Aus der
Medizinischen Klinik und Poliklinik I – Allgemeine Innere Medizin mit den Schwerpunkten Gastroenterologie und Hepatologie, Nephrologie, Infektiologie, Endokrinologie und Diabetologie
des Universitätsklinikums Bonn
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# Evaluation der Rolle der zirrhotischen Kardiomyopathie als Risikofaktoren für das Überleben bei Patienten mit fortgeschrittener Leberzirrhose

Habilitationsschrift
zur Erlangung der Venia Legendi
der Hohen Medizinischen Fakultät
der Rheinischen-Friedrich-Wilhelms-Universität Bonn
Für das Lehrgebiet
„Innere Medizin“
Vorgelegt von

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Wissenschaftlicher Mitarbeiter
an der Universität Bonn
Bonn 2024

Datum des Habilitationskolloquiums: 9. November 2023

## Übersicht

Der vorliegenden Habilitationsschrift mit dem Titel „Evaluation der Rolle der zirrhotischen Kardiomyopathie als Risikofaktoren für das Überleben bei Patienten mit fortgeschrittener Leberzirrhose“ liegen folgende publizierte Arbeiten zu Grunde:

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# 1 Inhaltsverzeichnis

# 2 Einleitung

## 2.1 Die Leberzirrhose

Bei der Leberzirrhose handelt es sich um das Endstadium vieler unterschiedlicher chronischer Leberschädigungen (Ginès et al. 2021; Zhou et al. 2014, 2014; Elpek 2014; Hernandez-Gea und Friedman 2011). Der Ursprung kann dabei in einer infektiösen, toxischen, autoimmunen, vaskulären oder metabolischen Ursache liegen (Augustin et al. 2017). Betrachtet man die Entwicklung des weltweiten Gesundheitssystems, so sieht man die wachsende Bedeutung der chronischen Lebererkrankungen und ihrer Folgen (Neff et al. 2011; Ginès et al. 2022; Younossi 2019).

Die häufigsten Ursachen für die Entwicklung einer Leberzirrhose in Deutschland sind die alkoholische und nichtalkoholische Fettlebererkrankung. Davon gefolgt, und das unterscheidet sich regional im Hinblick auf die weltweite Verteilung gravierend, die viralen Hepatitiden B und C. Interessant ist die Tatsache, dass die alkoholische Lebererkrankung mit 8619 Todesfällen und einer Sterbeziffer von 8,9/100 000 Einwohner im Jahr 2009 die 20. häufigste Todesursache der deutschen Allgemeinbevölkerung ausmachte (Gesundheitsberichterstattung des Bundes (2019).). Es ist in dem Wandel der Gesellschaft zu mehr Ernährungbewusstsein und Fitnessbewegung noch verwunderlich, dass sich zwischen 1980 und 2005 die Zahl der Leberzirrhose assoziierten Todesfälle pro 100 000 Einwohner von 5 auf 9,9 verdoppelt hat (Gesundheitsberichterstattung des Bundes (2019).). Dahingegen lässt die nun ubiquitär vorhandene Möglichkeit der Hepatitis C Therapie, besonders in den letzten Jahren, einen positiven Blick in die Zukunft wagen.

Der bestimmende Faktor in der Entstehung der Leberzirrhose ist die kontinuierliche Schädigung der Leber. Diese führt zu einem Untergang des funktionalen Lebergewebes, welches durch funktionsloses Bindegewebe ersetzt wird (Schuppan und Afdhal 2008). Die fortschreitende Vernarbung, welche als Fibrogenese des Lebergewebes beschrieben wird, stellt aber nur den Beginn dieser Erkrankung dar (Friedman 2008). Die Fibrogenese führt durch die reaktive Bindegewebsvermehrung bzw. der Ansammlung von extrazellulärer Matrix bereits zu einer Erhöhung des intrahepatischen Widerstandes. Die Zirrhose hingegen ist gekennzeichnet durch die Zerstörung der physiologischen Läppchen- und Gefäßstruktur der Leber. Es bilden

sich Regeneratknoten und es kommt zu einem weiteren drastischen Funktionsverlust des Organs.

Neben den Auswirkungen auf das Individuum an sich stellt die Leberzirrhose ein wachsendes klinisches und volkswirtschaftliches Problem dar (Pimpin et al. 2018). Publiziert ist, dass Europa die weltweit größte Anzahl an chronischen Lebererkrankungen aufweist (Global Health Data Exchange. (2020)). Ob dies so ist, oder die europäische Gesundheitspolitik nur die hinreichenden Diagnoseschritte ermöglicht und somit von einer Unterrepräsentation außerhalb der europäischen Gemeinschaft ausgegangen werden kann, sei dahingestellt.

