden Transplantation marginaler Organe, die Untersuchung einer medikamentösen Therapie mit Tacrolimus für den Ischämie-Reperfusionsschaden bei Transplantation von Lebern mit reduzierter Qualität.

**Studiendesign:**

Die Studie wurde durchgeführt als prospektive randomisierte Multicenterstudie (Phase III Studie), in der marginale Spenderorgane mit Tacrolimus perfundiert wurden (38). Teilnehmende Zentren waren neben München (LMU) Berlin, Heidelberg, Mainz und Regensburg.

- Offene, nicht verblindete Studie
- Zweiarmiges, Placebo-kontrolliertes Studiendesign:

**A:** Ex vivo Perfusion marginaler Transplantatlebern mit Tacrolimus (20 ng/ml) gelöst in 1000 ml HTK: Sequenzielle arterielle (500 ml) und portalvenöse (500 ml) ex vivo Perfusion mit HTK vor Implantation nach Ende der Kaltpräparation

**B:** Ex vivo Perfusion marginaler Transplantatlebern mit 1000 ml HTK

Haupteinschlusskriterium war das Vorliegen von zwei oder mehr Marginalitätskriterien beim Organspender bei Lebertransplantation in Anlehnung an die Definition von Eurotransplant (s. Tab. 1):

- Spenderalter > 65 Jahre
- Makrovesikuläre Steatose > 40%
- BMI > 30
- Natrium >165 mmol/l

- Intensivstation und/ oder Beatmung > 7 Tage
- Kalte Ischämiezeit > 13 Stunden
- AST > 99 U/l
- ALT > 105 U/l
- Bilirubin > 3 mg/dl (> 51 µmol/l)
- Gabe von Adrenalin

**Tab.1: Extended Donor Criteria (EDC) (nach: Eurotransplant manual (13))**

Beim Empfänger waren die Einschlusskriterien erstmalige Organtransplantation, chronische terminale Leberinsuffizienz sowie ein Alter > 18 Jahre.

Primärer Endpunkt der Studie waren die maximal gemessenen ALT-Spiegel im Serum als klinisches Maß für den Ischämie-Reperfusionsschaden.

Sekundäre Endpunkte:

- GPT, GOT postoperativ an den Tagen 1, 2, 4, 7
- Transplantatfunktion (Quick, Bilirubin)
- Organ- und Patientenüberleben
- Biopsiegesicherte Abstoßungen

Die maximalen ALT-Spiegel in den mit Tacrolimus behandelten Prüfungsteilnehmern wurden mittels nicht parametrischer Verfahren (Wilcoxon Rangsummentest) mit Placebo verglichen. In Anlehnung an frühere Daten von Peter et al. (39) wurde eine Effektgröße von 0,7 angenommen. Die statistische Power des Tests betrug 80% bei einem Signifikanzniveau von 0,05. Eine Fallzahlschätzung (NQueryAdvisor 6.1) für zwei unverbundene Stichproben mittels Wilcoxon Rangsummentest erbrachte bei einer erwarteten drop-out Rate von 15% eine Population von 86 Studienteilnehmern (43 Tacrolimus vs. 43 Placebo).

**Ergebnisse:**

Es wurden 25 Prüfungsteilnehmer randomisiert und in die Studie eingeschlossen. Ein Prüfungsteilnehmer (Zentrum 06, Mainz, Patient 003) wurde nach Studieneinschluss und Randomisierung aber noch vor Beginn der Studienbehandlung aus medizinischen Gründen nicht transplantiert. Diesen Patienten eingerechnet betrug die drop-out rate 4 % (25).

Die Dauer der Perfusion betrug im Median 19 (Range: 8-22) in der Tacrolimus/Intervention Gruppe und 15 (10-27) in der HTK/Placebo Gruppe (Wilcoxon rank-sum test, p=0,574).

Die ex vivo Perfusion von marginalen Lebern mit Tacrolimus (n=11; 20 ng/ml, gelöst in 1000 ml HTK) führte zu keinem statistisch signifikanten Effekt beim primären Endpunkt der Studie (p=0.207), dem maximal gemessenen GPT-/ ALT-Wert innerhalb der ersten beiden Tage nach Transplantation, gegenüber der Vergleichsgruppe (n=13), die mit 1000 ml HTK perfundiert worden war (U/l; Median (Range)): 812 (362; 3403) vs. 652 (147; 2034). Der Hodges-Lehman Schätzer für das 95% Konfidenzintervall des Medianen Unterschieds war (-178; 1166). Durch den vorzeitigen Studienabbruch kann aufgrund der geringen Patientenzahl keine Aussage zu Unterschieden zwischen den Zentren getroffen werden.

Der Vergleich der Behandlungsarme an den Tagen 1, 2, 4 und 7 mit Hilfe des multivariaten Rangsummentests nach O'Brien zeigte für die Parameter GPT, GOT, Quick und Bilirubin folgende p-Werte: 0,100, 0,011, 0,553 und 0,815.

Das Organ- und Patientenüberleben war gleich in beiden Behandlungsarmen. Die Anzahl der Abstoßungen war vergleichbar (p = 1,000).

Um mögliche Auswirkungen einer Spülbehandlung zu untersuchen, wurden die Ergebnisse der vorliegenden Studie in einer matched pairs Analyse mit 24 konsekutiven Lebertransplantationen (Spender ≥ 2 EDC) aus der Lebertransplantationsdatenbank unserer Klinik verglichen. Die präoperativen ALT- und AST-Werte unterschieden sich nicht zwischen Patienten der Studie und Placebo (p > 0,05). Bei den postoperativen ALT- / AST-Werten (Tag 1, 2, 4, 7) fanden sich keine Unterschiede zwischen den Patienten der TOP-Studie und Placebo (keine Ex-situ Perfusion erhalten hatte Placebo (Mann-Whitney-U-Test, p > 0,05).

Die Studie wurde daher 08/2013 bei fehlendem Nachweis der Wirksamkeit der Studienmedikation (Tacrolimus 20 ng/ml in 1000 ml HTK) gegenüber der Vergleichsgruppe (1000 ml HTK) beim primären Endpunkt der Studie (maximal gemessene GPT-/ALT-Werte an den ersten beiden postoperativen Tagen) vorzeitig beendet (25).

Ob andere Dosierungen, Darreichungsformen oder Zeitpunkte der Anwendung von Tacrolimus zu einem protektiven Effekt bei Lebertransplantation führen, bleibt abzuwarten.

## 4. Zusammenfassung

Der hepatische IRS ist eine zentrale Ursache für schlechte Organfunktion sowie reduziertes Organüberleben und ist verstärkt ausgeprägt in marginalen Organen. Diese Transplantate werden aufgrund des herrschenden Organmangels verstärkt verwendet.

Die vorliegende Arbeit zeigt klinische Risikofaktoren für einen verstärkten IRS auf. Gleichzeitig konnten anhand der Lebertransplantationsdatenbank unserer Klinik beeinflussbare Prognosefaktoren nach Lebertransplantation identifiziert werden, z.B. der intraoperative Blutfluss der Leberarterie und der Pfortader. Ein hoher Fluss ist eine unabhängige Variable für längeres Transplantatüberleben in einer Multivariat-Analyse. Auch die Anlage eines intraoperativen portosystemischen Shunts scheint das outcome nach Lebertransplantation zu beeinflussen: Interessanterweise war dieser Effekt besonders ausgeprägt bei Empfängern von Organen mit einem erhöhten DRI. Diese einfache Technik scheint daher eine sinnvolle Möglichkeit zur Protektion marginaler Organe.

Außerdem wurde ein experimentelles Modell der Lebertransplantation an der Ratte etabliert. Dabei wurde in einem Schädigungsmodell mit Fettlebern die Bedeutung von oxidativem Stress bei Transplantation dieser Organe gezeigt: Eine Behandlung mit dem ROS Scavenger Glutathion führte zu einer Reduktion der hepatozellulären Schädigung nach kalter Ischämie. Bei Transplantation nicht-geschädigter Organe konnte eine Reduktion des akuten IRS durch eine einmalige ex vivo Perfusion mit Tacrolimus gezeigt werden.

Diese Technik wurde übertragen in eine prospektive, randomisierte klinische Multicenter Studie (IIT) zur Behandlung marginaler Organe bei Lebertransplantation: Lebern mit ≥ 2 Marginalitätskriterien wurden vor Implantation mit Tacrolimus gespült. Diese Behandlung zeigte keinen Vorteil gegenüber Kontrolle, weswegen die Studie vorzeitig nach Einschluss von 25 Patienten abgebrochen wurde.

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# GSH Attenuates Organ Injury and Improves Function after Transplantation of Fatty Livers

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**Key Words**
Fatty liver · Glutathione · Organ injury · Transplantation

**Abstract**
Ischemia-reperfusion injury (IRI) is increased after transplantation of steatotic livers. Since those livers are increasingly used for transplantation, protective strategies must be developed. Reactive oxygen species (ROS) play a key role in hepatic IRI. In lean organs, glutathione (GSH) is an efficient scavenger of ROS, diminishing IRI. The aim of this study was to evaluate whether GSH also protects steatotic allografts from IRI following transplantation. Fatty or lean livers were explanted from 10-week-old obese or lean Zucker rats and preserved (obese 4 h, lean 24 h) in hypothermic University of Wisconsin solution. Arterialized liver transplantation was then performed in lean syngeneic Zucker rats. Recipients of fatty livers were treated with GSH (200 μmol/h/kg) or saline during reperfusion (2 h, n = 5). Parameters of hepatocellular damage and bile flow were measured. Transplantation of steatotic livers enhanced early reperfusion injury compared to lean organs as measured by increased aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase plasma levels. Bile flow was also reduced in steatotic grafts. Intravenous administration of GSH effectively decreased liver damage in fatty allografts and resulted in improved bile flow. Intravenous application of GSH effectively reduces early IRI in steatotic allografts and improves recovery of these marginal donor organs following transplantation.



## Introduction

In an attempt to overcome the discrepancy between liver organ availability and demand, livers with poor organ quality are increasingly accepted for transplantation. In deceased organ donors, the prevalence of steatosis ranges from 13 to 28%, approaching 50% when sensitive histological techniques are used [1, 2]. Demographic data suggest a strong increase of obesity in the population, which may have a negative impact on the quality of harvested allografts used for liver transplantation [3].

Several human studies have demonstrated an increased ischemia-reperfusion injury (IRI) following transplantation of steatotic liver grafts as evidenced by

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elevated plasma liver enzyme levels and delayed organ function [3, 4]. Zucker rats homozygotic for a leptine-receptor gene defect (fa/fa) develop macrovesicular hepatic steatosis. Studies have described increased reperfusion injury in fa/fa Zucker rats compared to animals with healthy livers following warm and cold ischemia [5, 6]. Therefore, fa/fa Zucker rats may represent a suitable animal model of IRI following transplantation of non-alcoholic fatty livers. Therefore, those rats may represent a suitable animal model of reperfusion injury following transplantation of non-alcoholic fatty livers.

Although the precise sequence of events leading to IRI in liver transplants has not been completely described, the generation of reactive oxygen species (ROS) and disturbances of the hepatic microcirculation may play key roles in the mechanisms following reperfusion injury [7, 8]. Administration of the antioxidant glutathione (GSH) has been shown to attenuate ischemia-reperfusion injury following warm ischemia or liver transplantation in healthy organs [9, 10]. However, the effect of GSH on hepatocellular damage caused by reperfusion of transplanted fatty livers remains largely unknown. Recent work has demonstrated differences in the cellular and humoral response in healthy and diseased livers following exposure to ischemia and reperfusion [11].

The purpose of the present study is to test the efficacy of post-ischemic intravenous GSH administration in preventing early reperfusion injury after transplantation of fatty livers. Despite promising results in several experimental studies, no agent has made its way into clinical practice [12]. This discrepancy between experimental studies and patient treatment may be in part due to the frequent use of healthy grafts in many animal studies [13, 14]. This is not representative of the clinical situation, in which organ shortage often necessitates the use of grafts of poor quality (i.e. steatotic grafts) from extended criteria donors. Thus, the results of our study may provide a novel and clinically relevant therapeutic approach for the protection of steatotic organs, thereby increasing the pool of transplantable livers.

## Methods

*Animals*

Male syngeneic Zucker rats (Charles River Wiga, Sulzfeld, Germany) aged 10–14 weeks were used in this study. Animals were housed in a temperature- and humidity-controlled room under a constant 12-hour light/dark cycle. Rats with a homozygote point mutation of codon 269 in the leptine receptor gene (fa/fa) develop massive obesity. In contrast, animals with a heterozygote defect of this gene (fa/–) remain lean. Donors (body weight: fa/fa 478 ± 63 g, fa/– 345 ± 72 g) and recipients (fa/– 318 ± 22 g) had free access to water and rat chow (standard diet; Altromin, Lage, Germany). Recipient rats were fasted 12 h prior to donor operation and liver transplantation. All procedures were carried out in accordance with the guidelines of the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. The institutional animal care and use committee of the Government of Upper Bavaria and the Ludwig-Maximilians-University (Munich, Germany) approved this project.

**Table 1.** Study groups

| Group | Donor | Recipient | Treatment | CIT, h |
|---|---|---|---|---|
| 1: fa/– veh | fa/– | fa/– | veh | 24 |
| 2: fa/fa veh | fa/fa | fa/– | veh | 4 |
| 3: fa/fa GSH | fa/fa | fa/– | GSH | 4 |

CIT = Cold ischemia time; veh = vehicle.

*Experimental Groups*

Male Zucker rats were randomly assigned to three groups (n = 5/group; table 1). In group 1 (fa/– veh) livers were explanted from lean male Zucker rats and subjected to 24 h of cold ischemia (4°C) in University of Wisconsin solution. Orthotopic arterialized liver transplantation was then performed on lean male Zucker rats (fa/–). During reperfusion, 6 ml of saline 0.9% (vehicle) were infused continuously at a rate of 3 ml/h starting 20 min before declamping of the portal vein and hepatic artery.

In group 2 (fa/fa veh) and 3 (fa/fa GSH) steatotic livers were explanted from obese male Zucker rats (homozygote, fa/fa). After a cold ischemia period of 4 h these livers were transplanted into lean Zucker rats (fa/–). This reduction in ischemic time from 24 h in lean organs to 4 h in steatotic grafts was applied based on previous studies by Amersi et al. [6]. During reperfusion, animals in group 2 received 6 ml saline (vehicle) by continuous infusion at a rate of 3 ml/h (vehicle), whereas animals in group 3 received GSH (Tationil 600, Roche, Italy) at a concentration of 200 μmol/h/kg dissolved in 6 ml saline. Reperfusion time was 120 min in all groups.

*Surgical Procedures*

Donor and recipient procedures were performed under spontaneous ether inhalation. For continuous monitoring of mean arterial blood pressure and for substitution of plasma volume, left carotid artery and jugular vein were cannulated with polyethylene catheters. Body temperature was kept between 36.5 and 37.5°C using a heating pad. Donor livers were preserved by retrograde aortal flush with 15 ml of University of Wisconsin solution and stored at 4°C for 24 h (group 1) or 4 h (group 2/3). Before implantation, livers were rinsed with 10 ml of Ringer's lactate solution at 4°C via portal vein (hydrostatic pressure: 10 cm $H_2O$). Orthotopic liver transplantation was performed according to the cuff technique described by Kamada and Calne [15]. Grafts were rearterialized and simultaneously reperfused via portal vein and

hepatic artery as described by Post et al. [16]. Portal clamping times never exceeded 20 min. Five minutes after declamping, all rats received 0.5 ml of albumin (5%) and 0.5 ml of sodium bicarbonate to maintain blood pressure and physiological pH values. After 120 min of reperfusion, animals were sacrificed and liver weight was determined.

*Quantification of Bile Synthesis*

To quantify liver function during the reperfusion period, bile flow was quantified as previously described [17]. The common bile duct was cannulated with a polyethylene tube during donor surgery. Cumulative bile flow (μl/g/min) was calculated from total bile volume secreted between start of reperfusion and end of experiment (120 min).

*Determination of GSH and GSSG*

As opposed to other plasma parameters, determination of GSH (reduced GSH prior to oxidation, administered form) and GSSG (oxidized form of GSH following radical scavenging or spontaneous oxidation) in plasma requires a separate isolation procedure as described previously by Jaeschke et al. [18]. A total of 500 μl of whole blood is required for determination of total GSH (GSH total = sum of GSH and GSSG). For GSSG analysis, an aliqot (200 μl) of blood was mixed immediately with 200 μl of 10 mmol/l N-ethylmaleimide (NEM) in 100 mmol/l phosphate buffer (pH 6.5) containing 17.5 mmol/l EDTA. The remaining blood was centrifuged for 1 min. An aliquot (100 μl) of plasma was pipetted into 100 μl sulfosalicylic acid (5%) for determination of total GSH. To separate GSSG from NEM and NEM-GSH, an aliquot of NEM-treated plasma was passed through a Sep-PakC18 cartridge (Waters, Framingham, Mass., USA) followed by 1 ml of 100 mmol/l phosphate buffer (pH 7.5). GSSG in the eluates and total GSH in acidified plasma samples was determined by an enzymatic test as described previously [19]. GSH plasma concentrations were calculated as difference between total GSH and GSSG.

*Plasma Collection and Storage*

Whole blood was obtained via arterial catheter line (approximately 1.5 ml) in microcentrifuge tubes (Microtainer, Becton Dickinson, Rutherford, N.J., USA) and centrifuged at 16,000 *g* for 15 min at 4°C. Plasma was separated, placed in pyrogen-free microcentrifuge tubes, immediately frozen, and stored (–80°C) until assayed for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

*Assessment of Serum Aminotransferases*

Serum aminotransferases were used as established markers of hepatic injury. AST and ALT were measured 2 h after reperfusion with a kinetic UV test using a serum multiple analyzer (Olympus AU 2700) [20].

*Assessment of Lactate Dehydrogenase*

Systemic cellular damage was determined by assaying serum levels of LDH 2 h after reperfusion with a kinetic UV test using a serum multiple analyzer (Olympus AU 2700) [21].

*Histology*

Representative histological sections (HE staining) of fatty livers were examined 2 h after reperfusion.

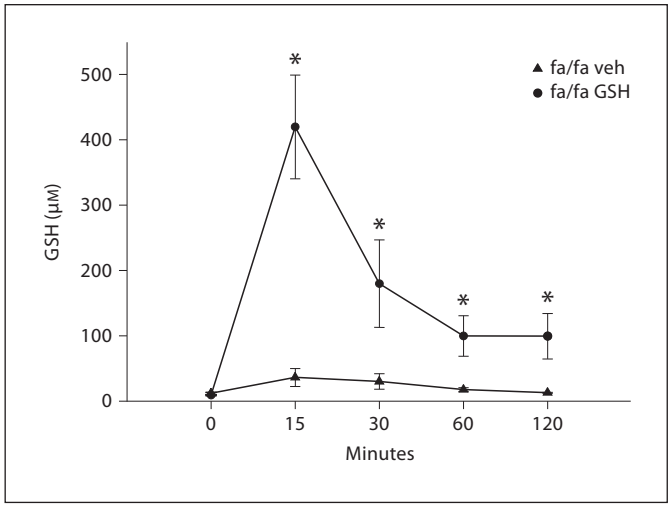


**Fig. 1.** Plasma GSH levels determined at time of reperfusion (0) and 15, 30, 60, and 120 min after liver transplantation. Livers were explanted from lean (fa/–) or obese (fa/fa) rats. Prior to declamping, recipient animals received vehicle or GSH (200 μmol/kg body weight/h). n = 5/group. Values are presented as mean ± SD. Data were analyzed by one-way ANOVA. * $p < 0.05$ vs. fa/– veh.

*Statistics*

The results are presented as mean ± SD. One-way ANOVA followed by the Student-Newman-Keuls test or Tukey test as a post hoc test for multiple comparisons was used to determine significance of the differences between experimental means. $p < 0.05$ was considered to be significant.

## Results

*Plasma GSH and GSSG*

Plasma GSH was determined in rats receiving steatotic organs and treated with vehicle or GSH at time of reperfusion (t = 0 min) and 15, 30, 60 and 120 min thereafter (fig. 1). GSH-treated rats displayed systemic peak GSH levels 15 min after reperfusion. Plasma GSH levels were significantly increased in GSH treated rats throughout the whole experiment compared to vehicle-treated animals ($p < 0.05$).

Plasma GSSG levels were significantly elevated 60 and 120 min after reperfusion in GSH-treated animals compared to non-treated rats ($p < 0.05$; fig. 2).

*Plasma Aminotransferases (ALT/AST)*

Alanine Aminotransferase

ALT levels were significantly increased in recipients of steatotic livers compared to recipients of lean livers after

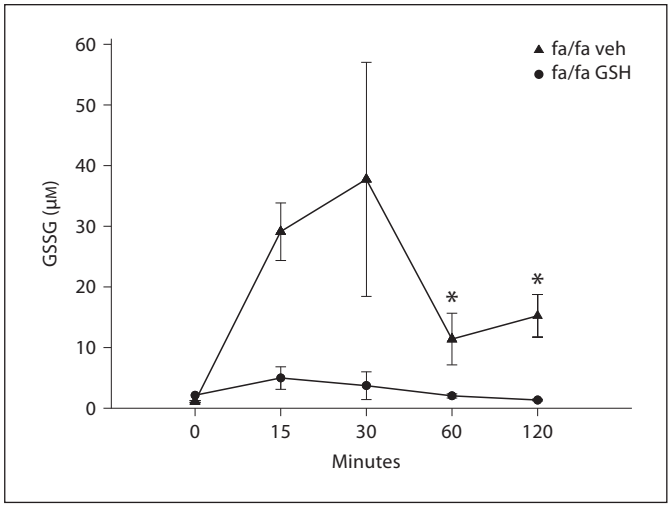


**Fig. 2.** Plasma GSSG levels determined at time of reperfusion (0) and 15, 30, 60, and 120 min after liver transplantation. Livers were explanted from lean (fa/–) or obese (fa/fa) rats. Prior to declamping, recipient animals received vehicle or GSH (200 μmol/kg body weight/h). n = 5/group. Values are presented as mean ± SD. Data were analyzed by one-way ANOVA. * $p < 0.05$ vs. fa/– veh.

reperfusion ($p < 0.05$; fig. 3). In recipients of steatotic grafts, administration of GSH significantly reduced plasma ALT levels compared to administration of vehicle ($p < 0.05$).

Aspartate Aminotransferase

Plasma AST levels were significantly enhanced in vehicle-treated recipients of fatty livers compared to rats receiving lean organs ($p < 0.05$; fig. 4). In recipients of steatotic livers, treatment with GSH was associated with a significant reduction of AST compared to administration of vehicle ($p < 0.05$).

*Lactate Dehydrogenase*

LDH, a non-organ-specific marker of IRI, was significantly higher in recipients of fatty livers treated with vehicle compared to recipients of lean livers following liver transplantation ($p < 0.05$; fig. 5). In recipients of steatotic livers, the administration of GSH significantly reduced serum LDH levels compared to the administration of vehicle ($p < 0.05$).

*Bile Flow*

Two hours after reperfusion, cumulative bile flow (a sensitive marker of liver function) was significantly diminished in steatotic livers receiving vehicle compared to lean grafts ($p < 0.05$; fig. 6). GSH treatment, however, significantly increased bile flow in steatotic grafts compared to treatment with vehicle ($p < 0.05$).

*Histology*

Histological sections (HE staining) demonstrated a 50–80% degree of steatosis (data not shown). Two hours after liver transplantation, no alterations between the groups and due to IRI were evident.

## Discussion

The critical organ shortage for liver transplantation has resulted in the routine acceptance of steatotic organs. In the general population, steatosis occurs with a prevalence of 13–50% [1, 2]. Steatotic livers substantially contribute to the donor pool, thereby partially compensating for organ shortage. Clinical and experimental studies have shown that steatotic livers are more susceptible to IRI than lean livers [3, 4, 22–25]. This results in increased plasma aminotransferases and diminished liver function in the early postoperative phase following transplantation [3, 4]. Other risk factors, including cold ischemia time and donor age [26, 27], have been shown to exhibit additive detrimental effects on organ function and survival following liver transplantation. Steatosis is a particularly relevant risk factor for IRI due to its increasing prevalence. Moreover, in contrast to risk factors such as cold ischemia time, steatosis cannot be affected by changing allocation procedures. Nonetheless, future experimental studies should consider other risk factors beside steatosis to better mimic the clinical situation.

Due to the clinical importance of steatosis for outcome following liver transplantation, animal models of steatosis have been established [2]. Genetically obese Zucker rats have been shown to develop liver steatosis mimicking non-alcoholic fatty liver disease [2, 28]. Interestingly, the pathophysiological mechanisms associated with reperfusion injury after orthotopic liver transplantation have been shown to be different between lean and steatotic Zucker rats [11]. Lean rats tend to develop hepatic apoptosis following ischemia-reperfusion whereas necrosis is the predominant form of cell death in steatotic organs [11]. Moreover, steatotic livers exhibit an increased production of reactive oxygen species (ROS) following warm and cold ischemia and reperfusion [5, 29]. These results collectively suggest that steatosis has to be considered when investigating new therapeutic strategies in liver transplantation.

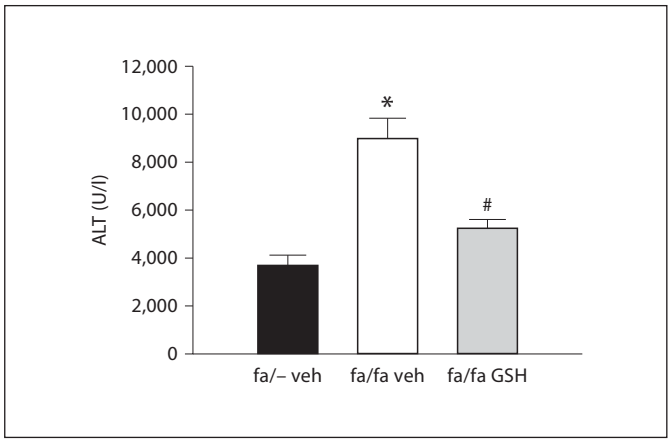


**Fig. 3.** Serum ALT levels determined 2 h after liver transplantation. Livers were explanted from lean (fa/–) or obese (fa/fa) rats. Prior to declamping, recipient animals received vehicle or GSH (200 μmol/kg body weight/h). n = 5/group. Values are presented as mean ± SD. Data were analyzed by one-way ANOVA. * $p < 0.05$ vs. fa/– veh; # $p < 0.05$ vs. fa/fa veh.

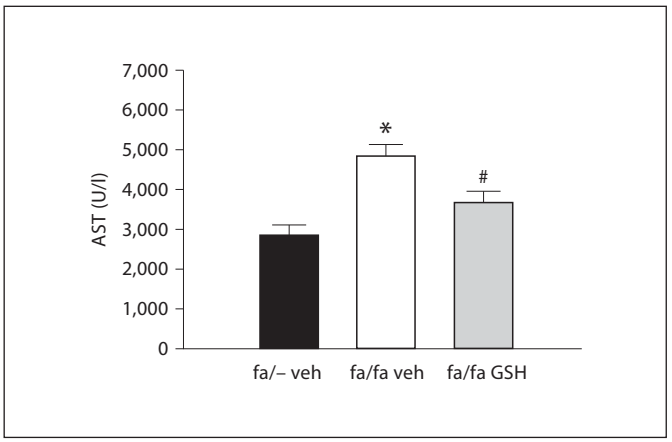


**Fig. 4.** Serum AST levels determined 2 h after liver transplantation. Livers were explanted from lean (fa/–) or obese (fa/fa) rats. Prior to declamping, recipient animals received vehicle or GSH (200 μmol/kg body weight/h). n = 5/group. Values are presented as mean ± SD. Data were analyzed by one-way ANOVA. * $p < 0.05$ vs. fa/– veh; # $p < 0.05$ vs. fa/fa veh.

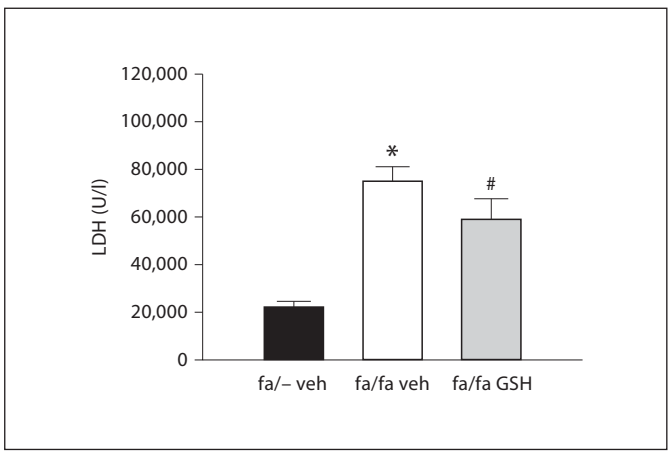


**Fig. 5.** Serum LDH levels determined 2 h after liver transplantation. Livers were explanted from lean (fa/–) or obese (fa/fa) rats. Prior to declamping, recipient animals received vehicle or GSH (200 μmol/kg body weight/h). n = 5/group. Values are presented as mean ± SD. Data were analyzed by one-way ANOVA. * $p < 0.05$ vs. fa/– veh; # $p < 0.05$ vs. fa/fa veh.

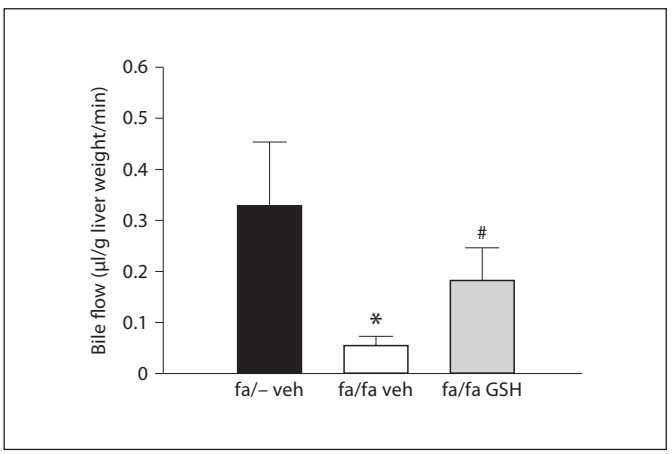


**Fig. 6.** Bile flow (μl/g liver weight/min) during 2 h of reperfusion. Livers were explanted from lean (fa/–) or obese (fa/fa) rats. Prior to declamping, recipient animals received vehicle or GSH (200 μmol/kg body weight/h). n = 5/group. Values are presented as mean ± SD. Data were analyzed by one-way ANOVA. * $p < 0.05$ vs. fa/– veh; # $p < 0.05$ vs. fa/fa veh.