Es ist publiziert, dass in Deutschland ca. 2 % der Bevölkerung an einer Leberzirrhose und deren Komplikationen leiden. Eine entsprechend hohen Morbidität und Mortalität findet sich in dieser Gruppe von Patienten (Chirapongsathorn et al. 2016). Man geht davon aus, dass die mittlere Überlebenszeit bei Patienten, welche sich im Krankheitszustand einer klinisch kompensierten Leberzirrhose befinden, etwa sieben Jahre beträgt (Franchis et al. 2022). Kommt es zu den ersten Dekompensationen ändert sich dies hingegen dramatisch. Nach der ersten Dekompensation sinkt das Zwei-Jahres-Überleben auf unter 50% (Franchis et al. 2022).

## 2.2 Die Komplikationen der Leberzirrhose

Eine Besonderheit der hepatischen Perfusion stellt die Tatsache dar, dass die Leber neben der arteriellen Durchblutung zum größten Teil durch das sauerstoffarme, aber nährstoffreiche Blut der Pfortader versorgt wird. Im Verlauf der Leberzirrhose ist die Entwicklung eines portalen Hypertonus zu erwarten (Abraldes et al. 2006, 2006; Augustin et al. 2014; Iwakiri 2014). Dieser ist bedingt durch einen Anstieg des vaskulären Widerstandes. Ab einem Gradienten zwischen Pfortader und Lebervene größer 5 mmHg spricht man von einem portalen Hypertonus. Ab 10 mmHg geht man von einer klinischen Relevanz des portalen Hypertonus aus. Ab 12 mmHg sind blutungsgefährdete Varizen zu erwarten (Burton et al. 2007). Die treibende Kraft, die zur Erhöhung des vaskulären Widerstandes führt, sind die architektonischen Veränderungen durch die fortgeschrittene Fibrose der Leber (Ginès et al. 2021).

Weiterhin kommt es zu phänotypischen Veränderungen in hepatischen Sternzellen sowie der sinusoidalen Endothelzellen der Leber. Die Aktivierung der Sternzellen begünstigen die Kontraktilität der Gefäße und erhöhen somit den Gefäßwiderstand

zusätzlich. Des Weiteren führt die Reduzierung der Bioverfügbarkeit von Stickstoffmonoxid in der zirrhotisch umgebauten Leber zu einem weiteren Anstieg des Gefäßtonus (Iwakiri und Trebicka 2021; Atucha et al. 2005; Shenoda und Boselli 2019). Es ist aber davon auszugehen, dass dieser Effekt nur 25 % des erhöhten Widerstands ausmacht. Der Großteil jedoch wird durch die architektonische Veränderungen bestimmt (Iwakiri 2014).

Die portale Hypertension bedingt im Laufe der Erkrankung der Leberzirrhose die gefürchteten Dekompensationen wie Aszites, Varizenblutungen oder hepatische Enzephalopathie (Franchis et al. 2022; Colombo 2020).

### 2.2.1 Aszites

Eine der Komplikationen im Verlauf der Leberzirrhose ist die Entwicklung von Aszites. Die treibende Kraft in der Pathogenese der Aszitesbildung ist die Entwicklung der portalen Hypertension und die Retention von Natrium und Wasser. Bereits bei Gradienten unter 8 mmHg konnte eine verringerte 24-Stunden-Natriumausscheidung im Urin dokumentiert werden (Morali et al. 1992; Casado et al. 1998).

Der erhöhte Pfortaderdruck wird vereinfacht von Rezeptoren des splanchnischen Einstromgebietes wahrgenommen, was wiederum zur Freisetzung von angiogenen Faktoren und Wachstumsfaktoren führt (Abraldes et al. 2006). Durch die Freisetzung dieser Faktoren wird dem portalen Hypertonus entgegengewirkt, indem es zu einer Etablierung von neuen, bzw. Ausbau von bestehenden portosystemischen Shunts kommt. Dies stellt eine mehr als adäquate Reaktion des Körpers dar, um einer Kongestion im splanchnischen Einstromgebiet entgegenzuwirken.

Im Rahmen der Zirrhose kommt es zu einem weiteren Anstieg des Portaldrucks, was zu einer weiteren Aktivierung der endothelialen Stickoxid-Synthase und Überproduktion von Stickstoffmonoxid führt. Dies wiederum bedingt eine splanchnische Vasodilatation, was zur Folge hat, dass das Herzzeitvolumen weiter steigt, wohingegen das effektive Blutvolumen sinkt. Daraus resultiert die effektive Hypovolämie im Laufe der Leberzirrhose (Schrier et al. 1988).

Die Mechanismen der Gegenregulation basieren auf einer Aktivierung des Sympathikus, was eine Stimulierung der Reabsorption von Natrium im proximalen und

distalen Tubulus, sowie in der Henle-Schleife und dem Sammelrohr zu Folge hat (Schrier 2006). Die Aktivierung des Sympathikus würde beim gesunden Menschen zu einer Erhöhung des Herz-Zeit-Volumens führen. Auf Grund der zirrhotischen Kardiomyopathie ist dies aber nur eingeschränkt möglich, worauf ich im Folgenden noch näher eingehen möchte. Weiterhin erfolgt eine Aktivierung des Renin-Angiotensin-Aldosteron-Systems. Dies führt wiederum zu einer Natriumabsorption aus dem distalem Tubulus und Sammelrohr (Cárdenas und Arroyo 2003).

Zusammenfassend ist davon auszugehen, dass die renale Natriumretention und die Hypovolämie bedingte Minderperfusion der Nieren zu Ödemen und der Aszitesbildung führen (Cárdenas und Arroyo 2003). Das Verständnis der Pathologie ist bei diesem Krankheitsbild besonders wichtig, um die laborchemisch bestehende Hyponatriämie als Verdünnungshyponatriämie zu interpretieren und entsprechend zu behandeln.

Die Therapie des Aszites erfolgt zunächst medikamentös durch eine Kombination von Aldosteronantagonisten und Schleifendiuretika, wobei Schleifendiuretika erst im Verlauf der Therapie bei fehlendem Ansprechen eingesetzt werden sollten (Gerbes et al. 2019). Ziel ist es, zwei wichtige Endpunkte im Verlauf der Leberzirrhose zu verhindern. Zum einen die spontane bakterielle Peritonitis und zum anderen das hepatorenale Syndrom (Ginès et al. 2004). In der Therapie der Patienten sind im Verlauf großvolumige Parazentesen häufig von Nöten. Auf Grund des großen Volumenverlustes und dem Verlust von Eiweiß im Transsudat sind hämodynamische Veränderungen und zirkulatorische Dysfunktion nach Parazentese häufig zu beobachten (Ginès et al. 1996; Ginès et al. 1988). Dieser Zustand der zirkulatorischen Dysfunktion wird unterstützt durch die zirrhotische Kardiomyopathie und der fehlenden Fähigkeit der adäquaten Anpassung der kardialen Auswurfleistung (Jansen et al. 2018a; Jansen et al. 2019a; Jansen et al. 2018b; Jansen et al. 2019b). In diesem Stadium der Erkrankung stellt die Anlage eines transjugulären intrahepatischen portosystemischen Shunts eine weitere Therapieoption dar.

Durch die Anlage des Shunts ist eine unmittelbare Dekomprimierung und Drucksenkung möglich. Kontraindikationen sind kardiale Vorerkrankungen, weit eingeschränkte Syntheseleistung und Entgiftungsfunktion der Leber sowie rezidivierende Episoden hepatischer Enzephalopathie. Die Anlage des transjugulären intrahepatischen portosystemischen Shunts stellt im Vergleich zu großvolumigen

Parazentesen eine besser Alternative für den Patienten bezüglich Lebensqualität und Mortalität dar (Allegretti et al. 2016; Rössle et al. 2000; Bureau et al. 2017).

### 2.2.2 Hepatische Enzephalopathie

Eine weitere Komplikation der Leberzirrhose stellt die hepatische Enzephalopathie dar. Hierbei handelt es sich um eine Störung des Zentralnervensystems, welche durch die akute oder chronische Lebererkrankung an sich als auch durch die Ausbildung portosystemischer Kollateralkreisläufe bedingt ist (Ferenci et al. 2002; Elias 1979; Kircheis et al. 2007; Vilstrup et al. 2014). Sie ist gekennzeichnet durch eine zunehmende psychomotorische Verlangsamung. Die Symptome reichen von einer oft schwer detektierbaren subklinischen Erscheinung bis hin zum Koma (Ferenci et al. 2002).