The present data indicate that steatotic livers harvested from genetically obese Zucker rats develop significantly more organ injury compared to lean allografts. Interestingly, increased organ injury following transplantation of fatty organs was evident despite a reduction in cold ischemia time from 24 h in lean to 4 h in steatotic organs. These findings are supported by previous results demonstrating increased organ damage and diminished organ function in fatty compared to lean organs following IRI [30, 31]. Cold ischemia time of 24 h for healthy donor organs was based on previous studies, demonstrating significant IRI in recipient animals [13, 17]. For stea-

totic organs, cold ischemia time was substantially reduced to avoid deleterious organ damage [7].

In healthy organs, continuous administration of GSH protects liver tissue from reperfusion injury after warm and cold ischemia [9, 10, 32]. Endogenous GSH is concentrated in the intracellular space [33]. It reacts with oxidants released during reperfusion resulting in formation of oxidized GSH (GSSG) [34]. Its effect on ischemia-reperfusion injury in steatotic grafts, however, remains unknown. In the present study, GSH was administered at a dosage of 200 μmol/kg body weight/h to provide steatotic organs with supraphysiological GSH levels. This dosage has been shown to ameliorate IRI following transplantation of lean liver allografts [30] and resulted in peak plasma GSH levels below the limit of toxicity in humans (500 μM) [35]. As has been found in lean organs, the administration of GSH at the beginning of reperfusion significantly decreased early liver injury (plasma aminotransferase levels) and ameliorated liver function (bile flow) in steatotic grafts.

Determination of the exact mechanisms which are responsible for the beneficial effects of GSH in steatotic organs was beyond the scope of our study. Nonetheless, GSSG levels, the oxidized form of GSH, were increased in GSH-treated recipients of fatty grafts. This suggests that intravenously applied GSH was mostly oxidized. Oxidation of GSH is known to be associated with detoxification of detrimental ROS [36, 37]. In addition, the metabolism of GSH produces glycine [35], which also has protective properties against liver reperfusion injury [14]. It has been proposed that the GSH-induced reduction of ROS is associated with improved sinusoidal perfusion, significant decrease of leukocytes sticking to sinusoids, as well as prevention of sinus endothelial cell injury in lean organs [10, 32]. Steatotic livers are characterized by decreased sinusoidal blood flow due to swollen hepatocytes which may induce chronic hypoxia [38] and ATP depletion [39]. Moreover, ischemia-reperfusion injury in fatty organs is associated with enhanced leukocyte adhesion and microcirculatory failure, potentially based on an increased ROS release [23, 25, 29, 40]. These studies collectively suggest that GSH may also decrease ROS in fatty organs following cold storage and transplantation, thereby improving microcirculation and preventing endothelial damage, at least during the early reperfusion period which was of particular interest in this study.

In summary, the present study demonstrated increased IRI in transplanted, fatty allografts. Continuous postischemic infusion of GSH, a ROS scavenger, ameliorated early hepatocellular injury and improved liver function in fatty organs. The protective effects of GSH were associated with an increase in oxidized plasma GSSG, indicating detoxification of ROS by GSH. Since no severe side effects of GSH administration have been reported, this peptide has significant potential to be a useful and safe means of improving the function of steatotic organs following liver transplantation in humans. A prospective randomized trial is required to verify these promising experimental results in the clinical arena.

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# Results of the TOP Study: Prospectively Randomized Multicenter Trial of an Ex Vivo Tacrolimus Rinse Before Transplantation in EDC Livers

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**Background.** Organ shortage results in the transplantation of extended donor criteria (EDC) livers which is associated with increased ischemia-reperfusion injury (IRI). Experimental studies indicate that an organ rinse with the calcineurin inhibitor tacrolimus before implantation protects against IRI. The tacrolimus organ perfusion study was initiated to examine the effects of ex vivo tacrolimus perfusion on IRI in transplantation of EDC livers. **Methods.** A prospective randomized multicenter trial comparing ex vivo perfusion of marginal liver grafts (≥2 EDC according to Eurotransplant manual) with tacrolimus (20 ng/mL) or histidine-tryptophane-ketoglutarate solution (control) was carried out at 5 German liver transplant centers (Munich Ludwig-Maximilians University, Berlin, Heidelberg, Mainz, Regensburg) between October 2011 and July 2013. Primary endpoint was the maximum alanine transaminase (ALT) level within 48 hours after transplantation. Secondary endpoints were aspartate transaminase (AST), prothrombine ratio, and graft-patient survival within an observation period of 1 week. After an interim analysis, the study was terminated by the scientific committee after the treatment of 24 patients (tacrolimus n = 11, Control n = 13). **Results.** Tacrolimus rinse did not reduce postoperative ALT peaks compared with control ($P = 0.207$; tacrolimus: median, 812; range, 362-3403 vs control: median, 652; range, 147-2034). Moreover, ALT ($P = 0.100$), prothrombine ratio ($P = 0.553$), and bilirubin ($P = 0.815$) did not differ between the groups. AST was higher in patients treated with tacrolimus ($P = 0.011$). Survival was comparable in both groups ($P > 0.05$). **Conclusions.** Contrary to experimental findings, tacrolimus rinse failed to improve the primary endpoint of the study (ALT). Because 1 secondary endpoint (AST) was even higher in the intervention group, the study was terminated prematurely. Thus, tacrolimus rinse cannot be recommended in transplantation of EDC livers.

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The organ shortage represents one of the biggest challenges in transplantation medicine today and has resulted in an increasing acceptance of extended donor criteria (EDC) grafts for liver transplantation.[1] In this respect, an increased ischemia-reperfusion injury (IRI) is associated with a diminished organ survival in these grafts.[2]

Thus, novel strategies have to be developed to attenuate IRI and improve the function and survival of EDC organs.

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Several models of experimental hepatic ischemia-reperfusion have revealed protective effects of tacrolimus preconditioning.[3,4] The authors recently demonstrated a reduction of IRI after an ex vivo tacrolimus rinse in a model of experimental liver transplantation in rats.[5] In the clinical arena, controversial data have been published on the effectiveness of a tacrolimus rinse before liver transplantation[6,7]: within a phase 1 trial, St Peter et al[6] showed a significant reduction of aminotransferase levels in the transplantation of non-EDC livers after a tacrolimus rinse. In contrast to these findings, Kristo et al[7] recently failed to show a reduction of alanine transaminase (ALT) levels through such a treatment after transplantation of nonmarginal livers. Nevertheless, the authors demonstrated a distinct reduction of precursors of proinflammatory enzymes on RNA level after a tacrolimus rinse in those patients. However, as both of these studies investigated the transplantation of nonmarginal organs, their relevance to the setting of EDC organ transplantation is limited.

Therefore, the present prospectively randomized multicenter trial (tacrolimus organ perfusion [TOP] study; Trial register: EUDRA CT number: 2010-021333-31, ClinicalTrials ID: NCT 01564095) included exclusively marginal livers with 2 or more EDC according to Eurotransplant's guidelines for marginal liver grafts. The aim of the TOP study was to determine whether an ex vivo rinse of such organs with tacrolimus results in a decreased IRI, thereby improving liver function and organ survival. The study outline has been published previously.[8]

## MATERIALS AND METHODS

### Study Design

The TOP study was designed as an investigator-initiated, prospectively randomized, multicenter trial according to German Medicines Act (Section 42b, Abs. 1 Arzneimittelgesetz German Pharmaceuticals Act). Patients were randomized into 2 groups within this placebo-controlled, nonblinded study:

(A) Ex vivo perfusion of marginal liver grafts with tacrolimus (20 ng/mL) solved in 1000 mL histidine-tryptophane-ketoglutarate (HTK) (tacrolimus + HTK)
(B) Ex vivo perfusion of marginal liver grafts with 1000 mL HTK (HTK-alone)

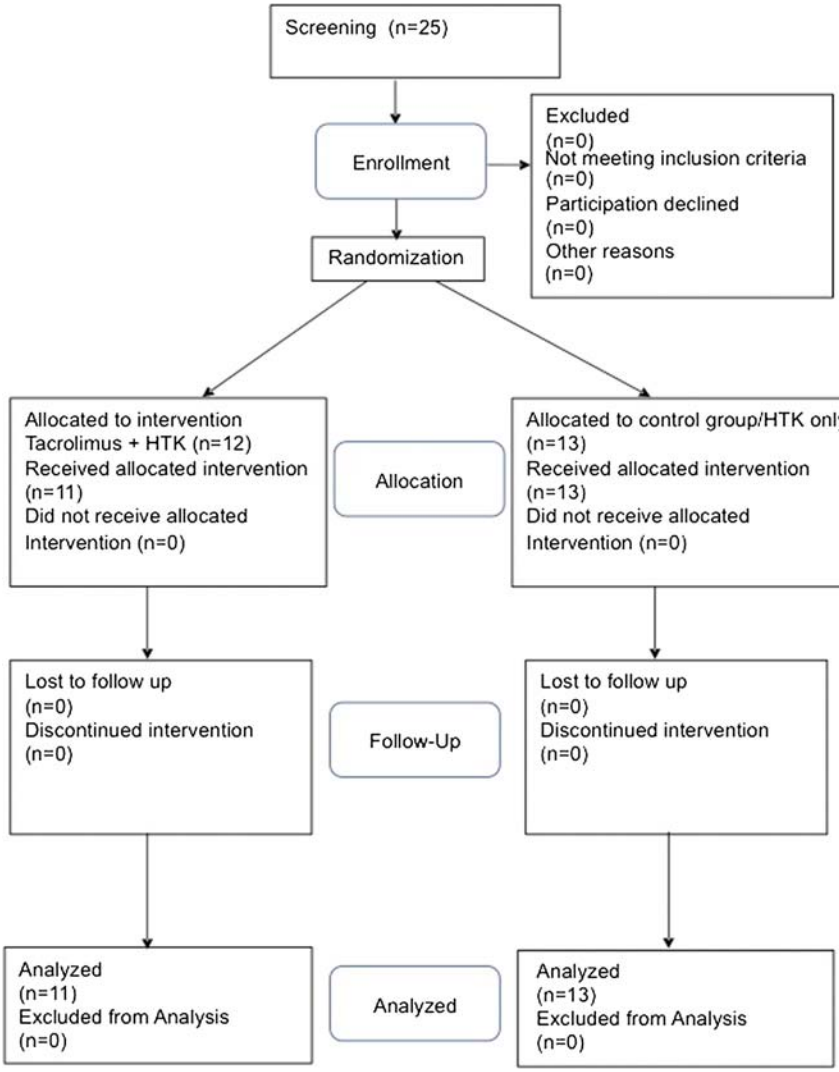


**FIGURE 1.** CONSORT flow diagram TOP study.

The observation period was 1 week. The inclusion period for this trial was between October 2011 and July 2013. Besides Munich Ludwig-Maximilians University as the initiating center, 4 German transplant departments participated in this trial: Berlin-Charité, Heidelberg, Mainz, and Regensburg.

Twenty-five patients were enrolled in the study from October 31, 2011 (first patient in) until July 9, 2013 (last patient out). One patient was included and randomized but not transplanted due to medical reasons. Eleven patients were treated with tacrolimus + HTK and 13 with HTK alone (Figure 1, CONSORT flow diagram). The study was terminated by the scientific committee in July 2013 after an interim analysis due to missing evidence of the effectiveness of the study medication (20-ng/mL tacrolimus in 1000-mL HTK) relative to the comparison group (1000-mL HTK) concerning the primary endpoint of the study, the maximum measured ALT values on the first 2 postoperative days. Moreover, potentially even harmful action was evident when analyzing another secondary endpoint of the study, postoperative aspartate transaminase (AST).

Protocol version 2.1 was approved by the local ethic committees of the ethics committee of the University of Munich. The study complied with the Declaration of Helsinki and Good Clinical Practice Guidelines. Informed consent was obtained from each patient in written form before randomization.

## Inclusion and Exclusion Criteria

Recipients with end-stage chronic liver disease older than 18 years receiving their first organ transplant were evaluated for inclusion in this trial. Only patients receiving livers with 2 or more EDC following the definition of EDC by Eurotransplant[9] (Table 1) were finally included in this trial. All recipient and donor inclusion and exclusion criteria are outlined in Table 1.

## Surgical Procedure/Perfusion Procedure

Tacrolimus was dissolved in 1000-mL HTK (concentration, 20 ng/mL) in the treatment group; in the control group, the rinse solution consisted of 1000-mL HTK-only. The rinse was administered sequentially to the portal vein and the common hepatic artery (500 mL each) at the end of back-table preparation via a 12-gauge cannula from a height of 100 cm without additional pressure using polyvinyl chloride–free infusion sets (Braun Melsungen AG, Germany). The duration of the perfusion procedure was 16 minutes (median) versus 18.1 ± 7.3 minutes (mean ± SD) and was similar between the groups ($P > 0.05$). Cava sparing liver transplantation was performed afterward. Immunosuppression and postoperative care were carried out according to center-specific standards.

## Primary and Secondary Endpoints

The primary endpoint was the maximum ALT level (U/L) within the first 48 hours after liver transplantation.

Secondary endpoints were ALT and AST levels (U/L) and graft function (prothrombine ratio/quick (%), bilirubin (mg/dL) on postoperative days 1, 2, 4, and 7. Within the follow-up, organ and patient survival was monitored.

## Donor and Recipient Characteristics

The following data were collected for each donor and recipient, respectively: height, bodyweight, body mass index (BMI)/graft steatosis, age and diagnosis. In addition, the duration of intensive care, cold ischemia time, donor risk index (DRI) (according to Feng et al[10]) and the number and type of EDC were captured (Tables 2 and 3).

Recipients' diagnoses were classified as follows: alcoholic cirrhosis, malignancy, viral hepatitis, primary biliary cirrhosis, and others. Based on the preoperative serum creatinine, bilirubin, and international normalized ratio levels, laboratory model for end-stage liver disease (MELD) scores were calculated as described previously.[11]

## Statistic Evaluation/Sample Size Calculation

The sample size estimation was based on previous findings published by St Peter et al[6] in which nonmarginal grafts had

**TABLE 1.**

**Inclusion/Exclusion criteria TOP study**

| Inclusion criteria | |
|---|---|
| Recipient | Donor |
| Chronic end-stage liver disease, age > 18 years, first organ transplant | • Donor age >65 years<br>• Macrovesicular steatosis >40% (secured macroscopically or by biopsy)<br>• BMI >30<br>• Na >165 mmol/L<br>• Intensive care and mechanical ventilation >7 d<br>• Cold ischemia time >13 h<br>• AST >99 U/L<br>• ALT >105 U/L<br>• Bilirubin >3 mg/dL (>51 μmol/L)<br>• Application of epinephrine |
| Exclusion criteria | |
| Recipient | Donor |
| • Multiorgan transplantation<br>• High urgency listing<br>• Extrahepatic tumor diseases<br>• Pregnancy<br>• Denial or withdrawal of consent by the patient or his relatives<br>• Accommodation in an institution due to governmental or judicial authorities<br>• Missing knowledge of German language, no understanding of information not guaranteed | Hepatitis B or hepatitis C infection |

**TABLE 2.**

**Donor characteristics/EDC (according to eurotransplant manual): portion of donors meeting inclusion criteria (n [%]) versus absolute number (median, mean ± SD) for each tacrolimus + HTK and HTK-only**

| | Tacrolimus + HTK | | HTK-only | | P [CI] (tacrolimus + HTK vs HTK-only) | | Overall |
|---|---|---|---|---|---|---|---|
| | Portion of donors meeting inclusion criterion, n (%) | Absolute number, median; (mean ± SD) | Portion of donors meeting inclusion criterion, n (%) | Absolute number, median, (mean ± SD | Portion (fisher exact test) | Absolute number (Mann-Whitney *U* test) | Median (mean ± SD) |
| Donor age, >65 y | 6/11 (54.5%) | 57 (56.7 ± 21.3) | 3/13 (27.3%) | 52 (52.4 ± 12.5) | 0.41 (0.51-2.9) | 0.43 | 55 (54.4 ± 16.8) |
| Graft steatosis >40% | 1/11 (9.1%) | n.a. | 1/13 (7.7%) | n.a. | 0.45 (0.0-32.5) | n.a. | n.a. |
| Serum sodium >165 mmol/L | 1/11 (9.1%) | 147 148.4 ± 9.1 | 0/13 (0%) | 144 143.2 ± 6.9 | 0.46 (0-33) | 0.32 | 147 (145.4 ± 8.3) |
| ICU stay, >7 d | 5/11 (45.5%) | 7 (6.8 ± 5.2) | 2/13 (15.4%) | 4 (7.2 ± 8.7) | 0.36 (0.024-3.08) | 0.93 | 4 (7.0 ± 6.9) |
| BMI >30 | 4/11 (36.4%) | 26.5 28.1 ± 6.3 | 6/13 (46.2%) | 29.5 29.6 ± 4.9 | 1 [0.16-7.97] | 0.56 | 28 22 ± 5.7 |
| Cold ischemia time >13 h | 1/11 (0.1%) | 10.1 (10.6 ± 2.8) | 2/13 (15.4%) | 9.1 (10.4 ± 2.9) | 1 (0.0098-71.84) | 1.00 | 10.1 (10.5 ± 2.9) |
| AST >99 U/L | 5/11 (45.5%) | 50 (100.4 ± 65.1) | 4/13 (30.8%) | 40 91.6 ± 125.2 | 1 (0.21-0.47) | 0.28 | 50 (96.8 ± 92.8) |
| ALT >105 U/L | 3/11 (27.3%) | 38 (83.3 ± 88.1) | 4/13 (30.8%) | 47 (115.5 ± 165) | 1 (0.15-10.64) | 1.00 | 40 (97.6 ± 123.6) |
| Bilirubin >3 mg/dL | 0/11 (0%) | 0.77 (0.79 ± 0.55) | 0/13 (0%) | 0.51 (0.57 ± 0.296) | 0.46 (0-33) | 0.44 | 0.74 (0.69 ± 0.44) |
| Adrenaline | 9/11 (81.8%) | n.a. | 10/13 (76.9%) | n.a. | 0.59 (0.0058-5.22) | n.a. | n.a. |

Portions of extended criteria are compared between groups using Fisher exact test, absolute numbers by using Mann-Whitney *U* test.
ICU, intensive care unit; n.a., not available.

been transplanted. An effect size of approximately 0.7 was considered appropriate for the sample size calculation presuming higher postoperative ALT levels and a more pronounced reduction in marginal liver grafts.[8] The power of the test was 80% at a significance level of 0.05. Therefore, sample size estimation (nQuery Advisor 6.1; Statistical Solutions, Saugus, MA) for 2 unpaired samples using the Wilcoxon rank-sum test with an expected dropout rate of 15% resulted in an estimated sample size of 86 patients (43 tacrolimus + HTK vs 43 HTK-only). The randomization was performed in blocks of variable length, stratified according to the transplant centers. The Hodges-Lehman estimator was used to estimate effects on the primary endpoint (ALT). Secondary endpoints were calculated using the multivariate rank-sum test by O'Brien. The portion of EDC in both groups was compared by Fisher exact test, the absolute numbers of these parameters were analyzed using the Mann-Whitney *U* test. The statistical analysis was performed using statistical software Predictive Analysis SoftWare statistics 18.0.0 (SPSS Inc., Chicago, IL).

For all statistical tests, a test wise α-level of 5% was used. *P* values less than 0.05 were considered statistically significant.

## RESULTS

### Donor Characteristics

The number and type of EDC as a basis for patient inclusion did not differ between the study groups. In this respect, the average number of EDC (which represent the inclusion criteria to the study) was 2.75 in both groups. The most prevalent EDC were high donor age older than 65 years (n = 9/24), an intensive care unit stay longer than 7 days (n = 7/24), obesity (BMI > 30) (n = 10/24), and elevated liver enzymes (AST > 105 U/L) (n = 9/24). The portion of donors which met these inclusion criteria was similar in both groups (Table 2). These factors also did not differ in absolute numbers (median vs mean ± SD; Table 2).

The donor risk index was not different whether grafts had been treated with tacrolimus or control/HTK (tacrolimus: median, 1.9; mean ± SD, 2.0 ± 0.4; control: median, 1.8; mean ± SD, 1.8 ± 0.3) (*P* = 0.35). When analyzing the donors' cause of death, 10 donors had died of cerebral hypoxia and 14 of an intracerebral bleeding. These diagnoses had the same portion in both groups.

### Recipient Characteristics

Recipient characteristics did not differ between the 2 study groups. Relevant prognostic factors were comparable in patients randomized to tacrolimus or control (ie, laboratory MELD scores, BMI, age; *P* >0.05) (Table 3). Fifteen patients were transplanted due to malignant diseases (hepatocellular carcinoma, n = 14; cholangiocellular carcinoma, n = 1) and 2 for a cryptogenic liver cirrhosis. The remaining indications were classified as follows: hepatitis C (n = 1), autoimmune hepatitis (n = 1), PSC (n = 1), and α1-AT deficiency (n = 1).

### Primary Endpoint: Maximum ALT

Perfusion of marginal livers with tacrolimus resulted in no statistically significant effect on the maximum ALT values measured within the first 2 postoperative days after transplantation compared with HTK-only (tacrolimus + HTK:

**TABLE 3.**

**Recipient characteristics: tacrolimus + HTK versus HTK-only is compared by using Mann-Whitney *U* test**

| | Overall | Tacrolimus | Control | *P* (tacrolimus + HTK vs HTK-only) |
|---|---|---|---|---|
| Recipient age | 60 (58.5 ± 7.7) | 63 (59.8 ± 8.1) | 55 (57.5 ± 6.5) | 0.26 |
| BMI | 27.4 (27.2 ± 4.9) | 25.3 (26.6 ± 4.7) | 27.4 (27.7 ± 4.8) | 0.52 |
| Laboratory MELD | 13 (14.3 ± 6.9) | 13 (13.3 ± 4.9) | 13 (15.2 ± 8.2) | 0.69 |

median, 812 U/L; range, 362-3403 U/L vs HTK-only: median, 652 U/L; range, 147-2034 U/L) ($P$ = 0.207). The Hodges-Lehman estimator for the 95% confidence interval for the median difference was (–178 to 1166).

On the first postoperative day, the maximum ALT was in median, 607 U/L (mean ± SD, 790.4 ± 714.4 U/L) in the tacrolimus group compared with 544 U/L (544.8 ± 326.9 U/L) in the control group, respectively ($P$ = 0.56). On the second postoperative day, the maximum ALT levels were in median, 726 U/L (mean ± SD, 1010.5 ± 634.9 U/L) for tacrolimus versus median, 425 U/L (mean ± SD, 613.9 ± 439.8 U/l) for control ($P$ > 0.05).

## Secondary Endpoints

### Alanine Transaminase

No statistically significant effect was evident when comparing tacrolimus + HTK versus HTK-only on postoperative days 1, 2, 4, and 7 ($P$ = 0.100, multivariate rank sum test according to O'Brien). For example, the median ALT was 607 U/L (tacrolimus + HTK) versus 497 U/L (HTK-only) on day 1 and 726.5 U/L (tacrolimus + HTK) versus 400 U/L (HTK-only) on the second postoperative day (Figure 2).

### Aspartate Transaminase

Patients whose grafts had been treated with tacrolimus showed increased systemic AST levels in the postoperative course. When comparing AST on postoperative days 1, 2, 4, 7, a $P$ value of 0.011 was evident (multivariate rank sum test according to O'Brien). For illustration, on postoperative day 1, the maximum AST was 1196 U/L (median) in grafts treated with tacrolimus versus 802 U/L (control). On the second postoperative day, the median AST was 1030 U/L (tacrolimus) compared with control: 390 U/L (Figure 3).

## Bilirubin

A tacrolimus rinse did not alter the postoperative bilirubin levels during the observation period of 1 week ($P$ = 0.815). In grafts flushed with tacrolimus, the median bilirubin was 4 mg/dL versus 2.75 mg/dL (control) on the first postoperative day and 3.3 versus 3.05 mg/dL on the second postoperative day, respectively (Figure 4).

## Prothrombine Ratio/Quick (%)

No statistically significant effect was evident when comparing tacrolimus and control on postoperative days 1, 2, 4, and 7 ($P$ = 0.553). For example, the median of this parameter was 53.5 (tacrolimus) versus 57.5 (control) on postoperative day 1 and 55.5 versus 63 on postoperative day 2 (Figure 5).

### Survival

Organ and patient survivals as well as the number of rejections were equal in both treatment arms ($p$ = 1.000). No study patient died or underwent retransplantation within the observation period of 7 days.

## DISCUSSION

Extended donor criteria liver grafts exhibit an increased IRI during liver transplantation resulting in decreased graft function and survival.[12-14] Due to the current shortage of organs from deceased donors and the declining number of liver transplantations in Germany (n = 1192 in 2010, n = 884 in 2013), these grafts are increasingly used for transplantation. In 2010, EDC organs made up more than 70% of all transplanted livers in Germany, and the proportion of liver grafts exhibiting 1 or more EDC increased from 29 % in 1997 to 73% in the year 2010 (data provided by Eurotransplant). Thus, new therapies have to be developed to reduce IRI in EDC organs.

The hypothesis of the TOP study was that a single ex vivo tacrolimus rinse reduces IRI in transplantation of marginal liver grafts. In this respect, experimental data demonstrate a protective role of the calcineurin inhibitor tacrolimus on the hepatic IRI after warm and cold ischemic periods.[5,15] For the present trial, an ex vivo tacrolimus rinse in EDC liver grafts has been chosen as study medication. This dosage form is based on previous experimental[5] and clinical[6,7] data indicating protective effects of a single ex vivo tacrolimus treatment in the cold liver graft: St Peter et al[6] described a significant reduction of postoperative aminotransferase levels after a tacrolimus rinse in transplantation of normal,

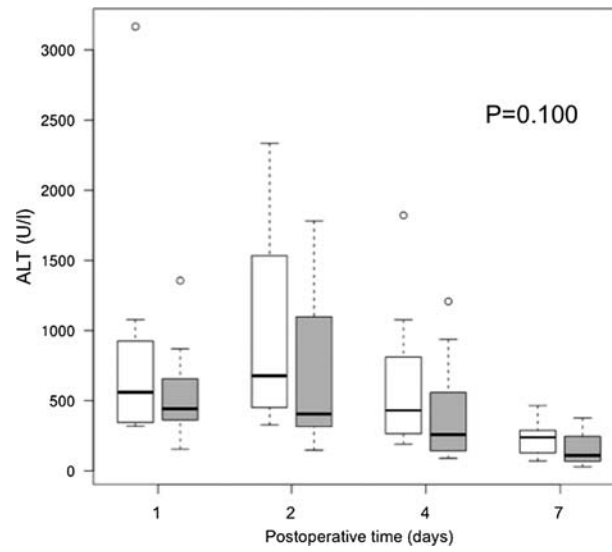


**FIGURE 2.** Serum ALT levels (U/L) on the first, second, fourth, and seventh postoperative days with respect to an ex vivo organ perfusion with tacrolimus (20 ng/ml) or with HTK-only. $P$ = 0.100; multivariate rank sum test by O'Brien. Tacrolimus + HTK, white column; HTK-only, grey column.

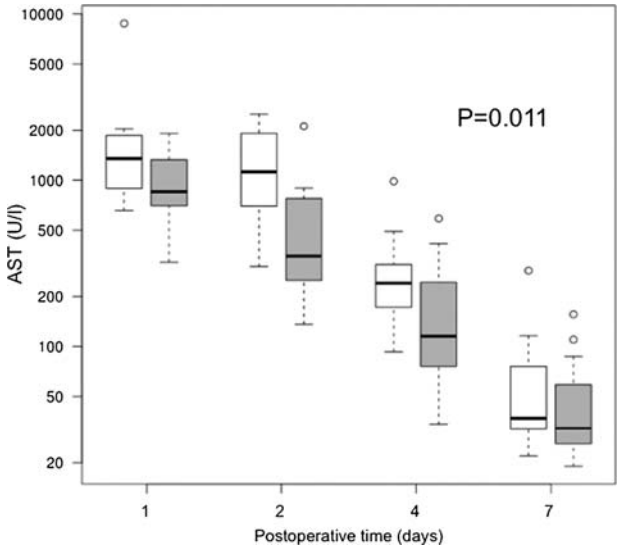


**FIGURE 3.** Serum AST levels (U/L) on the first, second, fourth and seventh postoperative days with respect to an ex vivo organ perfusion with tacrolimus (20 ng/ml) or with HTK-only. $P = 0.011$; multivariate rank sum test by O'Brien. Tacrolimus + HTK, white column; HTK-only, grey column.

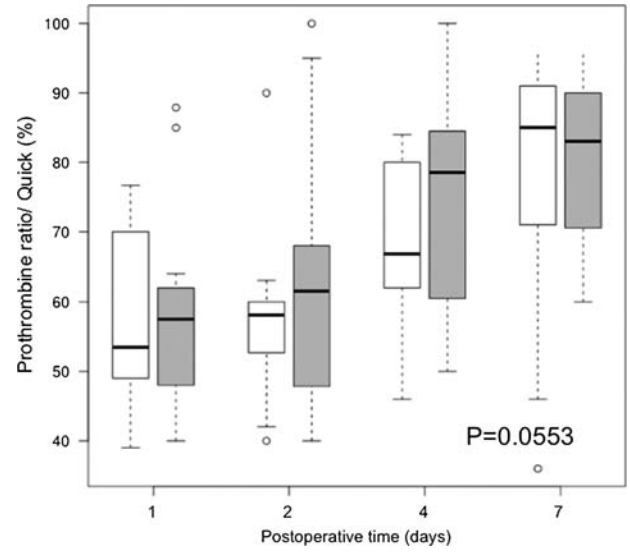


**FIGURE 5.** Serum ALT levels (U/L) on the first, second, fourth, and seventh postoperative days with respect to an ex vivo organ perfusion with tacrolimus (20 ng/mL) or with HTK-only. Tacrolimus + HTK, white column; HTK-only, grey column.

nonmarginal liver grafts. The second trial by Kristo et al[7] could not reproduce a clinical reduction of IRI but showed a decreased expression of inflammatory cytokines through such a treatment. In both studies, however, nonmarginal liver grafts were included. Thus, the results of these trials do not reflect the current clinical problem of organ shortage and marginal liver grafts.