Die zugrunde liegende Pathophysiologie liegt in dem Unvermögen der akut oder chronisch geschädigten Leber, die Ihre Funktion der Entgiftung nicht mehr wahrnehmen kann. Dabei ist davon auszugehen, dass es heterogene Faktoren sind unter denen sich auch das Ammoniak als Haupttoxin befindet. Ammoniak dient in der klinischen Praxis auf Grund der einfachen Messbarkeit als Surrogatparameter für alle weiteren Toxine.

Im Rahmen der hepatischen Enzephalopathie wird postuliert, dass ein geringgradiges Hirnödem und zerebraler oxidativer/nitrosativer Stress ausgelöst wird und diese Veränderungen die Astrozyten-/neuronalen Funktionen beeinträchtigt (Häussinger und Sies 2013; Görg et al. 2013; Görg et al. 2010; Häussinger und Schliess 2008; Häussinger und Schliess 2005; Häussinger et al. 1990). Der Literatur ist zu entnehmen, dass eine klinisch manifeste hepatische Enzephalopathie bereits bei 10-21 % der Patienten zum Zeitpunkt der Diagnosestellung einer Leberzirrhose vorliegt (Saunders et al. 1981; Romero-Gómez et al. 2001). Betrachtet man den weiteren Verlauf der Erkrankung, so ist diese schwere Komplikation bereits bei ca. 30 – 45 % der Patienten zu dokumentieren (Gundling et al. 2020; Labenz et al. 2017). Auf Grund der selektiven Vorstellung von Patienten in unsere Klinik mit bereits länger diagnostizierter Leberzirrhose, sind diese Zahlen nachvollziehbar.

### 2.2.3 Blutung

Im Rahmen der Entwicklung des portalen Hypertonus ist, wie bereits erwähnt, ab einem Gradienten zwischen Lebervenen und Pfortader von mehr als 12 mmHg das Auftreten blutungsgefährdeter Varizen zu erwarten (Burton et al. 2007). Die portosystemischen Kollateralkreisläufe führen wie oben beschrieben zunächst zu einer Entlastung des splanchnischen Einflussgebietes. Befinden sich diese Kollateralkreisläufe in unmittelbarem Bezug zu Hohlorganen, sei es Ösophagus, Magen oder Darm, besteht das Risiko einer Blutung. Die Blutung aus Varizen ist assoziiert mit einer hohen Mortalität (Franchis 2015).

Der Goldstandard zur Diagnose des portalen Hypertonus stellt immer noch die invasive Druckmessung dar. Auf Grund der fehlenden flächendeckenden Verfügbarkeit, dem invasiven Vorgehen und der damit verminderten Akzeptanz bei den Patienten, werden zunehmend nicht invasive Verfahren und Algorithmen etabliert. So sollten Patienten zur Vorbeugung einer Dekompensation im Sinne einer Blutung einer Endoskopie unterzogen werden, welche eine Lebersteifigkeit größer 20 kPa in der transienten Elastographie oder eine Thrombozytenzahl kleiner $50x10^9$ aufweisen (Franchis et al. 2022).

Die primäre Therapie der Ösophagusvarizen liegt in der medikamentösen Anwendung von nicht selektiven Betablockern, die der primär prophylaktischen Ligaturtherapie nicht unterlegen ist (Gluud et al. 2007). Ziel der Therapie ist die Reduktion der Blutungsereignisse (Cheng et al. 2003).

Eine Besonderheit stellen Patienten mit Hochrisiko-Varizen oder Patienten mit Kontraindikation/Intoleranz gegenüber nicht selektiven Betablockern dar. Hier kann die Ligaturtherapie als primäres Therapiekonzept diskutiert werden.

Kommt es hingegen zu einer Blutung, so steht nach hämodynamischer Stabilisation die Endoskopie im Vordergrund (Franchis et al. 2022). Auf Grund der schlechten Prognose und der hohen Wahrscheinlichkeit eines erneuten Blutungsereignisses ist eine Sekundärprophylaxe unabdingbar (Bari et al. 2021; Bari und Garcia-Tsao 2012). Wurde in der Vergangenheit darauf beharrt, sich für eine Therapie zu entscheiden, sehen die aktuellen Empfehlungen eine Kombinationstherapie aus nicht selektiven Betablockern und endoskopischer Ligaturtherapie vor (Franchis et al. 2022).

Eine kleine Gruppe von Patienten hingegen profitiert von einem transjugulären intrahepatischen portosystemischen Shunt innerhalb eines Zeitfensters von 72 Stunden. Dabei handelt es sich um Patienten mit einem Child-Pugh Stadium C aber weniger als 14 Punkten oder Patienten mit einem Child-Pugh Stadium B mit aktiver Blutung (Lv et al. 2019; García-Pagán et al. 2010). Auch bei rezidivierenden Blutungen sollte die Anlage eines transjugulären intrahepatischen portosystemischen Shunts erfolgen.

### 2.2.4 Akut-auf-chronisches Leberversagen (ACLF)

Betrachtet man in den letzten Jahrzehnten die einzelnen Dekompensationen im Rahmen der Leberzirrhose als eigenständige Ereignisse und deren Auswirkungen auf die Mortalität dieser Patienten, so etablierte sich in den letzten 10 Jahren mehr und mehr die wissenschaftliche Annahme eines neuen Krankheitsbildes.

Das akut-auf-chronische Leberversagen (ACLF) beschreibt die akute Dekompensation eines Patienten mit bekannter Leberzirrhose, begleitet durch die Entstehung der oben aufgeführten Komplikationen wie Aszites, hepatischer Enzephalopathie und gastrointestinalen Blutungen. Es wurde beobachtet, dass es im Zusammenhang mit diesen Komplikationen zu einem starken Anstieg des systemischen Inflammationsniveaus kommt und die Wahrscheinlichkeit eines Multiorganversagens mit entsprechender Steigerung der Mortalität vorliegt (Trebicka et al. 2021; Arroyo et al. 2020; Moreau et al. 2013).

Resultierend findet man diverse Kombinationen aus hepatischer, koagulatorischer, zerebraler, pulmonaler, renaler und zirkulatorischer Dysfunktion. In diesem breiten Arbeitsfeld zeigten sich mehr und mehr die Abhängigkeiten der einzelnen Organversagen.

Ziel dieser Arbeit ist es die Rolle der zirkulatorischen Dysfunktion unter Berücksichtigung der zirrhotischen Kardiomyopathie in der Entwicklung des ACLF zu beleuchten.

## 2.3 Risikostratifizierung der Patienten mit fortgeschrittener Leberzirrhose

### 2.3.1 Scores zur Risikostratifizierung

#### *2.3.1.1 MELD* (Model for End-stage Liver Disease)

Interessanterweise wurde dieses Modell das erste Mal 2001 beschrieben, jedoch unter dem Titele „A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts“ von Michael Malinchoc (Malinchoc et al. 2000). Heute dient der MELD-Score dazu, den Schweregrad einer Lebererkrankung zu erfassen und die 90-Tage Überlebenswahrscheinlichkeit abzuschätzen (Freeman et al. 2006).

Der MELD-Score wurde 2002 durch das United Network for Organ Sharing (UNOS), einer US-amerikanischen Organtransplantationsgesellschaft, in die Transplantationsmedizin eingeführt und 2003 von Eurotransplant übernommen. In den Eurotransplant angehörigen Staaten wird er weiterhin angewendet, um Patienten zu identifizieren, die aufgrund der Schwere der Lebererkrankung am dringendsten ein Spenderorgan benötigen.

Der MELD-Score basiert auf drei Laborparametern. Es erscheint einfach, aber beweist im Vergleich zu neuen Scores immer wieder seine Wertigkeit. Der Score wird berechnet aus den Parametern Bilirubin, Kreatinin und dem International Normalized Ratio. Weitere Punkte erhält der Empfänger bei Dialysepflichtigkeit, welche durch die hepatische Grunderkrankung bedingt ist. Der Score wird auf Ganzzahlen gerundet und liegt zwischen 6 und 40 Punkten. Es existieren Anpassungen für Patienten mit klar definierten Grunderkrankungen oder Zusatzkriterien, da diese Patienten durch den MELD in ihrem Überleben auf der Warteliste, laut aktueller Datenlage benachteiligt sind.

#### 2.3.1.2 Child-Pugh-Score

Die Child-Pugh-Kriterien wurden 1964 erstmals von Charles Gardner Child und Jeremiah G. Turcotte publiziert (Child und Turcotte 1964) und 1972 von Pugh modifiziert (Pugh et al. 1973). Im Gegensatz zum MELD-Score wird er durch das Heranziehen von fünf Parametern berechnet. Diese sind Bilirubin, Albumin, INR,

Aszites und hepatische Enzephalopathie. Es können zwischen 5 und 15 Punkte vergeben werden. Die erreichten Punkte werden den Child-Pugh Stadien A (5–6 Punkte), B (7–9 Punkte) und C (10–15 Punkte) zugeordnet. Auch wenn der Score nicht in der Vergabe von Organen angewendet wird, stellt er für den klinischen Alltag eine wichtige Entscheidungshilfe dar.