With respect to the current study design it must be stated that the metabolism of the graft is certainly reduced during the ex vivo rinse treatment of the cold liver. As a calcineurin inhibitor, tacrolimus requires active T cells to develop its effect which are not present during the ex vivo perfusion. Nevertheless, tacrolimus may exert its therapeutic effects

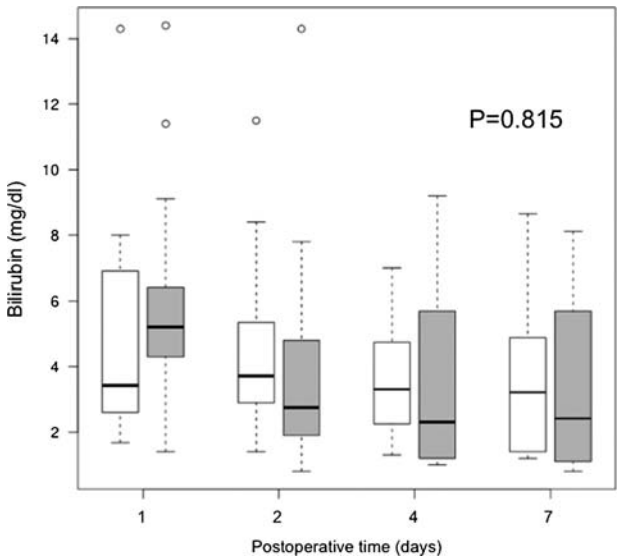


**FIGURE 4.** Serum bilirubin levels (mg/dL) on the first, second, fourth and seventh postoperative day with respect to an ex vivo organ perfusion with tacrolimus (20 ng/mL) or with HTK-only. $P = 0.815$; multivariate rank sum test by O'Brien. Tacrolimus + HTK, white column; HTK-only, grey column.

throughout the gradual warming of the graft during reperfusion. Furthermore, a local treatment is supposed to supply higher concentrations of tacrolimus compared with a systemic treatment of the recipient. In an experimental setting, the authors were also able to demonstrate a relevant decline of the tacrolimus concentration in the perfusate after passing the ischemic graft.[5]

In an attempt to provide protective action of tacrolimus from the beginning of reperfusion, most of the previous experimental models have performed a systemic donor preconditioning.[16,17] Despite their promising results, the clinical implementation of these models is problematic due to the organ allocation policy of Eurotransplant generating a confusing group of recipients. Whether a systemic recipient treatment before organ implantation would improve the effectivity of tacrolimus should be considered in further studies.

The hepatic IRI is triggered by innate immune activation and is characterized by proinflammatory cytokines, neutrophil infiltration, and diminished microcirculation. In this respect, potential mechanisms for the effects of tacrolimus on the hepatic IRI have been described, including an impact on inflammatory processes, a decrease of neutrophil infiltration and apoptosis as well as an improved microcirculation.[4,16,18-21] Because tacrolimus has its effects primarily through a downregulation of IL-2 and a consecutively diminished activation of T cells, this pathway may also account for the postulated action of a tacrolimus rinse besides the well-known anti-inflammatory properties of this substance. In this respect, Khandoga et al have reported a novel concept for the development of IRI demonstrating a pivotal of T cells in those pathophysiological processes.[22-24]

In the TOP study, an organ rinse with tacrolimus dissolved in HTK was compared with HTK-only (control group). This study design was also chosen because the rinse procedure itself may have protective effects on ischemia-reperfusion in liver transplantation. In this respect, experimental and clinical studies demonstrate that perfusion with Carolina Rinse or warm Ringer lactate exerts protective effects in terms of

graft damage and survival.[25,26] In contrast to these findings, a recent prospective trial by Heise[27] incorporating 264 patients failed to show protective effects when performing an ex situ perfusion of the hepatic artery with HTK solution.

The inclusion criteria (Table 1) chosen for this trial were based on their association with decreased graft function and survival. The study proposal has been published previously.[8]

The prevalence of 2 or more EDC was the central inclusion criterion because some authors describe that even transplantation of grafts from extremely extended criteria donors may not negatively influence the long-term outcome as long as only a single-donor risk factor is present (ie, donor age >70 years).[28] Instead, certain combinations of donor and recipient factors seem to be of higher prognostic value than the existence of single risk factors. For instance, a high donor age and hepatitis C in the recipient[29] or the combination of a worsening MELD score (D-MELD) and the presence of $\geq 2$ EDC in organ donors[14] may decrease survival after liver transplantation. Therefore, the TOP study only included marginal liver grafts exhibiting 2 or more EDC.

The design of the TOP study resulted in a clinically relevant reduction of graft quality to prove therapeutic effects of the study medication. In this respect, the median DRI was 1.9 in all organ donors whose grafts had been used for the TOP study. The average number of EDC did not differ between the study groups. Although the DRI represents a rating system for graft quality developed specifically for the United States,[10] it is also a valid marker for graft quality within the Eurotransplant allocation system.[30] The relevance of the DRI is further emphasized by a previous analysis conducted by our group which included 448 patients from our institutional liver transplant database, which showed a DRI of 1.25 or greater to be a highly prognostic marker in multivariate analysis with a hazard rate ratio of 3.2.[31]

The prognostic relevance of the hepatic IRI and other nonimmunological pathomechanisms, however, is the subject of ongoing discussion.[32-34]

Acute effects of IRI include the generation of reactive oxygen species and the release of inflammatory cytokines which result in microcirculatory disturbance causing graft damage and potentially graft loss.[35] Ischemia-reperfusion injury also correlates with the initial organ function.[36,37] Therefore, clinical markers of hepatic IRI (ALT, AST) were chosen as endpoints in the TOP study. Although IRI is a diagnosis mainly made by histologic staining,[38] the primary endpoint of the study, ALT, correlates well with intrahepatic changes characteristic for reperfusion associated liver damage.[39]

Moreover, IRI may be associated with chronic alterations of the graft. In this respect, some authors suggest an interaction between nonimmunological and immunological factors thereby influencing the long-term outcome of liver grafts: O'Leary et al[40,41] have shown a correlation between organ damage and the generation of donor specific HLA-antibodies.

In the present study, a tacrolimus rinse at a concentration of 20 ng/mL did not reduce postoperative ALT levels. Even a trend toward slightly higher levels of ALT seems to be evident in grafts treated with tacrolimus, and this observation reaches statistical significance in AST in the postoperative course ($P = 0.011$). Nevertheless, based on the present results suggesting no therapeutic effect also if larger numbers of patients had been recruited or even harmful action of a tacrolimus rinse the TOP study was terminated in July 2013 according to a decision of the scientific committee.

Conflicting data on ALT and AST levels after tacrolimus rinse before liver transplantation have been published.[6,7] Similar to our results, Kristo et al[7] reported no effect of tacrolimus on AST and ALT levels in nonmarginal organs. On the molecular level, a reduction of precursors of inflammatory markers using highly sensitive gene chip array analysis after a tacrolimus rinse with 20 ng/mL in liver transplantation was evident. These findings argue against a hepatotoxic effect of tacrolimus.

Altogether, previous data suggest that tacrolimus could also exert protective effects in marginal liver grafts which exhibit an increased IRI.

To analyze potential effects of a rinse treatment in marginal liver grafts itself, the results of the present study were compared with placebo using the liver transplant database of our hospital. For this purpose, 24 recent consecutive transplantations of livers exhibiting 2 or more EDC were analyzed. Preoperative ALT/AST values did not differ between study patients and placebo ($P > 0.05$). When analyzing postoperative ALT/AST values (days 1, 2, 4, 7), no differences were evident between study patients that had received an ex situ rinse and placebo (Mann-Whitney $U$ test, $P > 0.05$).

In summary, the study medication failed to decrease IRI and partially even increased graft damage in transplantation of marginal livers. Only a small number of patients (n = 24) has been included in this study, which limits its significance. Nevertheless, missing evidence of the effectiveness of the study medication must be presumed based on the present data. Whether varying the dosage of tacrolimus in the rinse solution or new approaches (ie, adding tacrolimus to a continuous machine perfusion) will increase its effectiveness should be addressed in future trials.

## CONCLUSIONS

Critical organ shortage contributes to decreased graft quality which is associated with an increase in IRI as well as reduced survival after liver transplantation. Experimental and clinical data indicate a protective role of a tacrolimus rinse in healthy, nonmarginal liver grafts. The aim of the TOP study was to reduce hepatic IRI with a single ex vivo tacrolimus rinse before reperfusion in EDC liver grafts. The present data do not indicate a protective role of tacrolimus rinse of marginal organs in liver transplantation rather demonstrating harmful effects. Thus, ex vivo tacrolimus rinse is contraindicated as an approach to decrease IRI after transplantation of marginal livers.

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OPEN

# The Authors' Reply

Sebastian Pratschke, MD[1] and Martin K. Angele, MD[1]

Thank you very much for your comment on our manuscript "Results of the TOP Study: Prospectively Randomized Multicenter Trial of an Ex Vivo Tacrolimus Rinse Before Transplantation in EDC Livers."[1] We would like to take the opportunity to respond to the valuable comments of Kobayashi.[2]

In the tacrolimus organ perfusion (TOP) study marginal liver grafts exhibiting ≥ 2 extended donor criteria (EDC) were randomly flushed ex situ with Tacrolimus (20 ng/mL) dissolved in histidine tryptophane ketoglutarate (HTK) solution or with HTK only (control) to reduce ischemia reperfusion injury. Kobayashi suspects a potential warming of the grafts and harmful effects through this procedure. Facing this criticism, the authors state that the temperature of the rinse solution was 4°C at the beginning of the treatment (though it was not measured at the end of the perfusion as suggested by Kobayashi) and the procedure took on an average of 18 minutes only. Although the plastic bags containing the rinse solution were not cooled during the procedure (analogously with the systemic perfusion at organ harvesting), grafts undergoing the study treatment were continuously stored on ice until the beginning of implantation. Therefore, the authors assume a warming of the graft and a subsequent initiation of the hepatic metabolism to be extremely unlikely. Moreover, Kobayashi questions the anastomosis time and assumes that warm ischemia could counteract the protective effects of a rinse treatment. In this respect, he suggests a new in situ technique of organ perfusion. The authors would like to state that changes in the surgical procedure were beyond the scope of the TOP Study. Moreover, the rinse treatment itself was not the point of interest in our study, but to investigate the effects of Tacrolimus in a rinse of 1000 ml HTK compared to 1000 ml HTK only. Cava sparing transplantation including anastomoses was performed after the rinse treatment according to center specific standards in both groups. Therefore, the study treatment did not affect the time for vascular reconstruction. To the authors' best knowledge, a simple study treatment in combination with routine surgery is required to generate valid data in a multicentric trial in liver transplantation.

Kobayashi presents an interesting concept of an in-situ perfusion. The authors greatly appreciate any attempts (ie, organ preperfusion) to improve the utilization of EDC grafts. In this respect, an additional in situ flush of the graft may have protective effects. Nonetheless, this procedure does not represent an established procedure and would need to be evaluated in a prospective trial. Since the TOP study failed to show protective effects of Tacrolimus compared with control, this concept must be reevaluated and the authors suggest further analyses of this substance due to its proven anti-inflammatory effects. Therefore, an adjunct of Tacrolimus to an in-situ perfusion as described by Kobayashi or to machine perfusion could be an option to reduce ischemia reperfusion injury especially in EDC grafts thereby increasing the limited donor pool, which represents the most pressing problem in today's transplantation medicine.

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*S.P. participated in the writing of the article. M.A. participated in the writing of the article.*

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# Tacrolimus Preconditioning of Rat Liver Allografts Impacts Glutathione Homeostasis and Early Reperfusion Injury

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***Objective.*** **To characterize the immunosuppressant tacrolimus as a protective antioxidant in rat liver transplantation.**

***Methods.*** **Livers of male Lewis rats underwent 24 h of hypothermic preservation in UW solution and were rinsed with tacrolimus or placebo directly before transplantation. Markers of liver injury, such as enzymes and bile flow, were determined during a 2 h reperfusion period. Concentrations of reduced (GSH) and oxidized (GSSG) glutathione were analyzed in plasma, bile, and liver tissue for estimation of oxidant stress caused by reactive oxygen species (ROS).**

***Results.*** **Administration of tacrolimus (10 ng/mL) resulted in decreased ALT plasma levels (1740 ± 1169 U/l *versus* 3691 ± 1144 U/l; $P < 0.05$) at 2 h of reperfusion. While endogenous intracellular GSH concentrations remained unchanged, GSSG, the oxidation product of GSH, was markedly decreased at 2 h of reperfusion in preconditioned livers (47.0 ± 10.4 nm/g *versus* 71.8 ± 30.6 nm/g; $P < 0.05$). Correspondingly, GSSG bile concentrations (0.19 ± 0.04 mM *versus* 0.13 ± 0.04 mM; $P < 0.05$) as well as plasma GSSG levels (2.4 ± 0.3 mM *versus* 1.4 ± 0.2 mM; $P < 0.05$) were significantly increased upon reperfusion. These findings suggest that tacrolimus impacts post-ischemic GSH metabolism when administered as a rinse solution for liver allografts through an unknown pathway.**

***Conclusion.*** **Hepatocellular injury following transplantation was significantly decreased by preconditioning with tacrolimus. One possible mechanism of action is the detoxification of ROS through the preservation of cytosolic and extracellular GSH/GSSG ratios.** 

***Key Words:*** **ischemia/reperfusion injury; liver transplantation; glutathione; tacrolimus.**

## INTRODUCTION

Ischemia-reperfusion injury (IRI) as a consequence of liver preservation has considerable effects on the outcome of patients after liver transplantation. Primary allograft dysfunction remains, therefore, a serious problem, resulting in high patient morbidity and mortality rates [1–3]. There is substantial evidence that activation of Kupffer cells (KC), the generation of intra- and extracellular reactive oxygen species (ROS), and disturbances of the hepatic microcirculation are involved in reperfusion injury [4, 5]. Therefore, therapeutic strategies which reduce IRI remain to be established.

A large number of investigations using antioxidant interventions support the pivotal role of ROS in the development of reperfusion injury following warm or cold liver ischemia [3, 6, 7]. Recent studies examined the therapeutic potential of the endogenous antioxidant glutathione (GSH), a compound which is of particular clinical interest due to its low toxicity in humans [8]. Furthermore, GSH is able to react spontaneously with nearly all oxidants formed during inflammation and reperfusion, resulting in the formation of oxidized glutathione (GSSG). In liver cells, the concentration of GSH is 10 mM and thus 1000-fold higher than in the extracellular space (10 $\mu$M), i.e., the sinusoids [9]. Recently, we could demonstrate that GSH effectively

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reduces IRI in livers following warm and cold ischemia when given intravenously during the reperfusion period [10, 11]. Upon reperfusion of ischemic livers, intracellular GSH is released into the sinusoidal space as well as into the bile through active transporters found on hepatocytes, and can thus act as an endogenous defense system against ROS in these compartments [12, 13].

Since the mid-1980s, the calcineurin inhibitor tacrolimus (FK 506) has been a mainstay in immunosuppressive regimens following organ transplantation [14, 15]. Specific T-cell inhibition seems to be responsible for the immunosuppressive properties of tacrolimus. In addition, tacrolimus has been shown to result in amelioration of ischemia-reperfusion injury in various organs. Its ability to reduce IRI is the cumulative result of effects on free radical metabolism, microcirculation, calcium-activated pathways, inflammatory cascades, and others [16]. Several experimental studies have demonstrated a beneficial impact on ROS-mediated cell damage upon ischemia and reperfusion [17, 18]. Although it seems plausible that the protective effect of tacrolimus is due to the suppression of free radicals following liver ischemia and reperfusion, the molecular mechanisms responsible for this effect remain largely unclear. Recently, an interesting human study was able to demonstrate a protective effect on reperfusion injury, when liver allografts were flushed with tacrolimus directly before the implantation procedure [19]. This procedure resulted in a significant decrease of postoperative peak ALT levels; however, this study did not investigate potential mechanisms underlying this important observation.

On the basis of these results, and the knowledge that glutathione is an important endogenous antioxidant in IRI, we hypothesized that the application of tacrolimus as a rinse solution for liver allografts may have an influence on the GSH/GSSG metabolism during rat liver transplantation.

## METHODS

### Animals and Preparation

All studies were performed with the permission of the government authorities and in accordance with the German legislation on laboratory animal experiments. Syngeneic, male Lewis rats (donors: 210 ± 14 g; recipients 284 ± 18 g body weight) were fasted 12 h prior to donor and recipient operations, which were performed as described earlier [20]. Prior to the implantation procedure, the livers were either rinsed with cold saline solution alone or with the addition of tacrolimus (Astellas Inc., Deerfield, IL) *via* the portal vein. Portal clamping time during transplantation was less than 20 min in all experiments (17 ± 1.5 min).

The common bile duct of the graft was cannulated with a PE-tube and bile was collected in Eppendorf cups. Plasma samples (500 μL) were obtained in the recipient before hepatectomy and during 120 min of reperfusion of the transplanted liver. The volume of the blood drawn was replaced by saline. Five min after starting reperfusion, all rats received 0.5 mL of albumin (5%) and 0.5 mL sodium bicarbonate in order to maintain blood pressure and physiologic pH. To avoid major fluid loss and drying of the liver, the abdominal cavity was covered with cling wrap throughout the operation. After 120 min of reperfusion, experiments were terminated and the liver weight was determined.

### Experimental Groups

Two intervention groups ($n = 6$ each) were compared with a control group ($n = 6$) and a sham-operated group (only laparotomy; $n = 5$). In control animals, saline (20 mL at 4°C; constant pressure 10 cm $H_2O$) was infused *via* the portal vein to wash the preservation solution from the liver allograft before implantation. Intervention groups received 10 or 50 ng/mL of tacrolimus, which was added to 20 mL of saline.

### Analytical Methods

#### *GSH and GSSG*

Total soluble glutathione (GSH and GSSG) was measured in plasma and bile as well as in the acidic homogenate from freeze-clamped livers as described in detail elsewhere [21]. For GSSG analysis an aliquot (200 μL) of blood was mixed immediately with 200 μL of 10 mM N-ethylmaleimide (NEM) in 100 mM phosphate buffer (pH 6.5) containing 17.5 mM EDTA [21]. The remaining blood (300 μL) was centrifuged at full speed for 1 min. An aliquot of (100 μL) of plasma was pipetted into 100 μL sulfosalicylic acid (5%) for determination of total glutathione. To separate GSSG from NEM and NEM-GSH adduct, an aliquot of NEM treated plasma was passed through a Sep-PakC18 cartridge (Waters, Framingham, MA) followed by 1 mL of 100 mM phosphate buffer (pH 7.5). GSSG in the eluates and total glutathione in plasma and acidic homogenates were determined by an enzymatic test as described previously [21, 22]. GSH plasma concentrations were calculated as the difference between total glutathione and GSSG. Bile concentrations of GSH and GSSG were measured as described above; however, these samples underwent dilution (1:1000 in phosphate buffer) before analysis.

#### *Serum Markers of Liver Cell Damage*

Serum aminotransferases were used as established markers of liver injury. Aspartate aminotransferase (AST) and alanin aminotransferase (ALT) were measured 2 h a fter reperfusion using a serum multiple analyzer (Hitachi917; Roche, Mannheim, Germany).

### Liver Function

Liver function upon transplantation was estimated from the amount of bile produced during the 2 h reperfusion period. Bile flow was determined by collecting the bile in 30 min intervals (μL/min/g) and the cumulative bile flow was calculated from total bile volume produced during 2 h of reperfusion per gram of liver (μL/2 h/g).

### Statistical Analysis

All data are expressed as mean and standard error. Statistical differences between groups were calculated using paired or unpaired Student's *t*-test for randomly distributed data and the Mann-Whitney U test for nonparametric data following analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$.

## RESULTS

### Tacrolimus Absorbance in the Liver Allograft

It was important to document the extent to which tacrolimus can be absorbed by an isolated graft when it was flushed by 20 mL of saline with 10 and 50 ng/mL of the immunosuppressant at physiologic hydrostatic pressure. For that purpose, the tacrolimus concentration in the effluate from the hepatic veins was measured after rinsing the livers with the tacrolimus solution. Interestingly, we found that very little of the tacrolimus in the rinse solution remained unabsorbed. Approximately 100% and 88% of the administered tacrolimus dosages of 10 and 50 ng/mL, respectively, were absorbed during rinsing, indicating successful incorporation by the liver tissue (Fig. 1).

### Cell Injury and Liver Function after Liver Transplantation

Injury of parenchymal liver cells after liver transplantation was assessed by the release of ALT and AST at 120 min of reperfusion. Parenchymal cell damage in untreated animals (controls) was indicated by a 34- and 40-fold increase of ALT- und AST serum levels, respectively, compared with sham-operated animals (Table 1). Rinsing of the allografts with tacrolimus at a concentration of 10 ng/mL resulted in a significant ($P < 0.05$) reduction in AST- and ALT-levels of almost 40% and 50%, respectively, whereas the administration of 50 ng/mL of tacrolimus seemed to be less effective (Table 1).

The function of the liver allografts was estimated by the recovery of bile flow during reperfusion. The bile flow of donor livers was approximately 1 $\mu$L/min/g (Table 1). Hypothermic preservation and the transplantation procedure dramatically decreased maximum bile flow upon reperfusion to approximately 50% of baseline values. The post-ischemic bile flow was significantly higher in those allografts, which had been rinsed with 10 ng/mL tacrolimus ($P < 0.05$). The administration of 50 ng/mL of tacrolimus showed no substantial effect on the functional recovery of the transplant as measured by the cumulative bile flow during 2 h of reperfusion (Table 1).

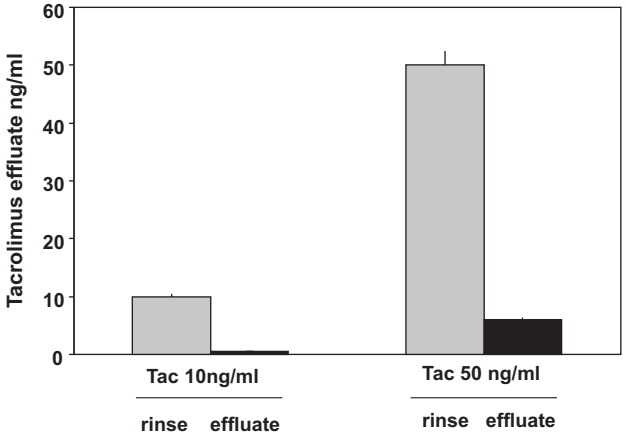


**FIG. 1.** Tacrolimus (Tac) concentrations in rinse solution with either 10 ng/mL or 50 ng/mL as substitute to 20 mL of saline. Black columns indicate tacrolimus concentration as measured in the effluate, sampled from liver veins. Almost 100% uptake following 10 ng/mL and about 88% uptake following 50 ng/mL of tacrolimus was determined.

### Effect of Tacrolimus on GSH and GSSG Tissue Levels

Samples of frozen liver tissue for determination of GSH and GSSG concentrations were taken at the end of the 2 h reperfusion period. Preconditioning with the tacrolimus flush solution did not significantly influence the total intracellular GSH content (GSH + GSSG) as shown in Fig. 2A. However, livers that had been rinsed with the 10 ng/mL tacrolimus solution showed significantly lower levels of GSSG following transplantation (47.9 ± 8.9 $\mu$mol/g *versus* 71.2 ± 12.2 $\mu$mol; $P < 0.05$). This decrease was less pronounced in livers which had been rinsed with the 50 ng/mL solution (63.8 ± 9.8 $\mu$mol/g; Fig. 2B). Moreover, similar results were evident when GSSG was normalized to percent of total GSH levels (data not shown). Tacrolimus seemed to mediate an increase in the reduced form of hepatocellular glutathione (GSH), in particular at the cytoprotective dose of 10 ng/mL.

### Effect of Tacrolimus on GSH and GSSG Bile Concentrations

Quantification of the bile concentrations of GSH and GSSG during the reperfusion period showed a substantial I/R-mediated decrease in controls compared with the sham group (Fig. 3). Tacrolimus preconditioning of liver allografts with 10 ng/mL significantly increased the GSH concentration compared with untreated controls (320 ± 123 mM *versus* 109 ± 19 mM; $P < 0.05$), whereas 50 ng/mL was less effective (191 ± 84 mM; Fig. 3A). An important fraction of the total GSH content in the bile was represented by the oxidized form of GSH (GSSG), as shown by GSSG concentrations of 0.19 ± 0.02 mM and 0.15 ± 0.03 mM after 10 ng/mL and 50 ng/mL tacrolimus flush, respectively, whereas controls exhibited GSSG bile concentrations of only 0.13 ± 0.04 mM (Fig. 3B), indicating a substantially accelerated export of GSSG from liver cells into the bile during reperfusion of transplants.

### Effect of Tacrolimus on GSH and GSSG Plasma Concentrations

Total plasma glutathione levels were significantly increased after the procedures of organ harvest, 24 h of hypothermic preservation, and orthotopic

**TABLE 1**

**Hepatocellular Injury and Liver Function Following Transplantation**

| | Sham [$n = 5$] | Control [$n = 6$] | Tac 50 [$n = 6$] | Tac 10 [$n = 6$] | $P$ value [*versus* control] |
|---|---|---|---|---|---|
| ALT [U/l] | 108 ± 12 | 3691 ± 1444 | 2213 ± 816* | 1740 ± 736* | <0.05 |
| AST [U/l] | 72 ± 9 | 2854 ± 676 | 2312 ± 412* | 1470 ± 812* | <0.05 |
| BF baseline [μL/min/g] | 1.03 ± 0.6 | 1.1 ± 0.4 | 0.9 ± 0.3 | 1.1 ± 0.5 | - |
| BF maximum [μL/min/g] | 1.05 ± 0.2 | 0.43 ± 0.1 | 0.52 ± 0.08 | 0.68 ± 0.12* | <0.05 |
| BF cumulative [μL/2 h/g] | 111.5 ± 4.8 | 34.7 ± 5.9 | 41.2 ± 4.2 | 53.4 ± 6.1* | <0.05 |

Sham operated animals underwent only laparotomy, but had open abdomen for the same time period as necessary for transplantation and reperfusion time. Tac 50 = 50 ng/mL and Tac 10 = 10 ng/mL tacrolimus added to rinse solution. ALT and AST serum levels were analyzed at the end of the 2 h reperfusion period. BF = bile flow; baseline values represent bile flow as measured in donors.

*$P < 0.05$ versus controls.

transplantation as shown in Fig. 4A (14.6 ± 1.2 μM in controls *versus* 9.4 ± 0.3 μM in sham-operated animals; $P < 0.05$). Preconditioning of allografts with 10 ng/mL tacrolimus resulted in a further increase in the total GSH concentration to 20.3 ± 1.2 μM ($P < 0.05$ *versus* controls), but only to 17.9 ± 0.9 μM following preconditioning with 50 ng/mL of tacrolimus. Moreover, plasma GSSG increased to significantly higher levels (2.4 ± 0.3 μM and 2.1 ± 0.4 μM, respectively) compared with untreated controls (1.4 ± 0.2 μm) or animals of the sham group (0.8 ± 0.1 μM) as demonstrated in Fig. 4B ($P < 0.05$ each). These findings suggest that tacrolimus also influences glutathione homeostasis in the extracellular space, i.e., the sinusoids of liver grafts.

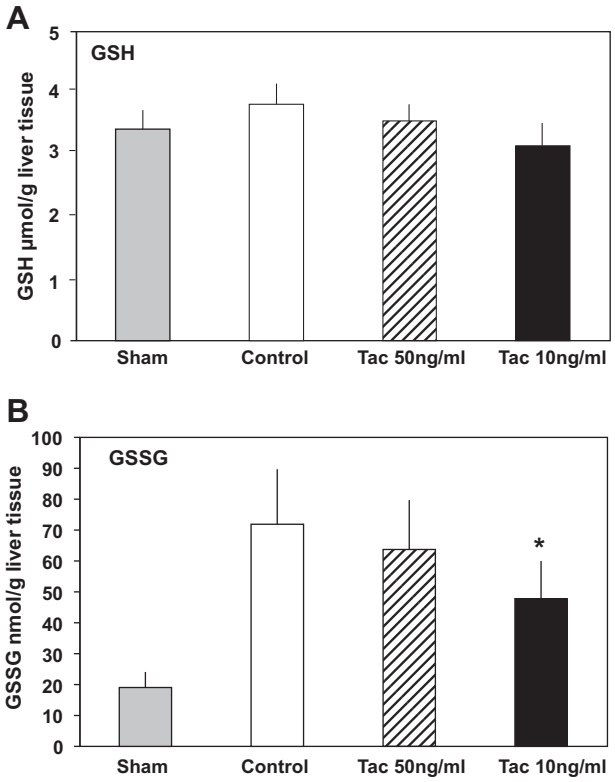


**FIG. 2.** Total GSH (GSH+GSSG) (A) and GSSG (B) concentrations in liver tissue of allografts at the end of reperfusion period (2 h). Donor organs were perfused and preserved with University of Wisconsin (UW) solution for 24 h at 4°C before transplantation. Significant increase of GSH and GSSG compared with sham group (grey column) indicates recruitment and oxidation of cytosolic GSH upon free radical formation. Pretreatment of livers with tacrolimus leads to a slight decrease of cellular GSH content, but to significantly reduced GSSG concentrations when 10 ng/mL were administered. Controls: white column; tacrolimus 50 ng/mL: striped column; tacrolimus 10 ng/mL: black column). *$P < 0.05$ *versus* control.

## DISCUSSION

There is substantial experimental evidence for ROS-mediated cell damage as a central pathomechanism of reperfusion injury of the liver after warm and cold ischemic periods [4, 7, 23]. Therefore, antioxidant strategies are thought to be a promising approach for the prevention of hepatic reperfusion injury in transplanted organs. In the present study, we used tacrolimus to precondition rat liver allografts directly before implantation, based on earlier work showing antioxidative properties of that immunosuppressant in liver I/R [16, 19]. Our results confirm the antioxidative properties of tacrolimus in the setting of liver transplantation. We found that (1) the use of tacrolimus as flush solution significantly reduces hepatocellular injury and enhances early transplant function within the first 2 h of reperfusion, (2) tacrolimus reduces levels of toxic GSSG in hepatocytes without affecting the total intracellular GSH content, and (3) tacrolimus augments the release of GSSG into the bile and also into sinusoids through a still unknown mechanism, as measured by plasma concentrations of oxidized GSH, resulting in preservation of GSH/GSSG ratios.

Liver transplantation is the therapy of choice in an increasing number of liver diseases, however the organ pool is limited and 5%–30% of human allografts exhibit severe postoperative dysfunction and frequently require retransplantation [2, 24]. A comprehensive understanding of the mechanisms underlying graft failure is essential to improving success rates in this clinical setting. From recent work, it seems clear that

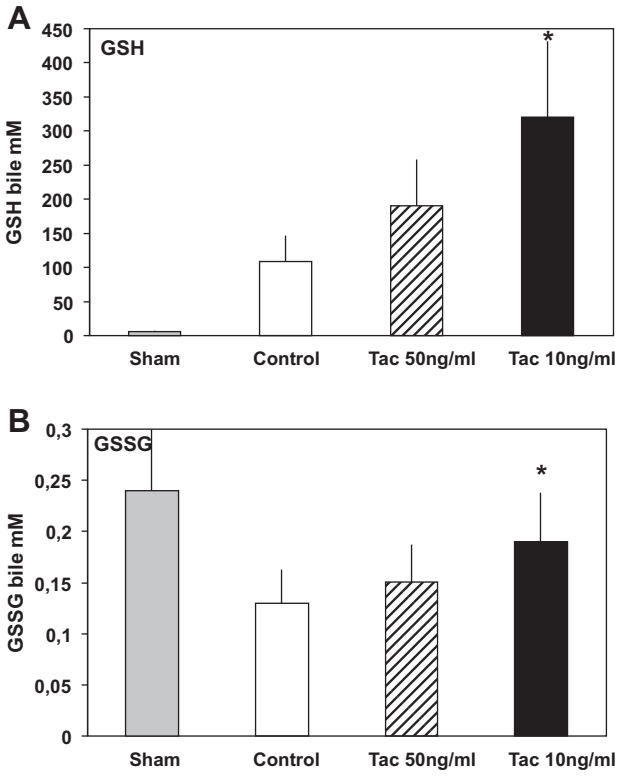


**FIG. 3.** Total GSH (GSH+GSSG) (A) and GSSG (B) concentrations in bile of allografts at the end of reperfusion period (2 h). In transplanted livers (controls, white column), bile concentration of GSH was substantially elevated whereas GSSG was markedly decreased compared with only laparotomy (sham, grey column). Significant increase of total GSH and GSSG export into the bile when livers were preconditioned with 10 ng/mL of tacrolimus (black column). *$P < 0.05$ *versus* controls.

reperfusion injury, and not ischemic cell damage during cold storage, presents the predominant problem [25]. During reperfusion of warm and cold ischemic livers, activated Kupffer cells (KC) produce mediators of inflammation, including tumor necrosis factor $\alpha$, interleukins, and chemokines, and release ROS into the sinusoidal space, which in turn result in liver damage [26]. There is no doubt that I/R-mediated intracellular oxygen radical formation may contribute to hepatic failure [6, 23, 27]. In addition, we were recently able to demonstrate that the generation of KC-derived ROS may act as extracellular signal molecules, rendering also the sinusoidal space to an "oxidative" environment [3, 8, 20]. Besides other effects, extracellular ROS action was associated with a dramatically impaired hepatic microcirculation upon reperfusion of transplanted livers which could be significantly improved by post-ischemic intravenous glutathione [11, 28] or glycine administration [29, 30].

Thus, we speculated that altered glutathione metabolism may contribute to the beneficial effects of tacrolimus rinse. Nonetheless, the possible role of other mechanisms, for instance a decreased inflammatory response, should also be considered in future studies.

The concept of flushing liver grafts after hypothermic storage with Carolina rinse solution containing antioxidants, adenosine, calcium blocker, energy substrates, and glycine resulted in new insights into preventive mechanisms of endothelial cell killing, Kupffer cell activation and ROS-release [31]. Because pharmaceutical preconditioning of liver grafts is more feasible in the clinical setting than donor preconditioning [32–35] or post-ischemic treatment [11, 36], we studied the addition of a calcineurin inhibitor (tacrolimus; FK506) to the rinse solution. We chose tacrolimus because of recent work demonstrating beneficial effects of this immunosuppressant on reperfusion injury in human liver transplantation [19].

In our study, we could observe a significant decrease in postoperative ALT- and AST-levels of 40% and 50%, respectively (Table 1), following 10 ng/mL of tacrolimus rinse. This effect was weaker after administration of the higher dosage of 50 ng/mL. The trend towards a reduction in efficacy at the higher 50 ng/mL dosage compared with the 10 ng/mL dosage did not reach statistical significance. However, the benefit of the 10 ng/mL dosage compared with the control group was statistically significant. The surprisingly poor protective activity of the higher tacrolimus dosage may be related to increased toxicity. In humans, the daily dosage of tacrolimus applied intravenously lies between 0.01 and 0.05 mg/kg/d. The corresponding tacrolimus dose calculated for a 300 g rat would be between 3 and 15 $\mu$g per day. For comparison, the total amount of tacrolimus applied with the flush solution is 0.2 $\mu$g (10 ng/mL) and 1 $\mu$g (50 ng/mL), respectively. In humans, plasma levels of tacrolimus range from 5 to 15 ng/mL, according to the patient's condition. Therefore, one reason for the more pronounced effects of tacrolimus at 10 ng/mL could be that this dosage better represents the therapeutic range of this drug. The 50 ng/mL dosage is clearly much higher than would be expected in a clinical setting, and may have resulted in toxicity in our model of liver transplantation.

Furthermore, tacrolimus-mediated preservation of hepatocellular integrity was associated with better bile flow during 2 h of reperfusion (Table 1), which is also consistent with postoperatively reduced bilirubin levels in the above mentioned study [19]. Although changes in aminotransferases better reflect early pathophysiologic alterations following ischemia-reperfusion injury, bile production also represents an important factor to characterize potential beneficial effects of tacrolimus rinse.

Looking at possible underlying mechanisms of graft protection by tacrolimus, it is relevant to note that similar results showing IRI protection were obtained when

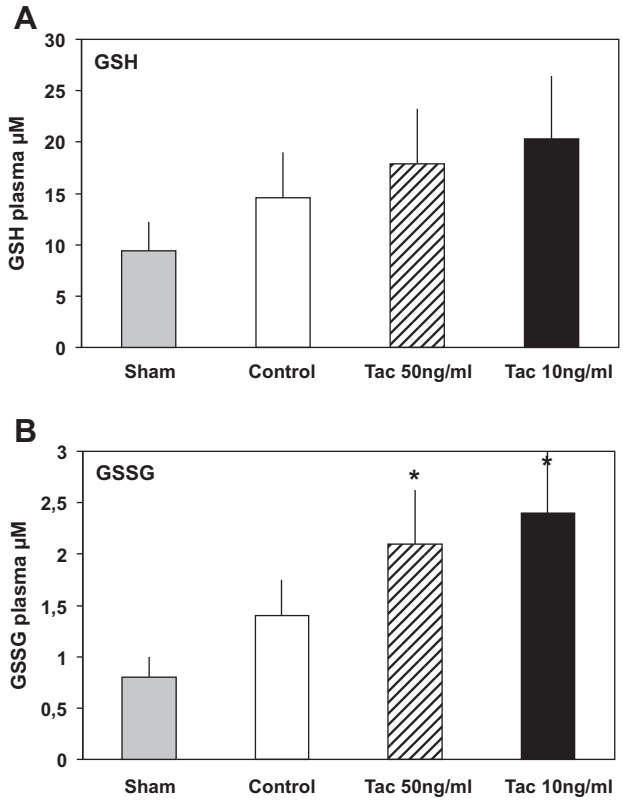


**FIG. 4.** Total plasma GSH (GSH+GSSG) (A) and GSSG (B) concentrations of transplanted animals at the end of reperfusion period (2 h). While post-ischemic GSH and GSSG increase in controls was significant compared with sham groups, preconditioning with tacrolimus did not lead to substantial elevated plasma GSH levels (A). However, plasma GSSG concentrations showed significant increase upon treatment with tacrolimus. Sham: grey column; controls: white column; tacrolimus 50 ng/mL: striped column; tacrolimus 10 ng/mL: black column $^*P < 0.05$ *versus* controls.

KC were blocked upon reperfusion [20, 37, 38], or when antioxidants were given at various time points during liver transplantation [11, 29, 39]. This suggests that ROS, either released intracellulary or in the sinusoidal space, might be a target for tacrolimus action [32, 40]. Currently, the most important clinical use of tacrolimus is as an immunosuppressant, based on its ability to inactivate T-cells [41]. However, additional effects of tacrolimus have been documented [32], in particular its ability to prevent ischemic damage in tissues when administered intravenously before ischemia, although the mechanisms underlying these effects are not known [42, 43]. Other investigators were able to demonstrate the preservation of microvascular perfusion following liver ischemia and reperfusion [44], and a substantial decrease in free radical production in association with improvement of IRI when tacrolimus is administered before ischemia [18, 45, 46]. Because tacrolimus is a highly lipophilic compound and, therefore, easily crosses the plasma membrane to gain access to intracellular spaces [47], it seemed likely that the primary antioxidative action of tacrolimus takes place within the hepatocytes. This theory was supported by our results, which showed an extraction rate of tacrolimus from the rinse solution *via* the portal vein of allografts between 88% (50 ng/mL) and 100% (10 ng/mL; Fig. 1), suggesting a nearly complete absorbance of the compound by the cells of the liver.

Our analysis of intracellular levels of the important endogenous antioxidant glutathione (GSH) revealed that compared with the dramatically increased concentrations of the oxidized form of GSH (GSSG) in control livers, GSSG levels could be significantly reduced by the administration of 10 ng/mL tacrolimus rinse, while the total GSH content remained unchanged (Fig. 2). Intracellular GSSG levels can be increased by the intracellular generation of $H_2O_2$ at the monoamine oxidase reaction upon reperfusion [9]. The prevention of a high reducing environment inside the cell, i.e., GSH/GSSG ratio by tacrolimus may thus contribute to free radical elimination during reperfusion [9, 48]. This is supported by earlier observations that demonstrated an improved hepatocyte oxidation/reduction following tacrolimus administration, with improved ketone body ratios, and the prevention of ATP content deprivation under hypoxic conditions [46].

The tacrolimus-induced preservation of hepatocellular GSH/GSSG redox state was likely the result of an enhanced excretion of GSSG into the bile (Fig. 3) and to the sinusoids (Fig. 4). Total glutathione (GSH+GSSG) bile concentrations were markedly increased in preconditioned animals, but based on the significantly higher cytosolic GSH levels [49], the relative fraction of biliary GSSG translocation seems to play the key role under these conditions [50], although ATP-dependent GSH release into the extracellular space under hypoxic conditions has also been previously reported [50, 51].

In contrast, it is well known that GSH is not taken up by cells [52], which in our study resulted in a slight post-ischemic increase in reduced GSH, including in the sinusoids that may enhance extracellular antioxidative capacity. As shown in Fig. 4, reperfusion of transplanted livers was associated with a significant increase in plasma GSH of about 35% compared with sham-operated animals, and tacrolimus preconditioning augmented total GSH plasma concentrations, even compared with untreated controls. Similarly, GSSG plasma concentrations were simultaneously increased upon reperfusion (Fig. 4). This observation is best explained by either an enhanced active release of GSSG into sinusoids as a consequence of hepatocellular detoxification of hydroperoxides [52], or by GSH oxidation upon free radical formation in the hepatic microvasculature [3, 4, 20], thus reducing extracellular vascular stress. Because both phenomena may be

critical to the preservation of liver cell integrity upon I/R, the ability of tacrolimus preconditioning to produce high GSH/GSSG ratios in liver cells as well as in sinusoids is likely an important feature leading to the efficacious defense of free radical action [9]. Nevertheless, the exact mechanisms behind this observation still remain unclear.

In summary, this study provides some new insights into the antioxidative properties of the immunosuppressant tacrolimus on I/R-mediated liver injury. However, the impact on GSH/GSSG metabolism by tacrolimus may only be one facet of multiple interconnected mechanisms involved in liver IRI, as tacrolimus is also known to have effects on the microcirculation, inhibition of calcium-dependent pathways, inhibition of inflammatory response, and modification of cellular responses to injury. Also, we are not able to fully explain the lower efficacy of the higher dosage (50 ng/mL) in the prevention of IRI, in particular as this dosage was effective in an *ex vivo* model of hypothermic I/R (Bilzer M., unpublished data). In this respect, further analysis of the underlying mechanisms of a tacrolimus rinse and its interconnections with the observed changes in glutathione metabolism must be performed. Moreover, those effects must be confirmed in a long-term model in the future. Nevertheless, liver allograft preconditioning with tacrolimus appears to be a promising alternative for the prevention of oxygen radical- mediated reperfusion injury after liver transplantation.

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TRANSPLANTATION RESEARCH

CLINICAL TRIAL PROTOCOL Open Access

# Protocol TOP-Study (tacrolimus organ perfusion): a prospective randomized multicenter trial to reduce ischemia reperfusion injury in transplantation of marginal liver grafts with an *ex vivo* tacrolimus perfusion

Sebastian Pratschke[1], Michael Eder[1], Michael Heise[2], Silvio Nadalin[3], Andreas Pascher[4], Peter Schemmer[5], Marcus N Scherer[6], Frank Ulrich[7], Heiner Wolters[8], Karl-Walter Jauch[1], Dirk Wöhling[9] and Martin K Angele[1*]

## Abstract

**Background:** Critical organ shortage results in the utilization of extended donor criteria (EDC) liver grafts. These marginal liver grafts are prone to increased ischemia reperfusion injury (IRI) which may contribute to deteriorated graft function and survival. Experimental data have shown that the calcineurin inhibitor tacrolimus exerts protective effects on hepatic IRI when applied intravenously or directly as a hepatic rinse. Therefore, the aim of the present study is to examine the effects of an *ex vivo* tacrolimus perfusion on IRI in transplantation of EDC liver grafts.

**Methods/Design:** The TOP-Study (tacrolimus organ perfusion) is a randomized multicenter trial comparing the *ex vivo* tacrolimus perfusion of marginal liver grafts with placebo. We hypothesize that a tacrolimus rinse reduces IRI, potentially improving organ survival following transplantation of EDC livers. The study includes livers with two or more EDC, according to Eurotransplant International Foundation's definition of EDC livers. Prior to implantation, livers randomized to the treatment group are rinsed with tacrolimus at a concentration of 20 ng/ml in 1000 ml Custodiol solution and in the placebo group with Custodiol alone. The primary endpoint is the maximum serum alanine transamninase (ALT) level within the first 48 hours after surgery; however, the study design also includes a 1-year observation period following transplantation. The TOP-Study is an investigator-initiated trial sponsored by the University of Munich Hospital. Seven other German transplant centers are participating (Berlin, Frankfurt, Heidelberg, Mainz, Münster, Regensburg, Tübingen) and aim to include a total of 86 patients.

**Discussion:** Tacrolimus organ perfusion represents a promising strategy to reduce hepatic IRI following the transplantation of marginal liver grafts. This treatment may help to improve the function of EDC grafts and therefore safely expand the donor pool in light of critical organ shortage.

**Trial register:** EudraCT number: 2010-021333-31, ClinicalTrials.gov identifier: NCT01564095

**Keywords:** Liver transplantation, Organ shortage, Extended donor criteria, Marginal grafts, Tacrolimus, Organ rinse, Graft function, Graft survival

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## Introduction

Organ shortage represents a critical problem in transplantation medicine. In 2010, 1192 liver transplantations were performed in Germany as opposed to 1846 new entries on the waiting list (German Organ Transplantation Foundation (Deutsche Stiftung Organtransplantation, DSO), Annual Report, 2010). As a consequence of this discrepancy, there is a noticeable trend towards the utilization of extended donor criteria (EDC) grafts. Data provided by the Eurotransplant International Foundation indicate that the proportion of liver grafts exhibiting one or more EDC increased from 29% in 1997 to 73% in 2010 (Axel Rahmel, Medical Director, Eurotransplant, personal communication). The proportion of grafts with two or more EDC increased from 4% up to 28% over the same time period.

Ischemia reperfusion injury (IRI) is a complex inflammatory, allogen-independent process commonly seen following graft transplantation; however, it is particularly pronounced in marginal organs [1-3], and may contribute to poor graft function and reduced survival in these recipients [4,5]. During ischemia and reperfusion, proinflammatory cytokines such as IL-6 or TNF-α are released into the systemic circulation by Kupffer cells and migrating neutrophils [6]. These molecules induce a complex inflammatory cascade and trigger the generation of reactive oxygen species (ROS) thereby affecting the redox status of the cell [7]. In turn, increased intracellular levels of oxidized glutathione contribute to impairment of the liver's antioxidative defense system [8]. In addition to sinusoidal congestion caused by endothelial sticking of migrating neutrophils, an imbalance between vasoconstrictive (endothelin-1) [9] and vasodilatatory substances (NO) [10] may directly disturb the hepatic microcirculation. This is considered to be a central pathomechanism for organ dysfunction and primary nonfunction [9,10], especially in marginal liver grafts [11,12]. Besides poor graft quality, the recipients' health status (model for end-stage liver disease (MELD) score) may also influence the outcome after liver transplantation [13]. The combination of extended criteria donors and poor recipient condition may be responsible for a reduction of graft survival following liver transplantation. In 2011, the 5-year graft survival rate in Germany was 52.6% compared to the international mean of 66.2% (data provided by DSO, Collaborative Transplant Study (CTS)).

Therefore, strategies must be developed to improve the function and survival of EDC organs. Several experimental models have shown that tacrolimus preconditioning before liver transplantation has protective effects (Table 1). The authors have recently demonstrated that an *ex vivo* tacrolimus flush reduces IRI in a model of experimental liver transplantation in rats [14]. Based on these experimental findings, a study protocol of a single *ex vivo* tacrolimus rinse prior to reperfusion in marginal livers was developed (Trial register: EudraCT number: 2010-021333-31, ClinicalTrials.gov identifier: NCT 01564095). The aim of the TOP-Study (tacrolimus organ perfusion) is to reduce hepatic IRI and improve long-term organ survival following transplantation of marginal livers.

## Hypothesis and endpoints

The hypothesis of the study is that a single *ex vivo* tacrolimus perfusion prior to reperfusion reduces IRI and improves long-term graft survival. The primary endpoint is the maximum alanine transaminase (ALT) level within the first 48 hours following liver transplantation. Secondary endpoints are ALT and aspartate transaminase (AST) levels, graft function (prothrombin time, bilirubin), and creatinine on days 1, 2, 4 and 7. In addition, the study documents graft and patient survival, histologically confirmed rejection, as well as ischemic-type biliary lesions (ITBL).

**Table 1 Tacrolimus and ischemia reperfusion injury experimental animal studies**

| Author | Cold vs warm ischemia | Ischemic time | Species | TAC-application: Systemic vs organ rinse | End points | *P* |
|---|---|---|---|---|---|---|
| Sakr *et al*., 1991 [15] | Warm | 45 minutes | Rat | Systemic | Survival, aminotransferases, LDH | <0.05 |
| Kawano *et al*., 1995 [16] | Warm | 60 minutes | Rat | Systemic | Microcirculation, aminotransferases | <0.05 |
| Kawano *et al*., 1996 [17] | Cold | 80 minutes | Rat | Systemic | Lipid peroxidation, aminotransferases | <0.05 |
| Garcia-Criado *et al*., 1997 [18] | Warm | 90 minutes | Rat | Systemic | Survival | <0.01 |
| | | | | | ROS, cytokines, aminotransferases, neutrophil infiltration | <0.05 |
| Takeichi *et al*., 2009 [19] | Warm | 50 minutes | Rat | Systemic | Aminotransferases, neutrophil activation | <0.05 |
| Huser *et al*., 2009 [20] | Cold | 120 minutes | Rat | Systemic | Aminotransferases, histology | 0.001 |
| Pratschke *et al*., 2012 [14] | Cold | 24 hours | Rat | Organ rinse | Aminotransferases, glutathione metabolism | <0.05 |

LDH, lactate dehydrogenase; ROS, reactive oxygen species; TAC, tacrolimus.

## Methods

The TOP-Study is an investigator-initiated, prospective, randomized trial comparing the *ex vivo* perfusion of marginal livers with tacrolimus to placebo prior to transplantation. The main inclusion criterion is the presence of two or more EDC. IRI is assessed by serum ALT and AST levels over a period of 7 days. Following this period, organ and patient survival, bile duct complications, rejections and organ function are monitored for 1 year. The TOP-Study is sponsored by the University of Munich Hospital with financial support provided by a grant from Astellas Pharma GmbH, München, Germany. Research and organizational support is provided by the contract research organisation (CRO) DABIO Gesellschaft für Auftragsforschung mbH, Höhenkirchen, Germany.

### Inclusion and exclusion criteria

The study includes patients undergoing liver transplantation in the participating centers who meet the following criteria: chronic terminal liver failure, over 18 years of age, first liver transplantation, and informed, signed consent by the recipient. Donor organs exhibiting two or more EDC according to the Eurotransplant Manual for extended criteria liver donors (Table 2) are included [21].

Patients receiving split liver and multiorgan transplantations are excluded as well as those undergoing retransplantation, high urgency transplantation or pediatric transplantation. In addition, recipients with extrahepatic malignant diseases, and organs from donors with hepatitis B or C infection, are excluded.

### Perfusion procedure

Livers are perfused with 1000 ml of the rinse solution from a height of 100 cm without additional pressure using polyvinylchloride (PVC)-free infusion systems with a 12 gauge cannula. The portal vein and the common hepatic artery are flushed sequentially, with 500 ml each. In the test arm, tacrolimus is added to 1000 ml Custodiol histidine-tryptophan-ketoglutarate (HTK) solution at a concentration of 20 ng/ml. A total of 20 μg tacrolimus is applied. In the placebo group livers are perfused with 1000 ml Custodiol. The perfusion procedure is performed at the end of the back-table preparation at least 1 hour before reperfusion. The duration of the perfusion does not exceed 15 minutes (Figure 1). Prior to reperfusion livers are flushed *in situ* with 500 ml of the recipients' blood.

**Table 2 Extended donor criteria (EDC)**

| Criteria | |
|---|---|
| Donor age | >65 years |
| Macrovesicular steatosis | >40% (macroscopy or biopsy) |
| BMI | >30 |
| Sodium | >165 mmol/l |
| ICU stay and ventilation | >7 days |
| Cold ischemia time | >13 hours |
| AST | >99 u/l |
| ALT | >105 u/l |
| Bilirubin | >3 mg/dl (>51 μmol/l) |
| Application of epinephrine | |

Criteria according to the Eurotransplant Manual for EDC in liver grafts [21].
BMI, body mass index; EDC, extended donor criteria; ICU, intensive care unit; ALT, alanine transaminase; AST, aspartate transamianse.

### Liver transplantation and postoperative immunosuppressant regimen

Liver transplantation is performed according to the standard clinical practice at each center. Immunosuppression during the first 7 postoperative days is tacrolimus-based. Thereafter, a tacrolimus-based immunosuppressive regimen is suggested but not mandatory. Additional immunosuppression, that is, corticoids, is administered at the discretion of the treating clinician.

### Follow-up

The present trial includes a 7-day interventional study regulated by the German Pharmaceuticals Act (Arzneimittelgesetz, AMG) and a non-interventional study (NIS) over 1 year (Figure 2). During the entire study period, the monitoring of safety and data is performed according to Good Clinical Practice (GCP) guidelines. Data management and CRO duties are performed by the DABIO Gesellschaft für Auftragsforschung mbH, Höhenkirchen, Germany.

### Study visits

To characterize IRI and graft function serum ALT/AST, prothrombin ratio and bilirubin are measured on postoperative days 1, 2, 4, 7, as well as 6 and 12 months following liver transplantation (Figure 2). Moreover, graft and patient survival, bile duct complications and histologically confirmed rejections are assessed.

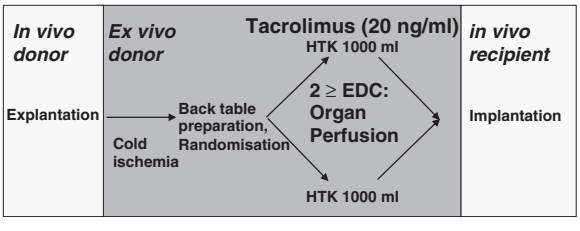


**Figure 1 Flow chart of tacrolimus organ perfusion.** EDC, extended donor criteria; HTK, histidine-tryptophan-ketoglutarate.

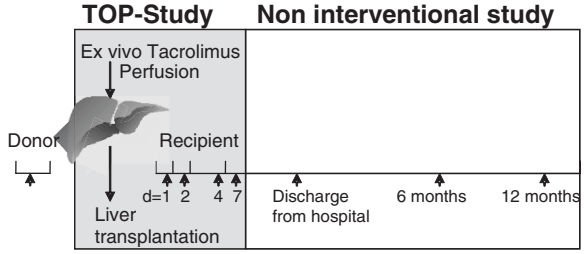


**Figure 2** Study visits TOP-Study (tacrolimus organ perfusion).

### Sample size, statistical analysis, randomization

The primary endpoint of the study is the maximum serum ALT level within 48 hours following liver transplantation, which reflects the degree of acute hepatocellular injury. Non-parametric analysis using Wilcoxon rank-sum test is performed to compare the maximum ALT levels in grafts treated with tacrolimus versus placebo. Based on a previous study using non-marginal healthy grafts, an effect size of approximately 0.5 was calculated [22]. In experimental studies, therapies for the treatment of IRI were more effective in steatotic livers [23,24]. Since recipients of marginal organs are incorporated in the present study, the predicted improvement in postoperative ALT levels should be higher than in non-marginal grafts. Thus, an effect size of 0.7 was considered appropriate for the sample size calculation. The power of the test is 80% at a significance level of 0.05. Therefore, sample size estimation (nQuery Advisor 6.1, Statistical Solutions, Saugus, MA, USA) for two unpaired samples using the Wilcoxon rank-sum test with an expected dropout rate of 15% results in an estimated sample size of 86 (43 tacrolimus vs 43 placebo).

To homogenize the patient collective only marginal organs with two or more EDC are included. Nonetheless, all EDC may affect the primary endpoint. Since documentation of EDC is required for patient inclusion, those parameters will be analyzed as potential confounders. Moreover, recipient age will also be registered.

### Participating centers

The Departments of Surgery of the following German university hospitals are participating in this trial: Charité Campus Virchow-Klinikum, Berlin; Johann Wolfgang Goethe-University, Frankfurt am Main; Johannes Gutenberg University, Mainz; Westphalian Wilhelms-University, Münster; Ruprecht-Karls-University, Heidelberg; University of Regensburg; Eberhard Karls University, Tübingen; and Campus Grosshadern, Ludwig-Maximilians-University, Munich.

### Ethics and safety

Protocol version 2.1 has been approved by the local ethic committees of the ethics committee of the university of Munich. The study complies with the Declaration of Helsinki and GCP guidelines. Informed consent is obtained from each patient in written form prior to randomization. The patient is informed about the nature, duration and possible consequences of the trial by an investigator specifically registered for this trial.

### Current status (October 2012)

Study permission by the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM) was received on 29 July 2011 and ethics committee approval on 23 August 2011. Version 2.1 of the protocol is active. To date (October 2012), seven centers (Berlin, Frankfurt, Heidelberg, Mainz, Munich, Regensburg and Tübingen) have been initiated and 17 patients have been recruited for the study. Estimated closure for recruitment for the study will be 31 December 2013. One year thereafter the study will be closed. Data calculation will require 6 months. A finalized report of the study is expected for July 2015.

## Discussion

Organ shortage and the consecutive transplantation of EDC grafts remain an unsolved problem in organ transplantation. Marginal organs are increasingly accepted, which is associated with increased acute IRI [4,23] and diminished graft survival [5,25,26]. An increased susceptibility of marginal organs to the pathomechanisms of IRI is discussed as a potential cause for the impaired outcome of these grafts [2,3]. Thus, clinically relevant strategies must be developed to prevent IRI in marginal organs.

Several experimental studies have demonstrated protective effects of tacrolimus on IRI following liver transplantation [14,17,20]. Despite their promising results, these models were based on systemic donor preconditioning, which is logistically difficult to incorporate into clinical practice due to the existing organ allocation practice in the Eurotransplant zone.

An *ex vivo* tacrolimus treatment may represent a solution to this problem. Recent experimental data indicates a protective effect of an *ex vivo* tacrolimus rinse in a model of experimental liver transplantation in rats [14]. Preservation of intracellular glutathione levels was suggested as a potential mechanism in this study. The calcineurin inhibitor tacrolimus acts through a blockade of the intracellular calcineurin-calmodulin complex. This blockade inhibits the calcium-dependent phosphorylation of the nuclear factor of activated T cells (NFAT). As a consequence, IL-2, which is normally involved in the activation of CD4+ and CD8+ T cells, and the IL-2 receptor are downregulated. Thus, the inactivation of T cells is regarded as the central mechanism in the immunosuppressant properties of tacrolimus [27,28].

In addition, tacrolimus might attenuate allogen-independent hepatic IRI, which is characterized by the release of a complex cascade of cytokines including IL-6 and TNF-α, the generation of ROS, the accumulation and transmigration of different cell types (that is, lymphocytes, neutrophils, platelets), as well as alterations of the microcirculation potentially causing graft dysfunction or even non-function [6]. In this respect, T cells have been shown to be critically involved in the induction of IRI of the liver [29-32]. A rapid recruitment of CD4+ T cells in hepatic sinusoids as early as 30 minutes after reperfusion is followed by their migration through the endothelial barrier to injured hepatic tissue [30]. Although CD4+ T cells themselves are not cytotoxic, they release a panel of cytokines, chemokines and adhesion molecules which are potentially harmful to the organ. Moreover, CD4+ T cells interact with platelets and Kupffer cells which further aggravate IRI [33]. However, it has yet to be determined whether tacrolimus affects IRI after liver transplantation via CD4+ T cells.

Neutrophils are also actively involved in hepatic IRI. The accumulation of neutrophils congests hepatic sinusoids and leads to the release of proinflammatory cytokines (that is, TNF-α and IL-6), as well as ROS [34]. Adhesion molecules such as P-selectin and ICAM-1 are involved in the process of neutrophil recruitment [35]. The application of tacrolimus decreases the expression of these adhesion molecules, thereby attenuating neutrophil recruitment [36,37]. In addition, direct suppressive effects of tacrolimus on the activation of Kupffer cells, which also release proinflammatory cytokines have been demonstrated *in vitro* [38]. This anti-inflammatory effect of tacrolimus was also evident in human liver biopsies after the transplantation of organs rinsed with tacrolimus [39].

With respect to the microcirculation, direct effects of tacrolimus on the expression of vasoconstrictive substances (endothelin-1) in endothelial cells have been shown, which might further improve hepatic microcirculation [40]. Increased levels of ROS are known to be involved in the pathogenesis of IRI. The application of tacrolimus *in vivo* is associated with a reduction of ROS [18]. Recently, a rat model of liver transplantation demonstrated that tacrolimus increases glutathione metabolism, which in turn may protect organ function by reducing ROS toxicity [14]. Tacrolimus has also been found to exert anti-apoptotic effects by preventing Fas-induced apoptosis in human hepatocytes *in vitro* [41], as well as in an *in vivo* model of IRI in rats [42]. A decrease in liver apoptosis may contribute to persisting protection of cellular integrity. In summary, several potentially synergistic mechanisms for the protective effects of tacrolimus in the setting of ischemia-reperfusion injury have been proposed.

Preliminary clinical data have shown beneficial results of tacrolimus preconditioning in human liver transplantation (Table 3). In addition, the tacrolimus rinse procedure has been tested clinically in a phase I trial (Table 3). In a previous trial, Peter *et al.* demonstrated a significant reduction of aminotransferase levels following the transplantation of normal livers rinsed with 20 ng/ml tacrolimus [22]. Although the results of this trial were promising, the clinical impact was limited by the small number of patients included (n = 20). In a similar clinical study, Kristo *et al.* recently failed to show a reduction in ALT levels on day 6 after transplantation [39]. However, the study population was relatively small, and, as most patients received healthy organs, the results cannot be directly compared to a study of marginal grafts. Postoperative aminotransferase levels in the Kristo *et al.* study were generally quite low, with serum ALT levels in the control group reaching almost normal levels 6 days after transplantation [39]. Nevertheless, the authors showed an impressive reduction in precursors of proinflammatory enzymes following tacrolimus rinse [39].

In the TOP-Study, livers are treated with a single *ex vivo* tacrolimus rinse prior to implantation, with the aim of reducing graft damage and secondarily improving the long-term course of EDC grafts. The maximum ALT level within the first 48 hours following liver transplantation was chosen as a clinical marker of hepatic injury and used to estimate the degree of IRI. Aminotransferases have been shown to be an appropriate marker of hepatic IRI in a number of studies. Puhl *et al.* demonstrated an inverse correlation between microcirculation, a key factor in the development of IRI, and serum ALT/AST levels in human liver transplantation [43]. Moreover, EDC organs, which are associated with increased levels of IRI, display significantly elevated ALT/AST levels [4]. In addition to assessing acute IRI, the TOP-Study assesses graft survival during a 1-year follow-up period. Although the impact of acute graft injury on long-term survival is discussed controversially in the literature, there is strong evidence that IRI correlates significantly with long-term graft survival [44].

The tacrolimus concentration of 20 ng/ml was chosen in the present trial based on safety data from previous

**Table 3 Clinical studies of tacrolimus rinse in liver transplantation**

| Author | Number of patients | Result | | *P* |
|---|---|---|---|---|
| | | Tacrolimus | Placebo | |
| St Peter *et al.*, 2003 [21] | 20 | AST (IU/l) | AST (IU/l) | 0.02 |
| | | day 1: 604 | day 1: 1294 | |
| | | day 2: 683 | day 2: 934 | |
| Kristo *et al.*, 2011 [38] | 26 | ALT (IU/l) | ALT (IU/l) | 0.88 |
| | | day 6: 79 | day 6: 101 | |

ALT, alanine transaminase; AST, aspartate transaminase.

studies [22,39]. At this dosage no adverse effects related to the tacrolimus treatment have been reported. The 20 μg of tacrolimus dissolved in 1000 ml of Custodiol to form the rinse solution represents a minute fraction of the $1.75 \times 10^3$ μg per day of tacrolimus administered intravenously to a 70 kg adult. If even 80% of the tacrolimus in the rinse solution reached the systemic circulation, the drug level would be below the detection limit of 3 ng/ml. Therefore, the rinse solution seems to have local effects in the liver graft, rather than contributing to systemic immunosuppression.

In summary, a tacrolimus rinse could represent a new strategy to reduce IRI and improve organ survival in EDC organs in liver transplantation. A reduction of organ damage in marginal grafts may allow the acceptance of more EDC organs, even in patients with high MELD scores, thereby safely expanding the donor pool in liver transplantation.

#### Abbreviations

ALT: alanine transaminase; AMG: German Pharmaceuticals Act (Arzneimittelgesetz); AST: aspartate transaminase; BfArM: Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte); CRO: contract research organization; CTS: Collaborative Transplant Study; DSO: German Organ Transplantation Foundation (Deutsche Stiftung Organtransplantation); EDC: extended donor criteria; GCP: Good Clinical Practice; HTK: histidine-tryptophan-ketoglutarate; IL: interleukin; IRI: ischemia reperfusion injury; ITBL: ischemic-type biliary lesions; LDH: lactate dehydrogenase; MELD: model for end-stage liver disease; NFAT: nuclear factor of activated T cells; NIS: non-interventional study; NO: nitric oxide; PVC: polyvinylchloride; ROS: reactive oxygen species; TAC: tacrolimus; TNF: tumor necrosis factor; TOP: tacrolimus organ perfusion..

#### Competing interests

The study is financed by a grant from Astellas Pharma GmbH, München, Germany.

#### Authors' contributions

SP performed experimental work, participated in the design of the study and wrote the manuscript. ME participated in the coordination of the study and helped to draft the manuscript. MH, SN, AP, PS, MS, FU and HW participated in performing the study (liver transplantation, organ rinse) and helped to draft the manuscript. KWJ participated in the study design and helped to draft the manuscript. DW participated in the study design, in coordination of the study and in statistical analysis. MA conceived the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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# Temporary intraoperative porto-caval shunt: useless or beneficial in piggy back liver transplantation?

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## Summary

The role of intraoperative porto-caval shunts in orthotopic liver transplantation (OLT) is controversial. Aim of this study was to analyze the effects of an intraoperative, porto-caval catheter-shunt on graft function and survival following cava sparing OLT. Four hundred and forty-eight piggy back liver transplantations with or without a temporary spontaneous porto-caval shunt between 1997 and 2010 were analyzed (shunt $n = 274$ vs. no shunt $n = 174$). Lab MELD scores and donor risk indices (DRI) were calculated. Hepatic injury (ALT, AST), -function (bilirubin, prothrombin ratio), postreperfusion liver blood flow and graft survival were registered [mean follow-up: 50.5 (0–163.0) months]. The impact of a shunt on graft survival was determined using multivariate analysis. Usage of a porto-caval shunt was associated with reduced hepatic injury (ALT, AST), whereas graft function was not affected. The shunt group showed a significantly increased portal venous blood flow after reperfusion. Retransplantation rate was decreased (7.7% vs. 20.1%, $P = 0.001$) and long-term graft survival was significantly increased with a porto-caval shunt (hazard ratio 2.1, $P < 0.001$). This effect was even more pronounced for marginal organs. Usage of intraoperative porto-caval catheter-shunts is beneficial in cava sparing OLT and is associated with reduced ischemia-reperfusion injury and improved organ survival in particular for recipients of marginal organs.

## Introduction

Ischemia-reperfusion injury following orthotopic liver transplantation (OLT) contributes to postoperative organ dysfunction and may result in graft loss [1,2]. The use of marginal livers, made necessary by an increasing shortage of organ donors, further aggravates ischemia-reperfusion injury [3]. The activation of Kupffer cells plays a pivotal role in the pathophysiology of reperfusion injury [4,5]. Several experimental studies demonstrate that gut-derived mediators are involved in the activation of Kupffer cells during reperfusion following temporary occlusion of the portal vein [6–9]. Portal hypertension during liver transplantation may cause intestinal edema leading to increased gut permeability and resulting in bacterial translocation and the release of various mediators, that is chemokines, cytokines, and endotoxin into the portal circulation [5,7].

Cava sparing surgical techniques for liver transplantation (i.e. piggy back technique or side-to-side cavo-caval anastomosis according to Belghiti [10]) have been developed to minimize blood flow stasis in the inferior caval vein during surgery [10–13]. This results in improved hemodynamic stability during transplantation and reperfusion [11–13]. Combining cava sparing OLT or piggy back technique with a temporary porto caval shunt [12,14,15] additionally reduces venous stasis by connecting the portal venous system with the inferior caval vein. This technique avoids splanchnic congestion and therefore may decrease the release of endotoxin and other mediators from the gut into the graft and consecutively into the systemic circulation

after reperfusion. Furthermore, a reduction in intraoperative blood loss through preservation of the retroperitoneum has been reported [15].

Tzakis and Belghiti described a temporary end-to-side porto-caval anastomosis to establish a shunt for patients with a lack of adequate portosystemic collaterals [14,15]. Alternatively, an extracorporeal spontaneous porto-caval shunt-catheter can be inserted using a plastic tube to connect the portal- to the femoral vein. This shunt technique, which does not require anticoagulation or an additional pump supply (Fig. 1), is commonly used at the transplantation center of the University of Munich.

The aim of this study was to determine whether the use of temporary porto-caval shunt-catheters reduces hepatic injury, improves cardiovascular stability and intraoperative blood loss, and improves short- and long-term organ survival during and after cava sparing OLT.

## Methods

### Study design

The study was performed at the surgical department of the University of Munich – Campus Grosshadern, Munich, Germany. The study period extended from January 1997 to April 2010. A retrospective search of the liver transplant database, including all consecutive patients who received a cava preserving OLT was performed. Pediatric and split liver transplantations were excluded. The retrospective data analysis of the liver transplant database was approved by the local institutional review board.

### Surgical procedures

All patients included in this study received a cava preserving OLT with an end-to-side or side-to-side caval anastomosis. For the piggy back technique, it is attempted to partially clamp the caval vein allowing blood flow to the right atrium through the inferior caval vein. Shunt application was performed by all transplant surgeons. Moreover, insertion of the femoral and portal catheter is standardized at our institution and carried out similarly by all surgeons according to an standard opening procedure (SOP). This minimizes the risk for heterogeneity within the groups because of the surgical procedure. The use of temporary intraoperative spontaneous extracorporeal porto-caval shunts was based on the transplant surgeons' assessment of the recipient's general condition and the presence of adequate porto systemic collaterals. In brief, a 17 F cannula (50 cm, CB 96535 015; Medtronic Inc., Meerbusch, Germany) was inserted into the femoral vein by direct puncture (Seldinger technique). Another catheter was placed in the portal vein and fixed by tourniquet ligation (24 F, 35 cm, CB 66124; Medtronic Inc.). The catheters were connected, allowing porto systemic blood pressure differences to establish spontaneous porto-caval blood flow. Insertion of the femoral and portal catheter accounts for approximately 20 min, which does not represent a relevant addition in operative time to the transplantation. It should be emphasized that this technique does not require a centrifugal pump or additional anticoagulation (Fig. 1).

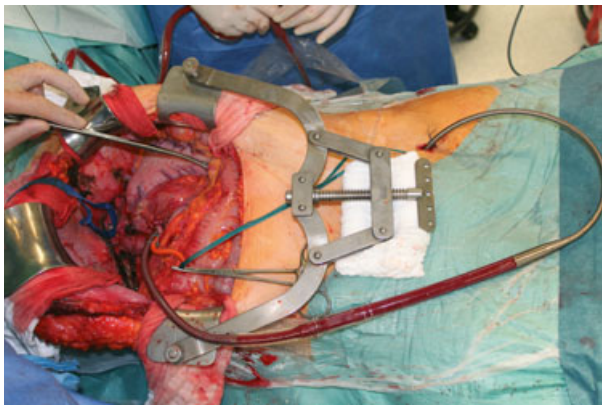

**Figure 1** Intraoperative picture of a temporarily inserted porto-caval shunt catheter after cava preserving hepatectomy prior to liver transplantation.

### Donor and recipient characteristics

The following data was collected for each donor and recipient: Age, sex, blood group, United Network for Organ Sharing (UNOS) status of the recipient [high urgency versus T-status listing on the transplant list], retransplantation, cold ischemia time, and type of graft preservation solution [University of Wisconsin (UW)- or Histidine-Tryptophan-Ketoglutarate (HTK)-solutions]. Based on the preoperative serum creatinine, bilirubin, and INR levels, the lab MELD Score was calculated as described previously [16]. Furthermore, the donor risk index (DRI) was calculated according to Feng *et al.* [17]. The indications for liver transplantations were classified as follows: alcoholic cirrhosis, malignancy, acute liver failure, viral hepatitis, primary biliary cirrhosis, and others.

For the detection of graft steatosis, liver biopsies of donor organs were routinely obtained after reperfusion using a Menghini needle (Hepafix; Braun, Melsungen, Germany) or wedge biopsy. In each biopsy, the percentage of hepatocytes showing macrovesicular steatosis, was determined. For analysis, steatosis was classified as mild (<30%) and moderate/severe (more than 30%). These categories were based on a previous study in which donor steatosis of more than 30% donor steatosis was associated with impaired postoperative organ function [18].

### Intraoperative parameters

Continuous intraoperative hemodynamic monitoring was performed according to standard hospital procedures. Hepatic arterial and portal vein blood flow were measured intraoperatively following reperfusion using transit time flow measurement as described previously [19]. The average consumption of catecholamines (norepinephrine or epinephrine) during the entire surgical procedure was used as a surrogate marker for hemodynamic stability [20], and intraoperative transfusion requirements were recorded (substitution of red cell units and fresh frozen plasma concentrates).

### Serum parameters

The prothrombin ratio [Quick (%)], AST, ALT, bilirubin-, and serum creatinine levels were recorded on the first, second, and seventh postoperative day as a measure of postreperfusion liver injury, graft function, and renal function.

### Short- and long-term outcome

Graft survival and the frequency of primary graft nonfunction (PNF) resulting in acute retransplantation within the first postoperative month after initial transplantation were assessed. Long-term organ survival status was registered for all patients (median observation period: 32.0 months). In addition, retransplantation rates caused by chronic organ dysfunction were documented. To verify the effect of shunt usage on long-term graft survival, the data werealso analyzed excluding patients with PNF.

### Statistical analysis

The statistical analysis was performed using statistical software PASW statistics 18.0.0 (SPSS Inc., Chicago, IL, USA). For all statistical tests, a testwise $\alpha$ level of 5% was used. *P*-values of <0.05 were considered statistically significant.

The effect of variables on cumulative organ survival was assessed using the log rank test in Kaplan–Meier survival analysis. In addition to hepatic and portal venous blood flow other variables that may influence the outcome following liver transplantation according to the survey of the European database [21] were evaluated using univariate analysis. Continuous variables, such as recipient and donor age, were dichotomized based on the values published by Adam *et al.* [21]. Variables were considered as potential confounders in a multivariate analysis performed using Cox proportional-hazard regression using the forward Wald method. Besides gender and recipient and donor age, those variables with a *P*-value of <0.05 in the univariate analysis were entered into the multivariate analysis model.

The results of continuous variables are presented as mean ± SEM. To determine the differences between the values on day 1 and 2, the Mann–Whitney *U*-test was applied. Categorical parameters, such as retransplantation and complication rate, were compared using chi-square test or the Fisher′s exact test, as appropriate.

## Results

Within the observation period, a total of 448 liver transplantations were performed in 392 patients [mean age 51.0 (±11.0) years, sex ratio m:f = 2.05:1]. A porto-caval shunt was established in 274 patients (61%) vs. 174 patients (39%) without a shunt. The morbidity rate due to the insertion of a shunt was 0.73% with two lymphatic fistulas documented. The mean follow-up was 50.5 [0–163.0] months.

### Patient characterization with respect to shunt application

The median Lab MELD score and the rate of high urgency transplantations did not differ whether a shunt was inserted or not (Table 1). The average recipient age was lower in patients receiving a shunt than in those without a shunt: 46.0 [10.0–84.0] vs. 52.0 [11.0–79.0] years, $P < 0.001$. Furthermore, the percentage of indications within the compared groups did not differ with respect to the insertion of a shunt, except in the group of transplantations not classifiable to those categories (Table 1).

### Intraoperative course

*Transfusion requirement, vasopressor support, organ blood flow*

The number of transfused packed red blood cells did not differ between the groups: 5.0 ± 4.0 (shunt) vs. 4.4 ± 5.0,

**Table 1.** Recipient characteristics.

| | Shunt | | |
|---|---|---|---|
| | Yes (*n* = 274) | No (*n* = 174) | *P*-value |
| Recipient age | 46.0 (10.0–84.0) | 52.0 (11.0–79.0) | <0.001 |
| MELD Score | 20 (2–40) | 21 (5–40) | 0.103 |
| High urgency-transplantation (%) | 11 (44) | 14 (56) | >0.05 |
| Indications for liver transplantation: *N* (% within groups shunt versus no shunt) | | | |
| Alcoholic cirrhosis | 57 (20.8) | 37 (21.3) | 0.907 |
| Malignancy | 65 (23.7) | 29 (16.7) | 0.074 |
| Acute liver failure | 18 (6.5) | 16 (9.2) | 0.306 |
| Viral hepatitis | 65 (23.7) | 32 (18.4) | 0.182 |
| Primary biliary cirrhosis | 30 (10.9) | 16 (9.2) | 0.551 |
| Others | 39 (14.2) | 44 (25.3) | 0.003 |

(no shunt), $P = 0.80$. Moreover, the number of fresh frozen plasma concentrates transferred could not be correlated with the use of a shunt: 21.1 ± 1.2 (shunt) vs. 19.6 ± 1.1 (no shunt), $P = 0.806$.

Continuous infusion of vasopressors was significantly reduced in patients receiving a shunt: The infusion rate of norepinephrine in patients receiving a shunt was 1.60 ± 0.8 mg/h vs. 1.88 ± 1.0 mg/h without shunt, $P = 0.012$. Similarly, the infusion rate of epinephrine was decreased in patients receiving a shunt: 0.08 ± 0.03 mg/h vs. 0.09 ± 0.02 mg/h, $P = 0.002$).

Intraoperative portal venous blood flow following reperfusion significantly correlated with the usage of a temporary porto-caval shunt-catheter and was elevated to 1727 ± 48 ml/min in patients with a shunt compared with 1431 ± 63 ml/min in patients without a shunt ($P < 0.001$). In contrast, no such correlation was evident with respect to hepatic arterial blood flow ($P = 0.792$) (Table 2).

## Postoperative course

*Hepatic cellular injury*

ALT and AST levels were significantly decreased on the first, second, and seventh postoperative day in patients transplanted with a temporary porto-caval shunt ($P < 0.001$) compared to patients without a shunt (Fig. 2a and b).

*Hepatic- and renal function*

Establishment of an intraoperative shunt was associated with a significant reduction in serum bilirubin levels on the first day following liver transplantation ($P = 0.023$) (Fig. 2c). In contrast, prothrombin ratio [Quick (%)] was not affected by the usage of a shunt (Fig. 2d).

Serum creatinine levels measured on the first, second, and seventh postoperative day were also not affected by the application of a shunt ($P > 0.05$).

*Causes of early graft loss*

Within the study period, a total of 13 grafts failed within the first postoperative month with consecutive retransplantation. While 12 cases of early graft loss occurred in grafts without a shunt, only one graft loss was apparent following transplantation with a shunt ($P < 0.001$). The causes of early graft loss were categorized into the following subgroups: PNF, vascular and others. Graft losses were distributed as follows: No shunt: PNF 7 (58%), Vascular 4 (33%), others 1 (8%); shunt: PNF 1 (100%).

**Table 2.** Application of a shunt and correlation with liver blood flow.

| | Shunt | No shunt | *P* (Mann–Whitney *U*-test) |
|---|---|---|---|
| *n* (%) | 274 (61.2) | 174 (38.8) | |
| Portal vein | 1724 ± 48 | 1431 ± 63 | <0.001 |
| Hepatic artery | 205 ± 8 | 205 ± 10 | 0.792 |

A subgroup analysis also indicates that patient and donor characteristics were equal in patients undergoing retransplantation with respect to the insertion of a shunt. DRI did not differ significantly in patients retransplanted whether a shunt was utilized or not ($P = 0.484$): 1.63 ± 0.08 (shunt) vs. 1.74 ± 0.07 (no shunt) (mean ± SEM; Mann–Whitney *U* test.). The lab MELD score did also not show differences between the subgroups: 26 ± 2 (shunt) vs. 27 ± 2 (no shunt), $P = 0.729$. According to univariate, not multivariate regression analysis, the insertion of a shunt significantly reduced the risk of graft loss within the early phase following liver transplantation ($P = 0.04$). DRI and lab MELD score did not affect early graft loss.

*Retransplantation*

Forty-three retransplantations were performed during the whole observation period after the first month. Within this period, significantly less retransplantations were evident in patients receiving a shunt compared to those transplanted without a shunt [*n* (%)]: 20 (7.3) vs. 23 (13.2), $P = 0.038$.

Altogether, 56 patients underwent retransplantation over the entire observation period. Within this group, 35 reoperations (20%) were performed in patients that had been initially operated without a shunt compared with 21 reoperations in patients that had not been receiving a shunt.

*Graft survival*

Univariate analysis revealed an increased long-term graft survival when a porto-caval shunt was applicated with a mean survival (CI) of 106 [98.0–115.7] months vs. 86.5 [73.5–99.5] months, $P = 0.001$ (Table 3; Fig. 3a). When patients with PNF were excluded from the analysis, these results did not change: 108.0 [99.1–116.9] vs. 88.5 [75.4–101.6] month, $P = 0.002$ (Table 3).

In 2007, the MELD score was introduced in the Eurotransplant allocation system. Therefore, the study period was divided into periods from 1997 till 2006 and 2007 till 2010. In this respect, the mean survival did not differ between the periods (Table 3).

A subgroup analysis with respect to recipients' lab MELD scores (MELD <35 vs. $\geq$ 35) and the application of a shunt revealed an increased graft survival in patients with a MELD Score $\geq$ 35 when a shunt was established. Mean survival increased from 39.9 [21.5–58] months to 101.2 [73.2–129.1] month, $P = 0.049$ (Fig. 3b; Table 3). Moreover, in recipients with a MELD Score <35 graft survival rose from 70.9 [60.8–80.9] to 110.0 [100.1–113.9] month, when a shunt was inserted, $P$ (log rank) = 0.001 (Fig. 2b).

According to CRT-analysis, grafts were divided into groups with a donor risk index < or $\geq$ 1.25 with regard to

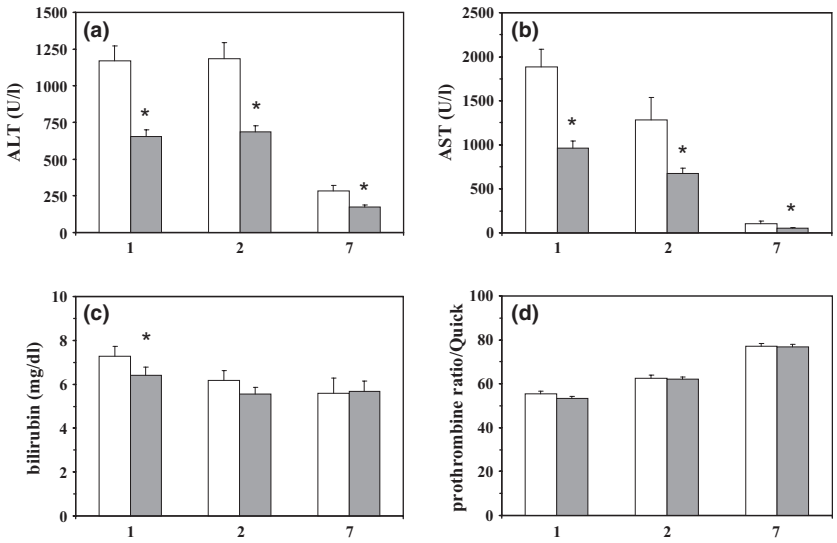


**Figure 2** Serum ALT levels (U/l) (a), AST levels (U/l) (b), bilirubin levels (ml/dl) (c), and prothrombin ratio [Quick (%)] (d) were determined on the first, second, and seventh postoperative day following liver transplantation with respect to the insertion of a porto systemic shunt (white column: no shunt, gray column: shunt). Values are presented as mean ± SEM. (a–c): *$P < 0.001$ shunt versus no shunt.

the application of a shunt. In this respect, graft survival increased from 77.7 [62.7–92.7] to 99.6 [88.9–110.3] months in grafts with a donor risk index $\geq 1.25$ if a shunt had been applicated, $P = 0.002$ (Fig. 3c).

*Other potential confounders: multivariate analysis*

Associations of collected variables with long-term graft survival (Cox model) in the univariate analysis are shown in Table 3. Donor age (>65 years), recipient age (>60 years), degree of steatosis, type of preservation solution (UW versus HTK), high urgency transplantation, malignancy, epinephrine treatment in donor, total ischemic time $\geq 12$ h, or a lab MELD Score $\geq 35$ did not affect survival ($P > 0.10$).

Potential confounders with a p value <0.05 in the univariate analysis were included in the multivariate model: retransplantation, arterial flow <100 ml/min, no use of a shunt and a DRI $\geq$ 1.25. No shunt, hepatic arterial blood flow <100 ml/min as well as a donor risk index $\geq 1.25$ were identified as independent risk factors for decreased graft survival in the covariate–adjusted model (Table 4).

## Discussion

Cava sparing liver transplantation in piggy back technique in combination with partial cava clamping during implantation may provide better hemodynamic stability as compared with full cava clamping in conventional technique [10]. Early division of the recipient portal vein substantially facilitates hepatectomy in piggy back technique. However, prolonged portal venous clamping during hepatectomy may lead to portal venous hypertension and splanchnic congesting with this technique. To decompress the portal venous system during hepatectomy, two principal shunting techniques have been established: (i) *In situ* portal venous shunt by an temporary end-to-side anastomosis of the the PV to the infrahepatic vena cava (ii) a spontaneous *ex situ* shunt. In animal models, an interruption of portal flow for up to 90 min resulted in increased permeability of splanchnic vessels, intestinal edema of the gut, and the accumulation of acute inflammatory cells with evidence of mucosal cell damage [22,23]. In light of those pathophysiological changes caused by an acute rise in portal venous pressure, the use of a temporary porto-caval shunt has been described by Tzakis *et al.* [14]. In those studies, a temporary end-to-side anastomosis was formed between the recipient's portal vein and the infrahepatic inferior vena cava. In contrast, an extracorporal shunt catheter is used at our institution, which is directly placed in the portal vein after dissection and connected to a catheter previously placed in the femoral vein to establish a spontaneous temporary porto-caval blood flow. Utilization of temporary porto-caval shunts was initially recommended for patients with portal hypertension caused by acute or subacute liver failure who are expected not to have adequate portosystemic venous collaterals [14]. Surprisingly, a subgroup analysis of patients transplanted for acute liver failure revealed no statistical difference in organ survival compared with patients transplanted for chronic liver diseases (data not shown). The small number of transplantations for acute liver failure ($n = 34$) may account for the lack of statistical significance. Therefore, experimental studies are required to further

**Table 3.** Univariate analysis.

| | *n* (%) | Mean survival [months] [CI] | *P* (log rank) univariate analysis |
|---|---|---|---|
| Total | 448 (100) | | |
| Recipient age | | | |
| <60 years | 377 (84.2) | 99.6 [91.7–107.5] | 0.494 |
| ≥60 years | 71 (15.8) | 92.9 [74.8–111.0] | |
| High urgency | | | |
| No | 423 (94.2) | 98.4 [90.9–105.8] | 0.720 |
| Yes | 25 (5.8) | 105.4 [78.0–132.7] | |
| Retransplantation | | | |
| 1. LTx | 381 (85.0) | 102.8 [95.0–110.6] | 0.003 |
| Re-LTx | 67 (15.0) | 73.6 [54.0–93.1] | |
| Shunt | | | |
| No | 174 (38.8) | 86.5 [73.5–99.5] | 0.001 |
| Yes | 274 (61.2) | 106.8 [98.0–115.7] | |
| Shunt (excl. primary graft nonfunction) | | | |
| No | 170 (38.5) | 88.5 [75.4–101.6] | 0.002 |
| Yes | | 108.0 [73.5–99.5] | |
| Time period | | | |
| 1997–2006 | 303 (67.6) | 97.6 [89.3–105.9] | 0.537 |
| 2007–2010 | 145 (32.4) | 31.8 [28.9–34.7] | |
| Total ischemia time | | | |
| <12 h | 339 (81.7) | 99.6 [91.3–108.0] | 0.831 |
| ≥12 h | 76 (18.3) | 98.3 [80.8–115.8] | |
| Donor age | | | |
| <65 years | 324 (81.4) | 100.4 [92.1–108.7] | 0.367 |
| ≥65 years | 74 (18.6) | 95.3 [75.1–115.6] | |
| Resuscitation donor | | | |
| No | 353 (86.9) | 100.0 [91.8–108.1] | 0.986 |
| Yes | 53 (13.1) | 90.8 [71.6–109.9] | |
| Shock donor | | | |
| No | 313 (77.5) | 101.2 [92.6–109.9] | 0.538 |
| Yes | 91 (22.5) | 87.9 [73.9–101.9] | |
| LabMELD Score | | | |
| <35 | 317 (79.4) | 101.4 [92.9–110.0] | 0.023 |
| ≥35 | 82 (20.6) | 85.6 [68.1–103.0] | |
| Shunt and LabMELD Score | | | |
| I: Shunt/MELD < 35 | 207 (51.9) | 110.0 [100.1–119.9] | I vs. II 0.001 |
| II: No Shunt/MELD < 35 | 141 (35.3) | 70.9 [60.8–80.9] | |
| III: Shunt/MELD ≥ 35 | 28 (7.0) | 101.2 [73.2–129.1] | III vs. IV 0.049 |
| IV: No Shunt/MELD ≥ 35 | 23 (5.8) | 39.9 [21.5–58.3] | |
| Donor risk indices (DRI) | | | |
| DRI < 0.125 | 88 (20.0) | 125.6 [112.3–138.9] | <0.001 |
| DRI ≥ 0.125 | 351 (80.0) | 90.4 [81.9–98.8] | |
| Shunt and DRI | | | |
| I. Shunt/DRI < 1.25 | 201 (45.8) | 123.7 [108.2–139.3] | III vs. IV 0.002 |
| II. No Shunt/DRI < 1.25 | 150 (34.2) | 106.3 [86.1–126.4] | |
| III. Shunt/DRI ≥ 1.25 | 66 (15.0) | 99.6 [88.9–110.3] | |
| IV. No Shunt/DRI ≥ 1.25 | 22 (5.0) | 77.7 [62.7–92.7] | |

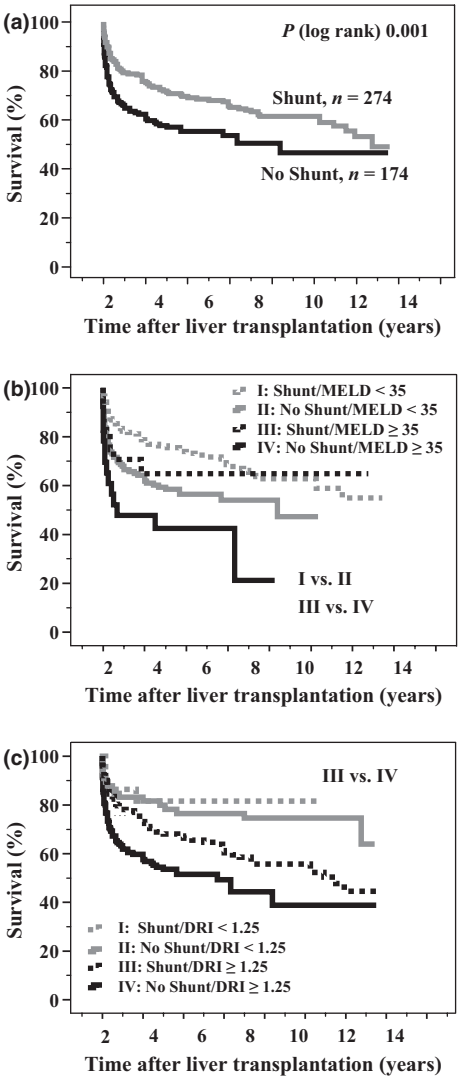


**Figure 3** (a) Cumulative graft survival (10 years) after liver transplantation with regard to the insertion of a spontaneous porto caval shunt. Shunt (gray line), no shunt (black line). 106.8 [98.0–115.7] vs. no shunt 88.5 [73.5–99.5] months; $P = 0.001$. (b) Cumulative graft survival after liver transplantation with regard to the insertion of a spontaneous porto caval shunt and the recipients' lab MELD Scores. I: Shunt and MELD < 35 (gray perforated line); II: shunt and MELD ≥ 35 (gray line); III: no shunt and MELD < 35 (black perforated line); IV: no shunt and MELD ≥35 (black line). I vs. III: $P = 0.001$; II vs. IV: $P = 0.049$. C: Cumulative graft survival after liver transplantation with regard to the insertion of a spontaneous porto caval shunt and the Donor Risk Index. I: Shunt and donor risk indices (DRI) < 1.25 (gray perforated line); II: no shunt and DRI < 1.25 (gray line); III: shunt and DRI ≥ 1.25 (black perforated line); IV: no shunt and DRI ≥ 1.25 (black line). I: Shunt and DRI < 1.25 (gray perforated line); II: no shunt and DRI < 1.25; III: shunt and DRI ≥ 1.25; IV: no shunt and DRI ≥ 1.25. III vs. IV: $P = 0{,}002$. Values are presented as mean ± SEM.

clarify the underlying mechanisms of the protective effects of intraoperative shunts. The routine use of temporary porto-caval shunts in liver transplantation, however, is

**Table 4.** Multivariate analysis.

| Prognostic factors | Hazard rate ratio [CI] | *P* |
|---|---|---|
| Transplantation without shunt | 2.1 [1.4–3.0] | <0.001 |
| Flow hepatic artery <100 ml/min | 2.1 [1.3–3.2] | 0.001 |
| DRI $\geq$ 1.25 | 3.2 [1.7–5.9] | <0.001 |

discussed controversially in the literature [24]. Despite the theoretical arguments in favor of a systematic use of porto-caval shunts, their clinical benefit remains the subject of controversy [25,26]. Hoffmann *et al.* [25] state in their review that shunts are not required for successful liver transplantation. Although this review analyzed utilization of shunts during en bloc transplantation with resection of the caval vein, it must be stated that the rationale for the insertion of a shunt, a reduction in venous stasis, is the same in both techniques. Nevertheless, basic differences between cava sparing and cava resecting liver transplantation may account for the discrepancy in the results. In particular, the mechanisms and effects of portosystemic shunts on hepatic injury remain unclear. Thus, it was the aim of this study to determine whether usage of a temporary porto-caval shunt-catheter may reduce liver damage after ischemia reperfusion and affect long-term graft survival.

The use of a portal venous shunt was associated with lower levels of aminotransferases for up to 7 days, suggesting a lowered degree of postischemic injury in this group of patients. In contrast, Ghinolfi *et al.* [26] could not show such effects in their retrospective analysis in 148 cava sparing liver transplantations. The smaller number of patients compared to this study may account for this discrepancy. Figueras *et al.* [12] also failed to demonstrate beneficial effects of porto-caval shunts on postoperative aminotransferase levels. Four-months graft survival rates in this study, however, were more than 97% in both groups suggesting differences in the patient collectives in terms of donor and recipient characteristics compared to the Eurotransplant allocation area [27]. Moreover, only 80 patients were included in this prospective trial. In addition to liver injury, beneficial effects of porto caval shunt utilization on blood product transfusion, intraoperative hemodynamics, and ease of retrohepatic dissection with a shorter operative time have been observed in liver transplantation with the use of a porto-caval shunt [24,26]. The subjective surgeon's impression at our institution suggests that establishment of a porto-caval shunt helps to control intraoperative blood loss. This impression, however, was not reflected in reduced blood product substitution in patients with porto-caval shunt.

A recent study reports beneficial effects of porto-caval shunting on postreperfusion hemodynamic instability, which is associated with significantly adverse postoperative outcome [28]. In this respect, increased organ survival in recipients transplanted with a temporary shunt using multivariate analysis considering all known potential confounders was shown. The absence of an intraoperative shunt in this study was identified as a significant risk factor for diminished organ survival with a hazard ratio of 2.1. As opposed to this finding, Ghinolfi *et al.* [26] failed to demonstrate such an effect in multivariate analysis. Even excluding patients with a primary nonfunction, this survival benefit was still present in our study. Our results imply that intraoperative portal-caval shunting may be associated with improved long-term survival.

Utilization of a temporary intraoperative porto-caval shunt was based on subjective assessment of the responsible surgeon, including personal preference or the presence of potentially adequate portosystemic collaterals. This represents an obvious limitation. Despite significant effects of the MELD score on organ survival in univariate analysis, this parameter failed to reach statistical significance in multivariate analysis. One potential explanation could be that the number of cases with a MELD score $\geq$35 is too small ($n$ = 82). Moreover, sick patients with a MELD score $\geq$35 generally receive grafts with good quality. This policy may to some extent compensate for the reduced health condition of those patients.

The recipient age was significantly higher in patients transplanted with a porto-caval shunt (52 vs. 46 years, respectively). In uni- and multivariate analysis, however, recipient age was not identified as a significant risk factor for organ survival. Nonetheless, a potential bias cannot be excluded in this study. In contrast to previous trials, a donor age $\geq$65 years as well as a recipient's age >60 years did not influence graft survival in this study ($P > 0.05$). In an analysis incorporating 22089 liver transplant patients, Adam *et al.* [21] showed statistical significance for these risk factors in multivariate analysis. A risk ratio of <1.3 was evident for a recipient age $\geq$60 years (RR 1.29) and for a donor age >55 years (RR 1.14). This relatively small effect may suggest limited clinical relevance in light of critical organ shortage. In this respect, the large number of patients included in the study of Adam *et al.* could explain the discrepancy in significance levels. Moreover, only a relatively small number of older recipients and grafts has been transplanted compared to the rest of the population in this study (recipients: 377 vs. 71; donors: 324 vs. 74), which may be another explanation of the lack of statistical significance.

Previous studies indicate the importance of portal blood flow and gradient measurements prior to hepatectomy in deciding who should be selected for shunt utilization [12,29]. Patients with high portal flow and elevated porto-caval gradient benefited particularly on post-transplant renal function when using a temporary porto-caval shunt. Margarit *et al.* defined the cut-off for high versus low portal flow prior to hepatectomy as 800 ml/min. Based on

those findings, measurement of portal blood flow prior to hepatectomy might represent an objective tool for selecting patients who should be transplanted with a temporary porto-caval shunt. This, however, should be clarified in a prospective trial.

As liver transplantation faces serious problems concerning extended criteria donors and recipients in poor conditions represented by high MELD scores, a subgroup analysis on the efficacy of shunt utilization was performed in this study with respect to the donor risk index and the recipients' MELD score. Ghinolfi *et al.* [26] however, failed to show such effects when stratifying the population by low and high MELD scores. Interestingly, the application of a shunt exhibited beneficial effects on graft survival especially in high risk transplantations, that is with poor graft quality (DRI $\geq$ 1.25) and a high Lab MELD score ($\geq$35). Although the relevance of poor graft quality and bad recipient conditions differs regionally, a general advice for shunt utilization might be supported by the present data. In contrast to our own results, Mehrabi *et al.* [30] postulated that usage of porto-caval shunts is not required when performing piggy back technique liver transplantation. This center, however, utilized porto-caval shunts in only 1.4% of 500 patients. Thus, this manuscript does not allow drawing valid conclusions of shunt utilization on organ outcome.

The exact underlying mechanisms for the protective properties of a temporary porto-caval shunt remain unknown. Nonetheless, in our series, application of a porto-caval shunt catheter was associated with significantly increased portal blood flow following reperfusion. This result may in part explain the beneficial effects of shunt usage, as enhanced portal blood flow has been shown to be associated with reduced liver injury previously [19]. Furthermore, incidence of postreperfusion syndrome was reduced after utilization of a temporary porto-caval shunt [28]. Whether solely those improvements in hemodynamics account for the ameliorated postoperative transaminase levels in patients with porto-caval shunt remains unknown. Alternatively, mediators released from the gut that is proinflammatory cytokines, endotoxin, chemokines etc. caused by splanchnic congestion, may be responsible for the observed liver injury in patients without a shunt. In this respect, induction of inflammatory responses in the liver, that is expression heat shock proteins [31] as well as remote organ injury following portal vein occlusion have been reported [32]. The implication of those potential mechanisms for the beneficial effects of maintained portal drainage versus portal occlusion during liver transplantation, however, has to be further investigated.

In summary, the insertion of a temporary porto-caval shunt catheter reduces cellular damage in patients with cava sparing liver transplantation. Shunt usage was associated with increased portal blood flow following reperfusion, which may in part explain the beneficial effects on hepatic injury. Moreover, the insertion of a shunt was associated with an improved graft survival. This effect was more pronounced in recipients with high MELD scores and recipients of marginal donor organs. Therefore, the application of a temporary porto-caval shunt catheter is advisable in cava sparing OLT especially for recipients of marginal organs. Nonetheless, a prospective randomized multicenter trial should be initiated to confirm this important observation in light of an increased frequency of transplantation of marginal grafts due to organ shortage.

## Authorship

SP: Participated in research design and writing of the manuscript. GM: Participated in data analysis. CJB: Participated in research design and writing of the manuscript. MK: Participated in research design. NP: Participated in data analysis. RZ: Participated in the writing of the manuscript. MG: Participated in research design. K-WJ: Participated in research design. FL: Participated in research design and writing of the manuscript. MKA: Participated in research design and writing of the manuscript.

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## Conflict of interest

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LETTER TO THE EDITORS

# Response to Gastaca

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Dear Sir,

Thank you very much for your comment on our manuscript 'Temporary intraoperative porto-caval shunt: useless or beneficial in piggy back liver transplantation?'. We would like to take the opportunity to respond to the valuable comments of Gastaca.

Gastaca pointed out that portal blood flow and pressure prior to hepatectomy should be considered as confounders when studying the effects of a temporary porto-caval shunt. Unfortunately, this information was not available in our prospectively conducted liver transplantation database. Moreover, information on portal blood flow and pressure prior to liver transplantation cannot be recruited retrospectively and therefore was not content of the present study. We certainly agree with Gastaca that this information would be of tremendous relevance to prospectively identify subgroups of patients who will have the highest benefit from an intraoperative shunt application. We would speculate that patients with low portal pressure and high portal blood flow prior to transplantation would profit most from temporary shunt usage. In this subgroup, patients are not used to portal venous stasis with consecutive intestinal congestion and have less porto-systemic collaterals. Nonetheless, we respectfully submit that investigating all consecutive patients transplanted with or without a porto-caval shunt at out institution in the period from 1997 to 2010 revealed a significant survival benefit in multivariate analysis. Potential confounders associated with poor graft survival, i.e., steatosis, cold ischemia time, donor age, etc., have been included in univariate and multivariate analysis.

Based on our manuscript, a prospective multicenter randomized trial within Germany will be initiated. In this trial, portal pressure and blood flow as well as shunt time will be considered as stratification criteria. This prospective study will address the valuable comments of Gastaca and potentially clarify whether the critical 90-min threshold for the development of organ injury owing to interruption of portal flow can be directly transferred into the clinical arena. Most important, liver tissue will be collected for molecular analysis further evaluating the underlying mechanisms for the amazingly positive results of intraoperative shunt application on liver injury and organ survival after liver transplantation.

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## Conflicts of interest

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ORIGINAL ARTICLE

# Arterial Blood Flow Predicts Graft Survival in Liver Transplant Patients

**Sebastian Pratschke,* Georgios Meimarakis,* Stephan Mayr, Christian Graeb, Markus Rentsch, Reinhard Zachoval, Christiane Josephine Bruns, Axel Kleespies, Karl-Walter Jauch, Florian Loehe, and Martin Kurt Angele**

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Proper liver perfusion is essential for sufficient organ function after liver transplantation. The aim of this study was to determine the effects of portal and arterial blood flow on liver function and organ survival after liver transplantation. The arterial and portal venous blood flow was measured intraoperatively by transit time flow measurement after reperfusion for 290 consecutive liver transplants. The graft survival, hepatic cell damage (alanine aminotransferase and aspartate aminotransferase), and liver function (prothrombin ratio and bilirubin) were determined. Grafts were stratified into groups according to arterial blood flow measurements [$<$100 mL/minute for arterial blood flow group I (ART I), 100-240 mL/minute for ART II, and $\geq$240 mL/minute for ART III] and portal venous blood flow measurements ($<$1300 mL/minute for portal venous blood flow group I and $\geq$1300 mL/minute for portal venous blood flow group II). With multivariate analysis, the impact of blood flow on graft survival was determined, and potential confounders were considered. Decreased portal venous blood flow was associated with significantly less organ survival in univariate analysis but not in multivariate analysis. In contrast, the arterial blood flow was significantly correlated with organ survival after liver transplantation in univariate and multivariate analyses [hazard rate ratio = 2.5, confidence interval = 1.6-4.1, $P < 0.001$, median survival = 56.6 (ART I), 82.7 (ART II), or 100.7 months (ART III)]. Moreover, low arterial blood flow resulted in impaired postoperative organ function and higher rates of primary nonfunction. Biliary complications were not affected by blood flow. Other risk factors for graft failure that were identified by multivariate analysis included retransplantation, histidine tryptophan ketoglutarate solution versus University of Wisconsin solution, and donor treatment with epinephrine. Impaired arterial blood flow after reperfusion represents a significant predictor of primary graft nonfunction and is associated with impaired graft survival. Whether the intraoperative measurement of hepatic arterial flow is predictive of graft survival should be evaluated in a prospective trial. ***Liver Transpl 17:436-445, 2011.*** © **2011 AASLD.**

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Postoperative liver graft function is influenced by extended donor criteria, such as prolonged cold ischemia times, graft steatosis, donor hypernatremia, and advanced donor age.[1,2] In addition, recipient-related risk factors, such as the underlying cause of liver failure and the recipient's age and general state of health, may contribute to an impaired outcome.[3-5] However, the extent of perioperative organ injury is not precisely predictable. In addition to extended donor criteria and recipient-related risk factors, postoperative intensive care interventions such as the use of vasoactive drugs and mechanical ventilation may alter the organ blood flow and contribute to poor graft function.[6,7]

Nasraway et al.[8] reported that liver graft performance depends on stable hemodynamics, sufficient

Abbreviations: ALT, alanine aminotransferase; ART, arterial blood flow group; AST, aspartate aminotransferase; HTK, histidine tryptophan ketoglutarate; ITBL, ischemic-type biliary lesion; PNF, primary nonfunction; PV, portal venous blood flow group; TTFM, transit time flow measurement; UW, University of Wisconsin.

Sebastian Pratschke, Axel Kleespies, and Martin Kurt Angele participated in the writing of this article. Georgios Meimarakis, Stephan Mayr, Reinhard Zachoval, and Florian Loehe participated in the data analysis. Sebastian Pratschke, Christian Graeb, Markus Rentsch, Reinhard Zachoval, Karl-Walter Jauch, Florian Loehe, and Martin Kurt Angele participated in the research design.

*These authors contributed equally to this work.

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organ blood flow, and prevention of venous stasis in the liver. Experimental studies have indicated that sufficient arterial blood flow is required for liver regeneration and recovery of organ function after transplantation.[9,10] In addition, disturbance of the arterial capillary blood flow is thought to play a key role in the pathogenesis of biliary complications.[11,12] Pathophysiological changes induced by ischemia/reperfusion injury may enhance microcirculatory perfusion failure via the activation of polymononuclear leukocytes and increased leukocyte-endothelial interaction.[13,14] The influence of microcirculatory changes on organ function was confirmed by Puhl et al.,[15] who demonstrated a correlation between initial microcirculation and early graft function in human orthotopic liver transplantation.

In order to evaluate the quality of the anastomosis, blood flow is determined intraoperatively after reperfusion with ultrasound-based transit time flow measurement (TTFM). The prognostic value of intraoperative blood flow measurements for graft survival has not yet been determined. Studies assessing whether intraoperative organ blood flow correlates with organ function are still lacking. The aim of the present study was to determine whether intraoperative blood flow measurements of the hepatic artery, portal vein, or both could predict graft function, graft survival, or both.

## PATIENTS AND METHODS

### Study Design

The study was performed at the Department of Surgery, Klinikum Grosshadern, Ludwig-Maximilians University (Munich, Germany). The study period extended from January 1997 to December 2007. In this retrospective analysis of our liver transplantation database, all consecutive whole organ liver transplants between 1997 and 2007 with documented arterial and portal blood flow measurements after reperfusion were included. Split liver recipients and pediatric recipients were excluded from the analysis. The retrospective data analysis was approved by the local institutional review board.

### Measurement of Arterial and Portal Blood Flow

Portal and hepatic blood flow was measured with TTFM at the end of the surgery (Medi-Stim AS, Oslo, Norway). This method generates valid data and provides reliable measurements that are independent of the examiner's experience as well as environmental conditions that cannot be controlled, such as the measurement angle, blood temperature, and hematocrit level.[16-18]

For data analysis, the grafts were divided into 3 arterial blood flow groups [<100 mL/minute for arterial blood flow group I (ART I), 100-240 mL/minute for ART II, and ≥240 mL/minute for ART III] and 2 portal venous blood flow groups [<1300 mL/minute for portal venous blood flow group I (PV I) and ≥1300 mL/minute for PV II]. The threshold between the groups was defined by classification and regression trees analysis according to the chi-square automatic interaction detector algorithm.

### Surgical Technique

All patients underwent cava-sparing liver transplantation. Operative procedures did not change during the observation period. In particular, anastomosis techniques related to the portal vein and hepatic artery were not modified. Thus, it appears unlikely that the results of this analysis were affected by changes in the arterial or portal venous blood flow related to changes in the surgical technique.

### Donor and Recipient Characteristics

The following information was collected for each donor-recipient pair: donor age, sex, and blood group; recipient age, sex, blood group, and United Network for Organ Sharing status; high-urgency listing versus regular listing for transplantation; underlying hepatic disease; cold ischemia time; and graft preservation solution [University of Wisconsin (UW) solution or histidine tryptophan ketoglutarate (HTK) solution]. The condition of the donor, which was based on the need for epinephrine, evidence of shock, or need for cardiopulmonary resuscitation, was also documented. The classification of the donor's condition was based on definitions derived from Eurotransplant's standardized donor data sheet, which was completed by the regional organ coordinator before organ retrieval. In this setting, *shock* is defined as a positive shock index over a prolonged period of time, *resuscitation* is defined by mechanical cardiopulmonary resuscitation, and *epinephrine donor* is defined as a donor requiring the continuous application of epinephrine in addition to norepinephrine to maintain adequate blood pressure. Moreover, donors ≥60 years and donors with ≥30% macrovesicular steatosis were flagged as having potential risk factors for poor graft function.[19] Because most authors regard macrovesicular steatosis in liver grafts to be more clinically relevant than microvesicular steatosis, only macrovesicular steatosis was included in the analysis.[20] In a previous article,[21] we conducted an extensive analysis of macrovesicular steatosis in the same patient cohort (1997-2005). For the purposes of the present study, the documented results of the more recent routine liver biopsies (2006-2007) were retrospectively confirmed by an additional pathologist. Donor organ biopsy samples were routinely obtained after reperfusion with a Menghini needle or by wedge biopsy. The tissue was stained with hematoxylin and eosin and was evaluated with light microscopy.

### Acute Graft Function, Hepatic Cell Damage, and Complication Rate

For the characterization of the postoperative liver function, the prothrombin ratios (Quick percentages) and bilirubin levels were registered on the first,

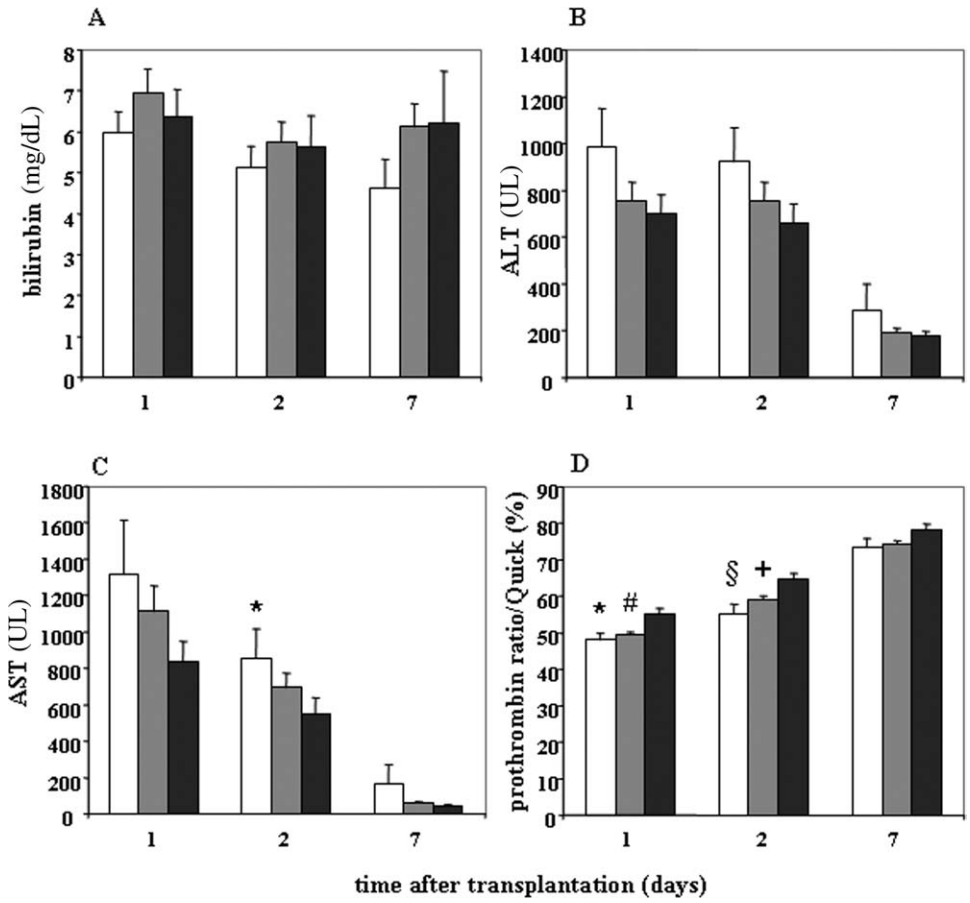


Figure 1. (A) Serum bilirubin levels, (B) ALT levels, (C) AST levels, and (D) prothrombin ratios (Quick percentages) were determined after liver transplantation on postoperative days 1, 2, and 7, and the grafts were categorized into 3 groups according to the arterial organ blood flow: <100 (white), 100 to 240 (gray), and ≥240 mL/minute (black). Values are presented as means and standard errors of the mean. (C) *$P$ = 0.019 for ART I versus ART III. (D) *$P$ = 0.020 for ART I versus ART III, #$P$ = 0.005 for ART II versus ART III, §$P$ = 0.006 for ART I versus III, and +$P$ = 0.005 for ART II versus ART III.

second, and seventh postoperative days. For the determination of hepatic cell damage, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured. Furthermore, the frequency at which primary nonfunction (PNF) of the graft resulted in acute retransplantation within the first 14 postoperative days was determined.

### Long-Term Results

Patient follow-up was continued until March 1, 2009. Biliary complications [ie, bile duct stenosis, leakage, or ischemic-type biliary lesions (ITBLs)] requiring reoperation or endoscopic retrograde cholangiopancreatography were documented prospectively at the time of occurrence, as was the need for retransplantation due to chronic organ dysfunction (ITBLs, hepatitis reinfection, or chronic rejection).

### Statistical Analysis

The statistical analysis was performed with SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL). For all statistical tests, we used a testwise $\alpha$ level of 5%. $P$ values less than 0.05 were considered statistically significant.

The effects of variables on cumulative organ survival were assessed with the log-rank test in Kaplan-Meier survival analysis. In addition to hepatic and portal venous blood flow, other variables that might influence outcomes after liver transplantation according to the survey of the European database[19] were evaluated by univariate analysis. Continuous variables (ie, recipient age and donor age) were dichotomized according to the values published by Adam et al.[19] Variables were considered potential confounders in a multivariate analysis performed by Cox proportional hazards regression with the forward Wald method. Variables with a $P$ value < 0.10 in the univariate analysis were entered into the multivariate analysis model.

The results of continuous variables are presented as means and standard errors of the mean. To determine the differences between the values on days 1 and 2, the Mann-Whitney U test was applied. Categorical parameters (ie, retransplantation and complication rates) were compared with the chi-square test or Fisher's exact test.

For the analysis of the effects of hepatic and portal venous flow on the occurrence of PNF or retransplantation, Fisher's exact test or the chi-square test and also binary logistic regression with the forward Wald method were performed.

## RESULTS

During the observation period, 290 liver transplants were performed in 261 patients [age = 48.7 ± 11.0 years (mean ± standard deviation), male/female ratio

= 2.1:1]. The median observation period was 43.0 months (range = 0-129.9 months).

## Effect of Organ Blood Flow on Acute Graft Injury and Function

### *Bilirubin*

Bilirubin levels were elevated above physiological levels on the first, second, and seventh postoperative days in all study groups (Figs. 1A and 2A). Variations in the intraoperative arterial and portal venous blood flow had no significant effect on the bilirubin concentration. An increase in the serum bilirubin level ≥ 20% was associated with diminished organ survival in multivariate analysis (Table 1).

**TABLE 1. Multivariate Analysis (Cox Proportional Hazards Regression) of Prognostic Factors for Graft Survival After Liver Transplantation (n = 290)**

| Prognostic Factor | Hazard Rate Ratio* | *P* |
|---|---|---|
| Retransplantation | 1.7 (1.0-3.1) | 0.054 |
| Preservation solution (risk with HTK) | 1.8 (1.1-3.1) | 0.022 |
| Postoperative bilirubin elevation ≥ 20% | 2.6 (1.7-4.0) | <0.001 |
| Epinephrine donor | 2.0 (1.2-3.4) | 0.011 |
| Hepatic artery flow < 100 mL/minute | 2.5 (1.6-4.1) | <0.001 |
| Total flow < 1400 mL/minute | 2.0 (1.3-3.1) | 0.002 |

*Confidence intervals are shown in parentheses.

### *ALT*

ALT levels were increased during the first postoperative week in all groups in comparison with physiological levels. There was a trend toward a reduction of ALT levels with increasing arterial (Fig. 1B) and portal venous blood flow (Fig. 2B), although this effect did not reach statistical significance ($P > 0.05$).

### *AST*

Peak AST levels on the first and second postoperative days were ameliorated with increasing arterial blood flow levels (Fig. 1C). This decrease was significant between ART I and ART III ($P < 0.05$). The portal venous blood flow had no significant effect on postoperative AST levels (Fig. 2C).

### *Prothrombin Ratio (Quick Percentage)*

In comparison with physiological levels, the Quick percentage was reduced on the first and second postoperative days in all groups (Figs. 1D and 2D). The Quick percentage significantly increased because of arterial blood flow in all study groups ($P < 0.05$ for ART I versus ART III and for ART II versus ART III) on days 1 and 2 (Fig. 1D). The portal venous blood flow was not associated with such differences (Fig. 2D).

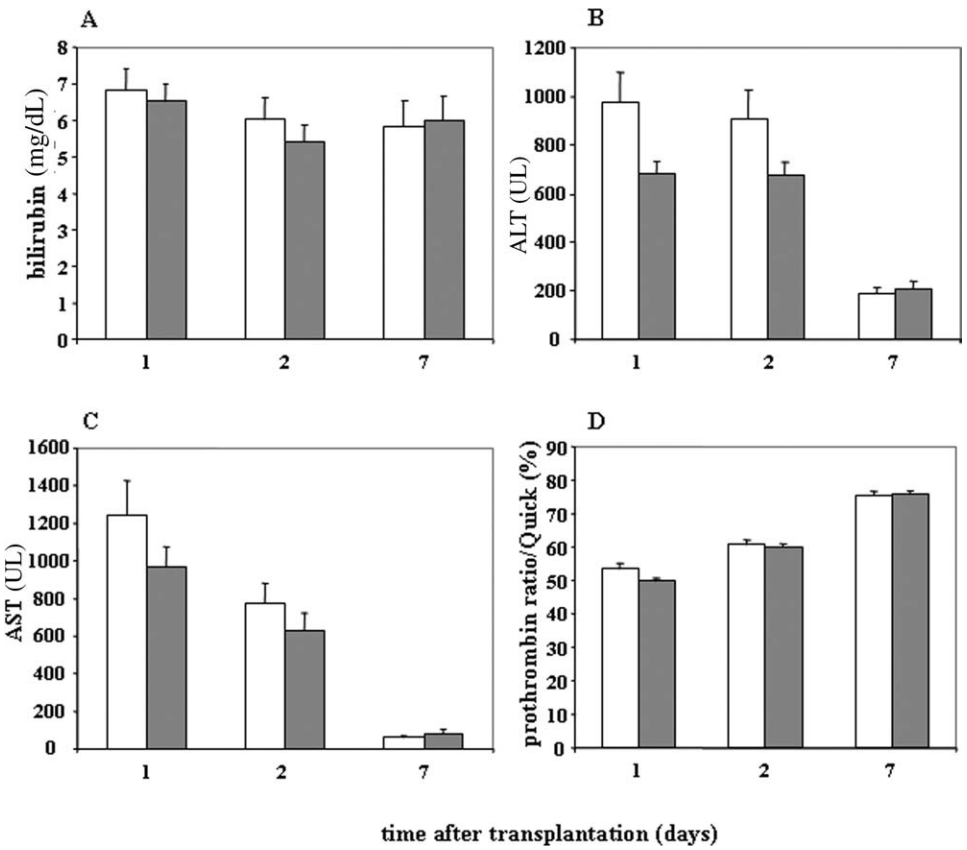


Figure 2. (A) Serum bilirubin levels, (B) ALT levels, (C) AST levels, and (D) prothrombin ratios (Quick percentages) were determined after liver transplantation on postoperative days 1, 2, and 7, and the grafts were categorized into 2 groups according to the portal venous blood flow: <1300 (white) and ≥ 1300 mL/minute (gray). Values are presented as means and standard errors of the mean.

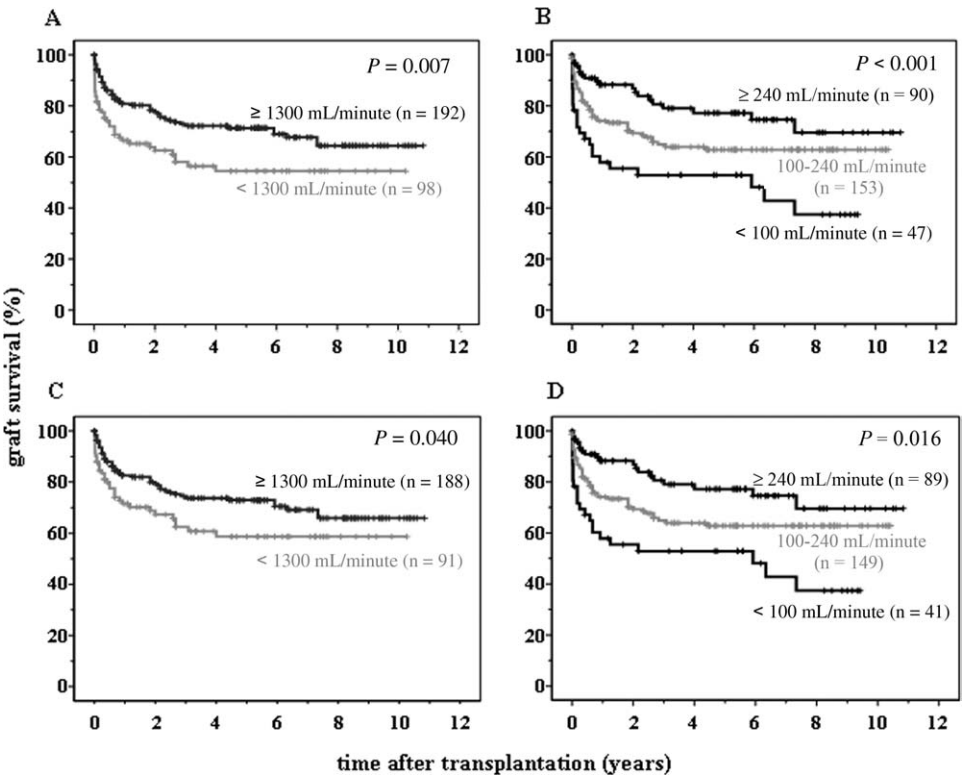


Figure 3. Cumulative graft survival 10 years after liver transplantation with respect to (A) the portal venous blood flow, (B) the arterial blood flow, (C) the portal venous blood flow (patients with PNF were excluded), and (D) the arterial blood flow (patients with PNF were excluded). (A,C) The portal venous blood flow was ≥1300 (black line) or <1300 mL/minute (gray line). (B,D) The arterial blood flow was ≥ 240 (top black line), 100–240 (gray line), or less than 100 ml/minute (bottom black line).

## Effect of Organ Blood Flow on Organ Survival

### *Graft PNF*

Low portal venous blood flow was associated with an enhanced risk for PNF (7.1% for PV I versus 2.1% for PV II, $P = 0.0487$). An arterial blood flow < 100 mL/minute was associated with significantly increased rates of PNF (12.8% for ART I) in comparison with an arterial blood flow ≥ 100 mL/minute (2.6% for ART II and 1.1% for ART III, $P = 0.022$). A similar result was observed for the total flow (8.2% for <1400 mL/minute versus 2.0% for ≥1400 mL/minute).

Multivariate analysis revealed a significantly increased risk of PNF in patients with an arterial blood flow < 100 mL/minute (hazard rate ratio = 7.4, confidence interval = 2.1-26.3, $P = 0.002$) and a total flow < 1400 mL/minute (hazard rate ratio = 4.8, confidence interval = 1.3-17.6, $P = 0.017$).

### *Long-Term Organ Survival*

**Portal Venous Blood Flow.** The portal venous flow, which was categorized into 2 groups (<1300 mL/minute for PV I and ≥1300 mL/minute for PV II), significantly affected long-term organ survival in univariate analysis (71.7 months for PV I versus 92.7 months for PV II, $P = 0.007$; Fig. 3A and Table 2). When the subgroup of patients developing PNF (n = 11) was excluded, the portal blood flow also significantly influenced mean organ survival (77.2 months for PV I versus 94.7 months for PV II, $P = 0.040$; Fig. 3C). Multivariate analysis did not display a significant effect of the portal flow on short- or long-term survival.

**Arterial Blood Flow.** Arterial blood flow, which was categorized into 3 groups (<100 mL/minute for ART I, 100 to <240 mL/minute for ART II, and ≥240 mL/minute for ART III), significantly affected long-term organ survival after liver transplantation (Fig. 3B and Table 2). The mean organ survival was 100.7 months for ART III, 82.7 months for ART II, and 56.6 months for ART I ($P < 0.001$). Diminished organ survival in recipients with an arterial blood flow < 100 mL/minute was reflected in decreased median follow-up (15.0 versus 36.5 months, respectively). Moreover, graft survival and blood flow rates did not change within the study period (data not shown). When patients developing PNF (n = 11) were excluded, the arterial blood flow still significantly affected survival (65.1 months for ART I versus 85.0 months for ART II), which reached 101.8 months in grafts with an initial arterial blood flow ≥ 240 mL/minute ($P = 0.016$; Fig. 3D). Multivariate analysis revealed a significant effect of an arterial blood flow < 100 mL/minute on organ survival (for ART I, hazard rate ratio = 2.5, confidence interval = 1.6-4.1, $P < 0.001$; Table 1). A total organ blood flow < 1.400 mL/minute was associated with significantly decreased survival (hazard rate ratio = 2.0, confidence interval = 1.3-3.1, $P = 0.002$).

**Other Potential Confounders: Multivariate Analysis.** Associations of collected variables with long-term graft survival (Cox model) in the univariate analysis are

**TABLE 2. Univariate Analysis of Prognostic Factors for Graft Survival After Liver Transplantation**

| Prognostic Factor | n (%) | Mean Survival (Months)* | $P^{\dagger}$ |
|---|---|---|---|
| Total | 290 (100.0) | 86.7 (79.7-93.8) | |
| Sex | | | 0.890 |
| Female | 95 (32.8) | 83.2 (5.9-71.7) | |
| Male | 195 (67.2) | 86.2 (4.4-77.6) | |
| Sex match | | | 0.485 |
| Yes | 113 (39) | 83.0 (5.7-71.8) | |
| No | 176 (61) | 87.5 (4.5-78.7) | |
| Recipient age | | | 0.836 |
| <60 years | 246 (84.8) | 86.5 (3.9-78.8) | |
| ≥60 years | 44 (15.2) | 84.4 (8.4-67.9) | |
| High urgency | | | 0.883 |
| No | 286 (98.6) | 95.0 (31.0-64.0) | |
| Yes | 4 (1.4) | 290.0 (94.0-196.0) | |
| Malignancy | | | 0.883 |
| No | 195 (67.2) | 86.8 (4.4-78.1) | |
| Yes | 95 (32.8) | 77.0 (5.3-66.6) | |
| Retransplantation | | | 0.030 |
| First transplantation | 252 (86.9) | 87.2 (3.7-80.0) | |
| Retransplantation | 38 (13.1) | 73.7 (10.7-52.8) | |
| Preservation solution | | | 0.072 |
| HTK | 69 (23.8) | 69.6 (8.4-53.2) | |
| UW | 221 (76.2) | 89.4 (3.9-81.8) | |
| Total ischemia time | | | 0.903 |
| <12 hours | 248 (85.8) | 86.5 (3.9-78.9) | |
| ≥12 hours | 41 (14.2) | 78.4 (8.0-62.6) | |
| Recipient blood group | | | 0.950 |
| 0 | 109 (38.1) | 85.5 (5.6-74.5) | |
| A | 115 (40.2) | 81.2 (5.6-70.2) | |
| B | 35 (12.2) | 83.4 (9.7-64.4) | |
| AB | 27 (9.4) | — | |
| Macrovesicular steatosis | | | 0.504 |
| <30% | 215 (79.9) | 87.9 (4.1-79.8) | |
| ≥30% | 54 (20.1) | 79.2 (7.9-63.8) | |
| Postoperative bilirubin elevation | | | <0.001 |
| <20% | 218 (75.2) | 93.1 (3.9-85.5) | |
| ≥20% | 72 (24.8) | 65.4 (7.0-51.6) | |
| Donor age | | | 0.499 |
| <60 years | 211 (77.6) | 85.4 (4.0-77.7) | |
| ≥60 years | 61 (22.4) | 85.1 (8.4-68.7) | |
| Resuscitation donor | | | 0.997 |
| No | 246 (86.3) | 86.8 (3.9-79.1) | |
| Yes | 39 (13.7) | 74.3 (8.3-58.0) | |
| Shock donor | | | 0.798 |
| No | 206 (72.8) | 86.1 (4.3-77.7) | |
| Yes | 77 (27.2) | 77.0 (5.8-65.6) | |
| Epinephrine donor | | | 0.047 |
| No | 246 (87.2) | 89.7 (3.8-82.1) | |
| Yes | 36 (12.8) | 67.1 (10.0-47.6) | |
| Hepatic artery flow | | | <0.001 |
| ART I: <100 mL/minute | 47 (16.2) | 56.6 (41.2-72.0) | |
| ART II: 100-240 mL/minute | 153 (52.8) | 82.7 (73.4-92.1) | |
| ART II: ≥240 mL/minute | 90 (31.0) | 100.7 (89.3-112.1) | |
| Portal venous blood flow | | | 0.007 |
| PV I: <1300 mL/minute | 98 (33.8) | 71.7 (6.2-59.6) | |
| PV II: ≥1300 mL/minute | 192 (66.2) | 92.7 (4.2-84.4) | |
| Total flow | | | 0.002 |
| <1400 mL/minute | 85 (29.4) | 69.1 (6.6-56.2) | |
| ≥1400 mL/minute | 204 (70.6) | 93.3 (4.1-85.3) | |

NOTE: For some factors, data were not available for all patients.
*Confidence intervals are shown in parentheses.
$^{\dagger}$Log-rank values from the univariate analysis.

shown in Table 2. Donor age, recipient age, gender mismatch, steatosis degree, recipient or donor age $\geq$ 60 years, high-urgency listing, malignancy, cold ischemia time $\geq$ 12 hours, and liver steatosis $\geq$ 30% did not significantly impact survival ($P > 0.10$).

Potential confounders with $P < 0.10$ in the univariate analysis were included in the multivariate model [retransplantation, bilirubin elevation $\geq$ 20%, type of preservation solution (UW versus HTK), donor treatment with epinephrine, arterial flow < 100 mL/minute, portal flow <1300 mL/minute, and total flow < 1400 mL/minute]. In addition to arterial and total blood flow, retransplantation, preservation solution (HTK), serum bilirubin elevation $\geq$ 20%, and donor treatment with epinephrine were identified as independent risk factors for decreased survival in the covariate-adjusted model (Table 1).

### *Retransplantation Rate*

The retransplantation rate correlated with arterial flow measurements (21.3% for ART I, 8.5% for ART II, and 5.6% for ART III, $P = 0.010$). Multivariate analysis revealed a significantly increased risk for retransplantation in patients with a blood flow < 100 mL/minute (hazard rate ratio = 3.4, confidence interval = 1.4-7.9, $P = 0.005$).

### *Biliary Complication Rate*

Within the observation period (mean = 43 months), 33.9% of all grafts developed biliary complications requiring intervention. Differences in the arterial flow did not significantly affect biliary complications (31.9% for ART I, 26.4% for ART II, and 48% for ART III).

## DISCUSSION

The organ blood supply is thought to play a pivotal role in organ function after liver transplantation. Experimental studies have indicated that sufficient arterial blood flow is required for liver regeneration and recovery of organ function.[22,23] However, the relevance of arterial and portal blood flow to liver function after transplantation remains unknown. TTFM reliably detects portal venous and arterial blood flow after the reperfusion of hepatic grafts. Previous studies have indicated that this method generates reproducible and valid data.[16-18,24] Therefore, the aim of the present study was to determine the effects of portal venous, arterial, and total blood flow on postoperative organ function, complication rates, and graft survival. Using a covariate-adjusted model, we considered other potential risk factors for diminished organ function, including liver steatosis, donor age, and prolonged cold ischemia times, as confounders of the blood flow effect.

From 1997 to 2007, TTFM was performed routinely within 2 hours after reperfusion on 290 liver grafts. When reduced arterial or portal flow macrovascular perfusion was optimized via resuturing of the anastomosis or thrombectomy, blood flow values after vascular reconstruction were used for further analysis.

This study failed to demonstrate a statistically significant correlation between organ blood flow and postoperative hepatic cell injury (AST and ALT levels) or graft function (Quick percentage and bilirubin level). In this respect, the consumption of coagulation products did not significantly vary between the study groups (data not shown). Thus, it appears unlikely that differences in the substitution of coagulation products accounted for the disparities in the postoperative Quick percentage measurements with respect to hepatic blood flow. In contrast, diminished arterial flow was associated with an increased rate of PNF and impaired survival. As cutoff points for arterial flow, 100 and 240 mL/minute were identified with classification and regression trees analysis. Most importantly, arterial blood flow was independent of other confounders and was associated with a clinically relevant hazard rate ratio of 2.5 for poor outcomes (Table 1). Although portal flow significantly correlated with graft survival, this parameter was not proven to be significant in multivariate analysis. This demonstrates the key role of arterial flow in graft function after liver transplantation and correlates well with data from Abbasoglu et al.,[24] who observed reduced initial arterial flow in patients who later developed vascular complications. In their study, an arterial blood flow < 400 mL/minute was chosen as a risk factor.[24] The authors focused on the impact of blood flow on arterial thrombosis; they excluded information on organ function and survival, and this may explain the discrepancy in the cutoff values versus those of the present study. Moreover, potential confounders within the groups were not considered. In contrast, Lisik et al.[25] suggested a correlation between portal flow and early liver function. This analysis, however, included only 15 patients, and this may limit its clinical relevance.

In this trial, information on organ blood flow was limited to macrovascular measurements, which potentially missed changes in microvascular perfusion and interactions between those factors. Microcirculatory disturbances occur during reperfusion and play a key role in the pathogenesis of ischemia/reperfusion injury.[26,27] A relevant study by Puhl et al.[15] demonstrated a significant correlation between the initial microcirculation and early graft function after human liver transplantation. Studies have demonstrated that measurements of the microcirculation via laser Doppler flowmetry on the liver graft surface correlate with macrovascular blood flow.[28] Moreover, systemic hemodynamics (eg, the mean arterial pressure, venous drainage, and right ventricular heart failure) may influence liver perfusion.[29] To analyze the effects of systemic cardiovascular function on hepatic blood flow, the intraoperative use of norepinephrine, epinephrine, and dopamine was examined with respect to hepatic arterial blood flow during liver transplantation. Although norepinephrine and dopamine did not significantly affect arterial hepatic blood flow, the use

of epinephrine was significantly correlated with diminished arterial blood flow (data not shown).

Potential interactions between macrocirculation and microcirculation after liver transplantation were beyond the scope of the present study. Despite the lack of information on microvascular perfusion, our data strongly emphasize the clinical relevance of macrovascular graft perfusion.

Nonetheless, further investigations analyzing the underlying causes of diminished hepatic flow are required, and they may contribute to the development of therapeutic approaches to optimizing organ blood flow. In this respect, the intraoperative application of nitric oxide, a microvascular vasodilator playing a key role in hepatic ischemia/reperfusion injury,[30,31] may represent a promising strategy.

It could be argued that stenosis and technical difficulties contribute significantly to diminished arterial flow after transplantation. Indeed, a previous study has demonstrated decreased arterial flow in patients developing long-term vascular complications.[32] In that study, however, the intraoperative arterial blood flow was >400 mL/minute, and this argues against an initial vascular stenosis. The present analysis demonstrates a detrimental effect of decreased arterial blood flow on long-term organ survival even when PNF is excluded. This further supports arterial blood flow as a relevant risk factor for poor graft survival beyond initial vascular difficulties. In our study, blood flow measurement was limited to a single time point. In addition, transcutaneous Doppler measurements were routinely performed on the first postoperative day and in patients with clinical symptoms. This procedure identifies vascular complications that may not be evident by intraoperative TTFM.

In the present study, biliary complications (including ITBLs, strictures, and leakage requiring intervention or retransplantation) were included; the overall rate was 33.9%. In the literature, biliary complication rates of 1% to 19% have been reported.[11,33,34] In those studies, the term *biliary complication* was mostly restricted to patients who developed ITBLs. Recently, Welling et al.[35] systematically reviewed 256 consecutive liver transplants and detected a bile leak rate of 18% and a stricture rate of 23%. Interestingly, no correlation was found between diminished arterial blood flow and the occurrence of biliary complications in the present study. Even though the biliary complication rate (48%) was highest in livers with an arterial blood flow > 240 mL/minute (ART III), no statistically significant correlation between biliary complications and hepatic blood flow could be found. Higher rates of early death or the need for retransplantation due to PNF in patients with an arterial blood flow < 100 mL/minute (12.8%) could bias this analysis because these patients undergo retransplantation or die before the development of biliary complications.

Several studies have found a correlation between ITBLs and diminished arterial blood flow.[11,36] Moench et al.[12] postulated that insufficient perfusion and the high viscosity of the preservation solution are potential mechanisms of biliary complications due to obstruction of the arterial microcirculation.

In addition to organ blood flow, the type of organ preservation solution, a postoperative increase in bilirubin > 20%, and donor treatment with epinephrine have been identified as risk factors for poor organ survival in multivariate analysis. Similarly, previous studies have demonstrated protective properties of UW solution versus HTK for organ function.[19] Because other studies have failed to demonstrate such effects, it appears unrealistic that the common use of HTK for organ preservation will be changed in the near future.[37,38] An increase in bilirubin levels > 20% within the postoperative course as a risk factor for poor organ survival is appealing because it represents an additional prognostic parameter during the postoperative course.

A recent study by Sainz-Barriga et al.[39] has shown negative effects of organ risk factors (eg, macrovesicular steatosis > 30% and warm ischemia time) on organ blood flow. In contrast, using TTFM, Angele et al.[21] demonstrated that macrovesicular steatosis does not affect hepatic blood flow. In the present analysis, the warm ischemia time did not influence the hepatic arterial blood flow (data not shown). Moreover, widely accepted risk factors such as donor age, cold ischemia times, and organ steatosis did not correlate significantly with diminished organ survival.[1,19] In contrast, an analysis of the Eurotransplant database, which incorporated 22,089 liver transplant patients, displayed statistical significance for those risk factors.[19] The risk ratio for most parameters in the analysis of Adam et al.[19] was less than 1.4, and this suggested limited clinical relevance in light of the organ shortage. Solely for the parameters retransplantation and transplantation for malignancy, a risk rate ratio > 2 was evident. In the present study, retransplantation was associated with a similar hazard risk ratio, which almost reached statistical significance ($P = 0.054$). The huge number of patients incorporated into the study of Adam et al. may explain the discrepancy in significance levels.[19] In this respect, single risk factors such as high donor age are less clinically significant than the combination of donor and recipient characteristics.[40] Therefore, marginal organs are primarily accepted for recipients without further risk factors, and this could result in a bias in the present data. Therefore, intraoperative blood flow measurement represents a predictive parameter combining donor and recipient characteristics after liver transplantation.

In summary, the results of the present study indicate that impaired arterial blood flow after reperfusion is a significant predictor of increased graft injury as well as PNF and is associated with diminished long-term graft survival. Thus, intraoperative TTFM of the hepatic artery may allow us to identify organs at risk for poor outcomes. Whether the intraoperative measurement of the hepatic arterial flow may predict graft survival should be evaluated in a prospective trial.

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ORIGINAL SCIENTIFIC REPORT

# Temporary Intraoperative Porto-Caval Shunts in Piggy-Back Liver Transplantation Reduce Intraoperative Blood Loss and Improve Postoperative Transaminases and Renal Function: A Meta-Analysis

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## Abstract

*Background* The value of temporary intraoperative porto-caval shunts (TPCS) in cava-sparing liver transplantation is discussed controversially. Aim of this meta-analysis was to analyze the impact of temporary intraoperative porto-caval shunts on liver injury, primary non-function, time of surgery, transfusion of blood products and length of hospital stay in cava-sparing liver transplantation.

*Methods* A systematic search of MEDLINE/PubMed, EMBASE and PsycINFO retrieved a total of 909 articles, of which six articles were included. The combined effect size and 95 % confidence interval were calculated for each outcome by applying the inverse variance weighting method. Tests for heterogeneity ($I^2$) were also utilized.

*Results* Usage of a TPCS was associated with significantly decreased AST values, significantly fewer transfusions of packed red blood cells and improved postoperative renal function. There were no statistically significant differences in primary graft non-function, length of hospital stay or duration of surgery.

*Conclusion* This meta-analysis found that temporary intraoperative porto-caval shunts in cava-sparing liver transplantation reduce blood loss as well as hepatic injury and enhance postoperative renal function without prolonging operative time. Randomized controlled trials investigating the use of temporary intraoperative porto-caval shunts are needed to confirm these findings.

M. Weniger and M. K. Angele have contributed equally to this work.

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**Abbreviations**

| | |
|---|---|
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| CI | Confidence interval |
| DCD | Donation after cardiac death |
| DRI | Donor risk index |
| FFP | Fresh frozen plasma |
| ICU | Intensive care unit |
| IV | Inverse variance |
| LOS | Length of hospital stay |
| MELD | Model for end stage liver disease |
| PNF | Primary non-function |
| PRBC | Packed red blood cells |
| RCT | Randomized controlled trial |
| TPCS | Temporary intraoperative porto-caval shunt |

## Introduction

For many years, liver transplantation was routinely performed with portal venous and cavo-caval bypass systems in order to reduce venous stasis in the splanchnic circulation and the lower half of the body, especially in orthotopic liver transplantation.

In this respect, cava-sparing liver transplantation (i.e., piggy back) was introduced to avoid interruption of blood flow in the inferior vena cava (IVC), to maintain venous return to the heart with consequent hemodynamic better stability and to reduce IVC pressure and renal congestion. This technique was first described by Calne in 1968 [1] and became clinical routine in the 1990s following publications by Tzakis [2] and Belghiti [3] on this topic.

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In order to decrease portal venous stasis during hepatectomy, cava-sparing liver transplantation can be combined with an additional spontaneous, extracorporeal porto-caval shunt catheter or alternatively an intracorporeal, surgical end-to-side porto-caval anastomosis. The combination of cava-sparing liver transplantation and a shunt has beneficial effects: A remnant caval backflow is achieved by partial clamping, and during hepatectomy this technique allows early portal dissection and decompression of the portal system and facilitates dissection of the retrohepatic vena cava. As a mechanism, a porto-caval shunt transplantation may alleviate gut edema, reduce bleeding by reduction of portal venous pressure and improve hemodynamic stability [4]. At our institution, a porto-caval shunt is routinely used to facilitate hepatectomy in cava-sparing liver transplantation [5].

In orthotopic, cava-resecting liver transplantation with replacement of the inferior vena cava, this vessel and the portal vein are usually clamped simultaneously and the time for a shunt to develop its full effect is usually too short.

Recent data from our group demonstrated beneficial effects of a temporary porto-caval shunt (TPCS): Patients undergoing cava-sparing liver transplantation showed reduced graft damage as well as enhanced long-term graft survival [5]. In the literature, however, heterogeneous data have been published on the effects of a TPCS [6–8].

The aim of this study was to analyze the effects of a TPCS in liver transplantation by performing a meta-analysis on selected outcomes from the current literature.

## Methods

### Search strategy and inclusion/exclusion criteria

A systematic search of MEDLINE/PubMed, EMBASE and PsycINFO was performed to identify suitable studies. Additionally, reference lists were hand-searched for the relevant literature. The study was performed according to the PRISMA statement for systematic reviews and meta-analyses [9]. The used search terms were "liver transplantation" combined with "portosystemic shunt," "porto-caval shunt" or "porto-caval anastomosis." Different spellings of the search terms were taken into account (e.g., portocaval vs. portacaval). Boolean operators ("AND," "OR," "NOT") were applied to combine the search terms. A protocol of the review process may be obtained from the contact author. The following outcomes of interest have been defined: (1) hepatic injury [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and primary non-function (PNF)], (2) characteristics of the surgical intervention [packed red blood cell (PRBC) requirement, fresh

frozen plasma requirement (FFP), duration of operation], (3) renal function (creatinine) and (4) health care utilization [length of stay (LOS)].

All studies published in English or German up to October 2014 were included for abstract screening. While randomized controlled trials, clinical trials, and observational studies comparing liver transplantation with and without TPCS were included, reviews, reports, comments, case studies and case series were excluded. Split liver transplantations, Domino- and DCD (donation after cardiac death) grafts were not included in the analyzed studies. All abstracts were independently screened by two researchers (A. R. and M. K.). In case of disagreement, the corresponding studies were discussed to achieve consensus. The same procedure was conducted again with the selected full texts. Here, studies were excluded in a first step if they did not apply a TPCS or did not compare outcomes for a TPCS versus no TPCS. In the final step, studies were excluded when they did not investigate the outcomes of interest.

### Appraisal of the quality of the studies and publication bias

The checklist developed by Downs et al. [10] was applied to rate the quality of the included studies. In this checklist, 26 items distributed over five subscales are used to assess the quality of the study type, reporting bias, external and internal validity, biases, and confounding. Both researchers rated the quality independently. In case of disagreement, a discussion led to consensus. Tests for publication bias were not performed since for all outcomes not more than six studies were included.

### Data extraction and management

For each of the included studies, all outcomes were extracted (type of outcome, measure of outcome, time-point of measurement). Subsequently, all specific outcomes were assigned to parent areas. In a consensus meeting, the authors selected those outcomes for which meta-analyses should be performed. For outcomes of interest for which data were not reported completely, the corresponding authors were contacted and asked to provide the missing data.

### Data synthesis

Depending on the type of data, effect sizes were calculated either as mean difference (for outcomes reported as continuous data using the same scale in all studies), standard mean difference (for outcomes reported as continuous data using different scales across the studies) and odds ratio (for dichotomous outcomes).

For all outcomes, a random-effect model as described by Borenstein et al. [11] was applied. This model takes into account that heterogeneity between the trials such as variation in the study population or the implementation of interventions exists, which was assumed for the included studies.

For all meta-analyses, the following statistics were calculated: For each single study, the effect size with its 95 % confidence interval (CI) was calculated. The combined effect size with its 95 % CI was calculated applying the inverse variance weighting method (IV). To test the significance of the overall effect size, a $Z$-transformation was performed. The significance level was set as .05. To describe heterogeneity among the studies, the variance of the true effect size between the studies ($\tau^2$), $\chi^2$, degrees of freedom ($df$) and a test for heterogeneity ($I^2$) were calculated. An $I^2 > 50$ % was considered as substantial heterogeneity [4]. For each meta-analysis forest plots were created. Statistical analyses and generation of figures were conducted using the statistic software RevMan 5.3. developed by the Cochrane Collaboration.

## Results

The systematic search of the literature revealed 900 potentially suitable publications. An additional nine studies were identified by hand search of the reference lists. After abstract screening, 28 studies were included in the full-text analysis. Of these, 11 studies were included in the qualitative analysis. Six studies addressed these outcomes and were selected to be included in the meta-analysis (Fig. 1).

Table 1 depicts the characteristics of the included studies. All studies were performed in Europe except one performed in the USA and were published from 1999 to 2012. Five studies were conducted as retrospective data analyses; one study was carried out as a randomized controlled trial. The quality score showed a range from 9 to 20 across the studies. The mean age of the patients included in the studies ranged from 46 to 58 years, and the total sample sizes of the studies ranged from 65 to 448.

### Effects of the temporary porto-caval shunt on selected outcomes

#### *Hepatic injury*

For ALT and AST, data from two studies including a total of 619 patients were available [5, 12]: The mean difference in ALT (Fig. 2) assessed within the first three postoperative days was considered. While Pratschke et al. [5] found significantly lower ALT values for utilization of a TPCS, Ghinolfi and colleagues failed to demonstrate such a

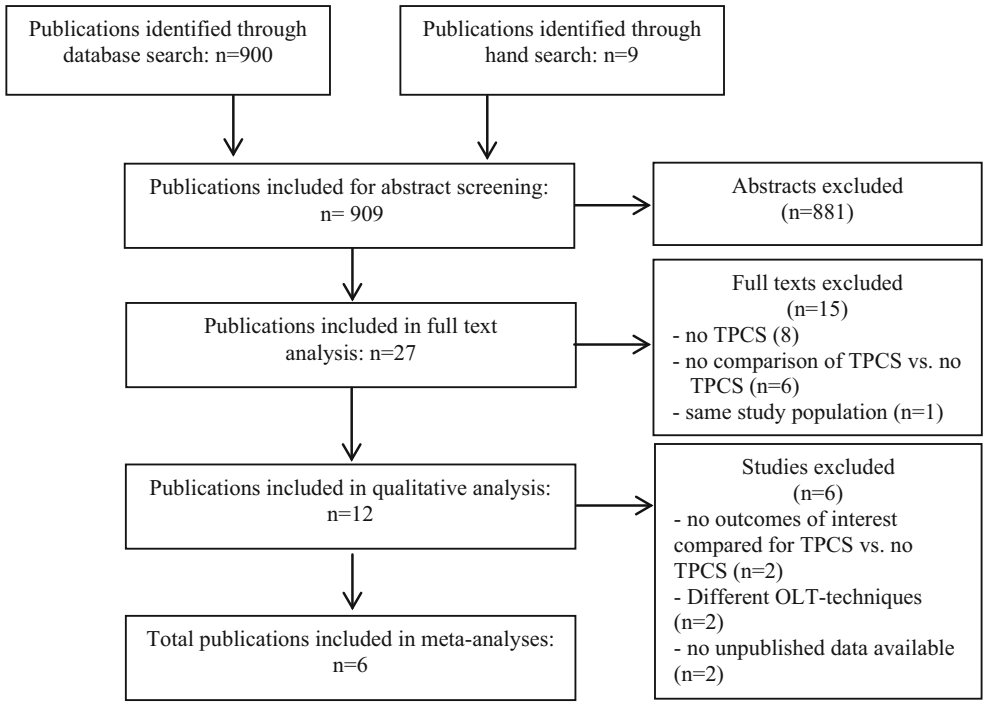


**Fig. 1** Flow diagram of systematic literature search according to the PRISMA statement [21]

difference [12]. Moreover, for the combined effect no significant reduction was found anymore (mean difference: −192.84; 95 % CI −801.97, 416.29; $p = .53$).

For AST (Fig. 3), the effect size was reported as the mean difference measured at the seventh postoperative day. In both studies [5, 12], lower AST values were found in the TPCS group. While this difference was not significant in each individual study, the combined analysis showed significantly lower AST values (mean difference: −25.96; 95 % CI −50.90, −1.03; $p = .04$) in the TPCS group.

For PNF (Fig. 4), data from three studies [5, 12, 13] including a total of 676 patients were included. The occurrence of PNF was compared using odds ratios. In each of three studies patients operated with a TPCS showed reduced odds for PNF; however, a significant difference was only found in the study by Pratschke et al. [5]. The combined odds for PNF was reduced in the TPCS group; however, statistical significance was not reached (odds ratio 0.32; 95 % CI 0.09, 1.16; $p = .08$).

## Characteristics of the surgical intervention

For duration of operation (Fig. 5), data from five studies [4, 5, 14–16] including a total of 880 patients were evaluated. The mean difference in the duration of the operation was used as effect size. The mean duration of operation was shorter in all studies, but only Arzu et al. [15] found a significant shorter duration for the TPCS group. The combined effect did not reach statistical significance (mean difference: −40.77; 95 % CI −83.48, 1.94; $p = .06$).

For analyses of the PRBC requirement (Fig. 6), data from six studies [5, 12–16] comprising 1328 patients were included. The standardized mean difference of transfused PRBCs was used as effect size. Raw data described either the number of packs or units of PRBCs required during surgery. In five of the six studies, fewer PRBCs were required in the TPCS group [5–9]. In three of these studies, the difference was statistically significant [12, 14, 16]. Only Pratschke et al. found a decreased number of transfused PRBCs in the group without TPCS [5]. Though in this study the TPCS group required more PRBCs than the group transplanted without a TPCS, the combined effect suggests a lower transfusion requirement (standardized mean difference: −0.30; 95 % CI −0.56, 0.03; $p = .03$) during surgery with TPCS.

To analyze the FFP requirement (Fig. 7), data from five studies comprising a total of 927 patients were included [4, 5, 12, 14, 15]. The standardized mean difference was used as effect size. Raw fundamental data described either the number of packs, units or number of concentrates, or milliliters of FFP required during surgery. While in three studies the mean difference was lower for the TPCS group [12, 14, 15], in two studies it was lower for the group without TPCS [4, 5]. However, the lower need for FFP in

**Table 1** Characteristics of studies included in the meta-analysis

| References | Country | Study design | Rating of evidence[a] | | | | *n* (intervention/control) | Transplantation type: (intervention/control) | Graft type | Severity of liver disease (intervention/control) | Patient age: intervention/control (Mean; SD) | Reported outcomes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Study type and total score | Quality | | | | | | | | |
| | | | | Reporting bias (max: 11) | External validity (max: 3) | Internal validity (max: 13) | | | | | | |
| Hesse et al. [29] | Belgium | Retrospective case–control study | Low | 5<br>Total: 9 | 0 | 4 | 16/49 | Laterolateral cavo-cavostomy with/without TPCS | n.r. | CHILD: 10.7 ± 3.1/10.4 ± 3.6° | 55 ± 8/52 ± 12 | 1, 2, 3 |
| Figueras et al. [13] | Spain | Randomized controlled trial | High | 9<br>Total: 20 | 3 | 8 | 40/40 | OLT (piggyback technique) with/without TPCS | n.r. | CHILD A, B, C: 13, 18, 9/10, 19, 11 | 58 ± 8/60 ± 7 | 1, 2, 3, 4, 8 |
| de Cenarruzabeitia et al. [15] | Spain | Retrospective case–control study | Low | 7<br>Total: 14 | 2 | 5 | 356/45 | OLT (piggyback technique) with/without TPCS | n.r. | CHILD A, B, C: 18, 19, 29/10, 17, 27 | 57 ± 8/54 ± 11 | 1, 3, 5 |
| Arzu et al. [30] | Italy | Retrospective case–control study | Low | 5<br>Total: 12 | 2 | 5 | 89/97 | OLT (cavo-cavostomy end-to-side anastomosis) with/without TPCS | Cadaveric | MELD: 23.0 ± 10.3/17.5 ± 7.6 | 52 ± 10/52 ± 11 | 1, 2, 3, 4, 5 |
| Ghinolfi et al. [12] | Spain | Retrospective case–control study | Low | 8<br>Total: 17 | 2 | 7 | 58/90 | OLT (piggyback technique) with/without TPCS | n.r. | MELD: only total sample: 29.1 ± 6.9 | Total: 56 ± 10 | 1, 2, 3, 4, 5, 6, 7, 8 |
| Pratschke et al. [5] | Germany | Retrospective case–control study | Low | 8<br>Total: 16 | 2 | 6 | 274/174 | OLT (piggyback technique) with/without TPCS | n.r. | MELD: 20 (2–40)/21 (5–40) | 46 ± 12/52 ± 16 | 1, 2, 5, 6, 7, 8 |

*n.r.* not reported, *OLT* orthotopic liver transplantation, *TPCS* temporary porto-caval shunt

1—Packed red blood cells requirement; 2—fresh frozen plasma requirement; 3—duration of operation; 4—length of hospital stay; 5—creatinine; 6—alanine aminotransferase; 7—aspartate aminotransferase; 8—primary non-function

[a] Based on the system developed by Downs et al. [9], maximum total score = 27

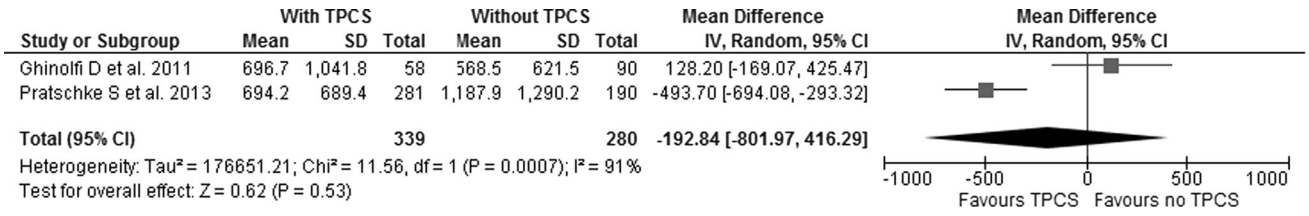


**Fig. 2** Forest plot for the meta-analysis of studies examining the effect of a temporary porto-caval shunt on alanine aminotransferase values. The size of the *squares* indicates the weighting of the studies. The *diamond* indicates the overall effect size

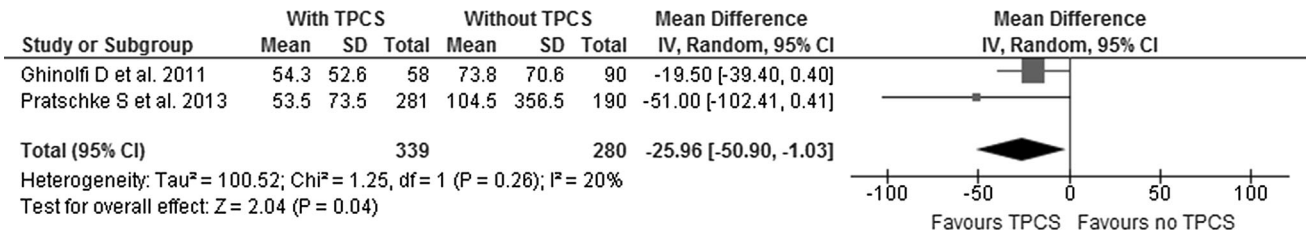


**Fig. 3** Forest plot for the meta-analysis of studies examining the effect of a temporary porto-caval shunt on aspartate aminotransferase. The size of the *squares* indicates the weighting of the studies. The *diamond* indicates the overall effect size

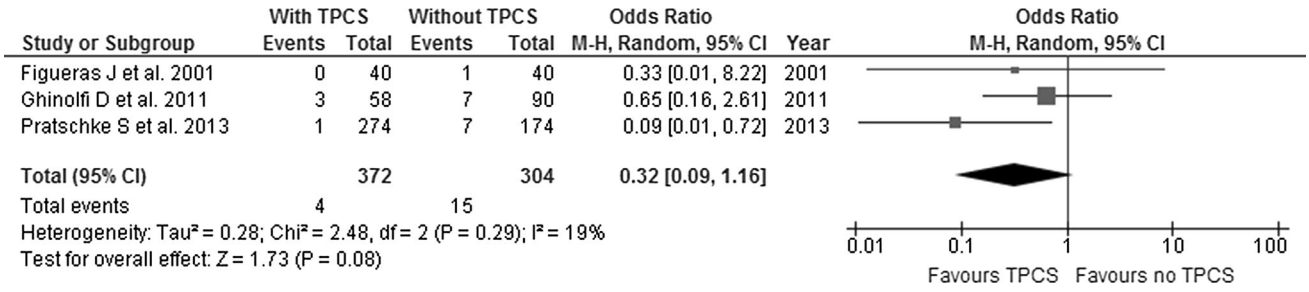


**Fig. 4** Forest plot for the meta-analysis of studies examining the effect of a temporary porto-caval shunt on primary non-function. The size of the *squares* indicates the weighting of the studies. The *diamond* indicates the overall effect size

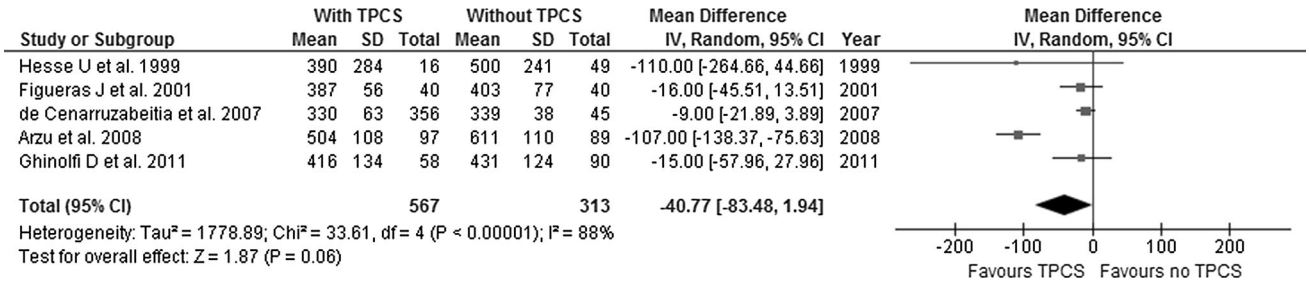


**Fig. 5** Forest plot for the meta-analysis of studies examining the effect of a temporary porto-caval shunt on the duration of operation. The size of the *squares* indicates the weighting of the studies. The *diamond* indicates the overall effect size

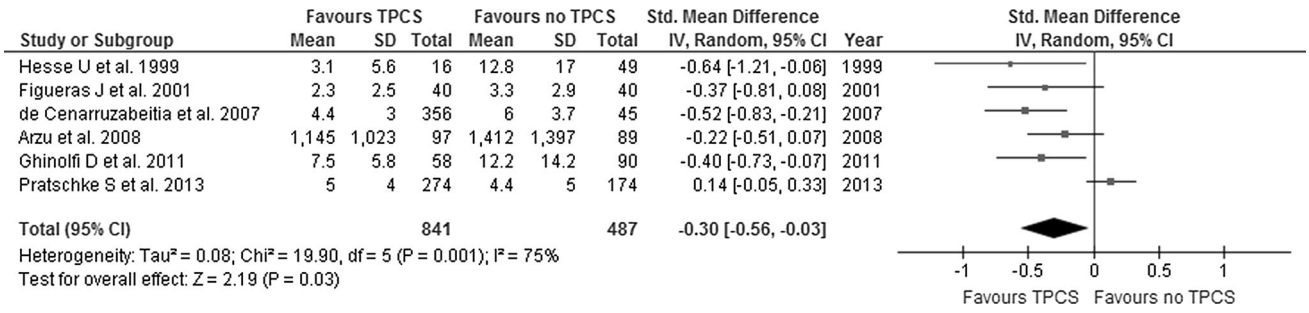


**Fig. 6** Forest plot for the meta-analysis of studies examining the effect of a temporary porto-caval shunt on the requirement for packed red blood cell transfusion. The size of the *squares* indicates the weighting of the studies. The *diamond* indicates the overall effect size

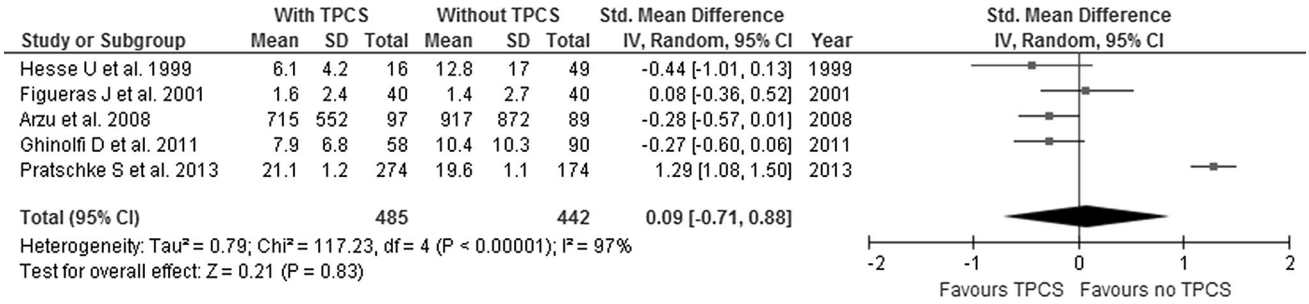


**Fig. 7** Forest plot for the meta-analysis of studies examining the effect of a temporary porto-caval shunt on the requirement for fresh frozen plasma transfusion. The size of the *squares* indicates the weighting of the studies. The *diamond* indicates the overall effect size

the group without TPCS was only statistically significant in the study by Pratschke et al. [5]. The combined estimate suggests no effect of TPCS on FFP requirement (standardized mean difference: 0.09; 95 % CI −0.71, 0.88; $p = .83$).

## Renal function

Data from four studies [5, 12, 15, 16] comprising a total of 1206 patients were included to investigate the effect on creatinine values (Fig. 8). The standardized mean difference was used as effect size. Raw data described either creatinine level (in mg/dl) or creatinine clearance in the early postoperative phase (3rd day). In all studies, lower creatinine values were found for the TPCS group, although this difference was not significant in the study by Pratschke et al. [5]. The combined data showed a significant (standardized mean difference: −0.29; 95 % CI −0.53, 0.04; $p = .02$) reduction of the creatinine values in the TPCS group. No information was available on the percentage of renal replacement therapy from the studies analyzed.

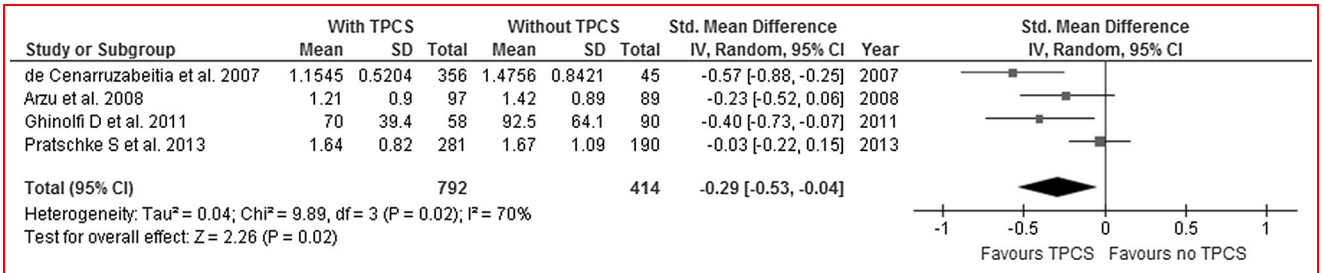


**Fig. 8** Forest plot for the meta-analysis of studies examining the effect of a temporary porto-caval shunt on creatinine levels on the 3rd postoperative day. The size of the *squares* indicates the weighting of the studies. The *diamond* indicates the overall effect size

### Length of hospitalization

For the LOS (Fig. 9), data from three studies [4, 12, 15] comprising a total of 414 patients were included. The mean difference for LOS in days was used as effect size. This mean was lower for the TPCS group in all studies; however, none of the studies showed statistical significance. The combined effect did not reach statistical significance (mean difference: −2.18; 95 % CI −5.60, 1.24; $p = .21$).

### Heterogeneity in the studies

For ALT, duration of operation, red blood cell requirement, FFP requirement and creatinine the $I^2$ values indicated substantial heterogeneity ($I^2 > 75$ %, $p < .05$).

## Discussion

Cava-sparing techniques as described by Belghiti et al. [3] have been developed to maintain the venous return to the heart, thereby improving hemodynamic stability and renal perfusion [8]. Additionally, the dissection of the retroperitoneum can be omitted which results in reduced blood loss. Nevertheless, ischemia–reperfusion injury still remains a considerable issue and cross-clamping of the portal vein with subsequent splanchnic congestion, gut edema, bacterial translocation and sudden cytokine release after release of the portal venous blood flow substantially contributes to ischemia–reperfusion injury [7, 17–19].

Tzakis [20] and Belghiti [21] described a temporary porto-caval shunt (TPCS), which was primarily designed for patients with a lack of sufficient porto-caval collaterals. Subsequently, this technique was also applied in patients with adequate porto-caval collaterals. A TPCS may be established using a temporary end-to-side porto-caval anastomosis [21] or using an extracorporeal porto-caval shunt catheter connecting the portal to the femoral vein [5]. The latter does not require anticoagulation or roller pumps and is the preferred procedure at our department. Due to the small number of reports, a separate analysis considering the technique (plastic tube or temporary anastomosis between portal vein and caval vein) was not performed. Only one study from the USA was included in this meta-analysis. The higher acceptance of a porto-caval shunt in Europe as compared to North America could be the result of regional differences in the average DRI and MELD score between both transplant communities. As the use of a TPCS still remains controversial [22–24], we sought to investigate the effect of a TPCS on posttransplant transaminases, primary graft non-function, duration of the operation, transfusion of blood products, posttransplant renal function and length of hospital stay (LOS).

All studies included the transfusion of packed red blood cells (PRBC) as a surrogate marker for intraoperative blood loss. The use of a TPCS significantly decreased the amount of transfused PRBCs. One may hypothesize that decompression of the portal vein would reduce blood flow in preexisting portosystemic collaterals and thereby facilitate intraoperative hemostasis. Although the determination of hemodynamic stability was beyond the scope of this study, one would speculate that lower blood loss would result in improved hemodynamic stability. This question, however, needs to be evaluated in a prospective trial. In contrast to PRBC substitution, there was no significant difference in the transfusion of FFPs between both groups. This suggests that the trigger for FFP substitution is multifactorial and does not solely depend on intraoperative blood loss. Use of a TPCS was associated with a nonsignificant trend toward a shorter operation time which might be due to multiple confounders influencing operation time which were not analyzed in this study. Differences in operation time may also affect other outcomes. For instance, a reduction in intraoperative blood loss could be the result of reduced operation time. Interestingly, none of the studies reported an increase in time of operation.

The length of hospital stay as a surrogate marker for postoperative morbidity was analyzed. The use of a shunt did not influence LOS. It must be stated that the mean LOS in the European studies was longer than in the American

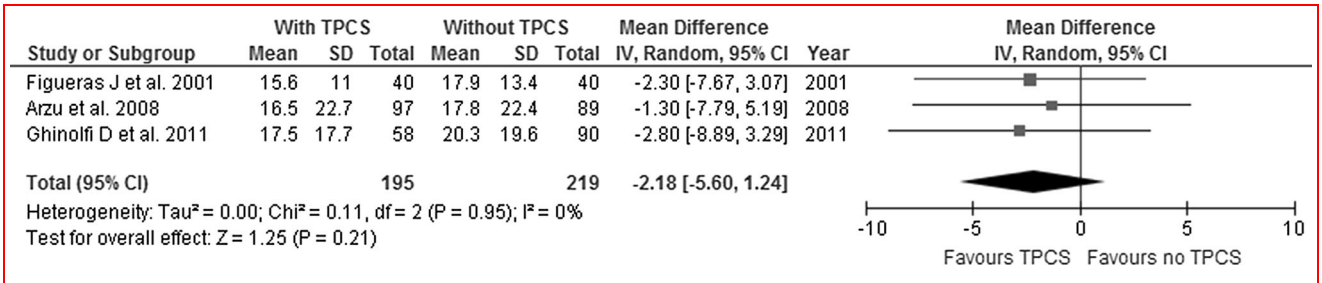


**Fig. 9** Forest plot for the meta-analysis of studies examining the effect of a temporary porto-caval shunt on the length of hospital stay in days. The size of the *squares* indicates the weighting of the studies. The *diamond* indicates the overall effect size

study by Ghinolfi et al. [12]. Therefore, it cannot be excluded that different health systems or other confounders may contribute to this observation. No sufficient information on the length of ICU-stay was available from the analyzed studies.

One may speculate that improved central venous filling and thus enhanced renal perfusion would contribute to improved kidney function in patients operated with a TPCS. In this respect, the meta-analysis of studies reporting creatinine values on the 3rd postoperative day revealed a significant advantage for patients operated with a TPCS. Despite this observation, it cannot be excluded that portal venous blood flow prior to hepatectomy may also influence this effect. Margarit et al. [25] have shown that renal function in patients with a portal venous flow greater than 800 ml/min prior to hepatectomy was particularly improved following the use of a TPCS. Two of the studies incorporated in this meta-analysis reported improved postoperative serum creatinine levels in patients with an increased portal venous blood flow, whereas the two others did not investigate portal venous flow in detail. The present studies do not provide information on renal replacement therapy. Nonetheless, patients with renal replacement therapy also exhibit elevated creatinine levels. Moreover, this systematic mistake accounts for both groups. Therefore, elevated creatinine levels reflect a diminished kidney function despite the above-mentioned limitation. However, no final conclusion can be drawn on the impact of the portal venous flow on postoperative renal function in liver transplantation with and without TPCS.

Hepatic injury and liver function must be considered as important parameters when analyzing the effects of TPCS. AST and ALT levels represent clinical indicators for hepatic injury as a result of ischemia–reperfusion injury [26, 27]. In the present analysis, significantly reduced AST values were evident in patients transplanted with a TPCS. This suggests that the utilization of a TPCS is associated with decreased liver injury in this setting. In contrast, ALT levels were not significantly improved. These parameters may also be subject to unspecific effects.

Although there was a trend toward a reduction of the rate of PNF in patients operated with a shunt, this observation only reached statistical significance in one study but not in the pooled analysis. Since other factors, i.e., reduced graft quality [28], ischemia reperfusion injury [6], etc., are known to affect the occurrence of PNF, those confounders should be considered in a prospective randomized trial evaluating the potentially protective effects of a portocaval shunt.

The results of this meta-analysis favor the use of a TPCS and indicate that important intraoperative parameters as wells as parameters of the early postoperative phase are improved. Examination of the underlying mechanisms for the protective effects of a TPCS in liver transplantation on cardiovascular stability and liver injury was beyond the scope of the present analysis. Several potential mechanisms may act synergistically in mediating the protective effects of a TPCS. Previously, we have been able to show that the use of a TPCS in liver transplantation is associated with an enhanced portal venous blood flow [5]. This finding is clinically relevant as graft survival is impaired in patients with decreased portal venous blood flow [29]. Moreover, shunts have been shown to reduce the incidence of postreperfusion syndrome which may reflect improved central venous filling and thus improved cardiac function and oxygenation [30]. Alternatively, decreased release of endotoxin and pro-inflammatory mediators from the gut and liver due to reduced bacterial translocation in the gut may contribute to hemodynamic stability and reduced liver injury [17–19]. These findings need to be evaluated in prospective trials.

This study has several limitations. The number of identified studies is low which is due to the fact that the evidence on the application of a TPCS in liver transplantation is scarce. Moreover, no sufficient information on long-term survival eligible for this meta-analysis was available in the included studies. Furthermore, only one randomized controlled trial (RCT) could be included. Due to the paucity of available publications, the authors had to combine non-RCTs with the one available RCT. In this respect, the identified heterogeneity for some of the outcomes suggests diversity regarding clinical, i.e., donor and recipient characteristics (donor risk index, MELD score), and methodological parameters among the studies which cannot not be clarified from the present data. To address this heterogeneity, we applied random-effect models, which only allow the identification of an average intervention effect compared to a best estimate of an intervention effect when applying fixed-effect models. The meta-analysis, however, contains heterogeneous data which may bring a potential bias to the results but also underlines the need for a prospective trial on this issue.

The present data indicate similar donor and recipient conditions whether a shunt had been inserted or not. In this respect, three studies demonstrate similar MELD scores [5, 12, 15], three manuscripts indicate similar CHILD Pugh scores [4, 14, 16], and all studies give more or less detailed information on indications for liver transplantation which do not seem to differ between the groups. Nevertheless, the scarcity of information on potential risk factors for the outcome after liver transplantation in all studies clearly represents a limitation of this meta-analysis. Two studies incorporated the DRI in their analysis [5, 12] suggesting that no differences in the DRI between patients with or without shunt were evident. Interestingly, these studies also indicate a more pronounced impact of a shunt in transplantation of grafts with high donor risk indices, but not in

patients with high MELD scores. This suggests that besides those risk factors mentioned above usage of a shunt affects outcome after liver transplantation.

Nevertheless, this meta-analysis cannot provide information about the rationale and the indications for the use of a shunt: This is mainly due to the retrospective study design of the included trials, and only one prospective trial by Figueras et al. [4] was available. One could speculate that patients with a lack of adequate collaterals (i.e., in acute liver failure) would benefit most from a shunt. No evidence can be found for this hypothesis from the present data. Therefore, no recommendation can be given which indications should preferably be treated with a shunt (i.e., chronic vs. acute liver diseases) currently.

Despite these limitations, the application of a TPCS seems to exert beneficial effects in cava-sparing liver transplantation. Since a selection bias in any retrospective analysis cannot be excluded, the positive data of the present meta-analysis have to be confirmed within a prospective randomized trial.

## Conclusion

Temporary porto-caval shunts are a simple and effective technique to decrease blood transfusion, ameliorate postoperative renal function and reduce hepatic injury in liver transplantation. Since previous data show some limitations, this method must be analyzed in a prospective trial.

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**Authors' contributions** S Pratschke designed the study and wrote the manuscript. A. Rauch and M. Kirschneck performed the statistical analysis and provided important advice on writing the manuscript. M. Albertsmeier, M. Rentsch, J. Werner and M. Guba substantially contributed to the design of the study and critically revised the manuscript. J. Andrassy and M. Thomas designed the study and critically revised the manuscript. J. Figueras, N. De Ruvo and J del Rio Martin provided important data and critically revised the manuscript. M. Weniger and M. K. Angele conceived the idea of the study, majorly contributed to its design and critically revised the manuscript. All authors read and approved the submitted version of the manuscript.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.

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