#### 2.3.1.3 ACLF (akut-auf-chronische Leberversagen) Grad/Score

Um das ACLF zu detektieren und die Schwere zu bewerten wird das ACLF in Grade eingeteilt. Weiterhin ist es möglich einen Score zu berechnen. In die Kalkulation fließen die hepatische, koagulatorische, zerebrale, pulmonale, renale und zirkulatorische Funktion ein. Grad 1 entspricht einem isolierten Versagen von Leber, Gerinnung, Kreislauf oder Respiration mit einem Serumkreatinin zwischen 1,5 und 1,9 mg/dl. Gleichzeitig kann eine milde hepatische Enzephalopathie vorliegen. Ein ACLF Grad 2 liegt vor, wenn zwei Organsysteme betroffen sind, Grad 3 bei drei Organsystemen.

Um das ACLF in einem Score zu erfassen, werden den einzelnen Organsystemen 0 bis 4 Punkte zugeordnet. Um die einzelnen Organsysteme zu bewerten, benötig man die Parameter Bilirubin, Kreatinin (wenn kein Nierenersatzverfahren durchgeführt wird), den Grad der hepatischen Enzephalopathie, INR, den mittleren arteriellen Druck und die arterielle Sauerstoffsättigung. Zur Bewertung der arterielle Sauerstoffsättigung werden zudem die Informationen benötigt, ob eine mechanische Ventilation durchgeführt wird und welche Sauerstoffsättigung die Atemluft hat. Um den mittleren arteriellen Druck zu beurteilen ist die Angabe essenziell, ob kreislaufunterstützende Medikamente eingesetzt werden.

### 2.3.2 Neue in dieser Arbeit verwendete Methoden

#### 2.3.2.1 Langzeit EKG Messungen (Herzfrequenzturbulenzen/ Herzfrequenzvariabilität)

Im Rahmen der Studien, die dieser Habilitationsschrift zugrunde liegen, wurden die Herzfrequenzturbulenzen und die Herzfrequenzvariabilität untersucht. Für beide Verfahren ist die Aufzeichnung einer 24-Stunden-Langzeitmessung eines Elektrokardiogramms erforderlich. Für die weitere Analyse ist es essenziell, dass eine Abtastrate von mindestens 1000 Hz vorliegt. Klassische Holter EKG Geräte erfüllen diese Bedingung, die meisten stationären Monitorsysteme aber nicht.

Herzfrequenzturbulenzen

Für die Berechnung der Herzfrequenzturbulenz sind mindestens drei vorzeitige ventrikuläre Kontraktionen im Rahmen der Langzeitmessung erforderlich. Bestimmt werden im Rahmen der Analyse zwei Parameter: Turbulenzbeginn (TO) und Turbulenzsteigung (TS) (Barthel et al. 2003; Bauer et al. 2006; Schmidt et al. 1999; Stein und Deedwania 2009; Zhang et al. 2005). Drei R-R-Intervalle vor und bis zu 21 Intervalle nach jeder vorzeitigen ventrikulären Kontraktion werden analysiert. Der Turbulenzbeginn wird als Quotient definiert aus ((RR1 + RR2)-(RR-2 + RR-1)) und (RR-2 + RR-1) multipliziert mit 100 (%). TS ist definiert als die maximale positive Regressionssteigung, die sich über fünf beliebige aufeinanderfolgende QRS Komplexe nach der vorzeitigen ventrikulären Kontraktion ermitteln lässt.

Herzfrequenzvariabilität

Bei der Herzfrequenzvariabilität werden kontinuierlich die R-R-Intervalle ausgewertet und die Standardabweichung aller Schläge zueinander bestimmt (Barthel et al. 2003; Bauer et al. 2006; Schmidt et al. 1999; Stein und Deedwania 2009; Zhang et al. 2005).

#### 2.3.2.2 Speckle Tracking/Strain-Analyse

Die Speckle Tracking/Strain-Analyse ist eine neue Diagnostik, mit der sich die komplexe Kontraktilität des Herzens unabhängig von den volumenbezogenen Größen beschreiben lässt. Es werden mehrere Komponenten der komplexen Kontraktion analysiert.

Die Verkürzung des Ventrikels folgt nicht einer einzigen Richtung. Vielmehr ist die Kontraktion vergleichbar mit dem Auswringen eines Waschlappens. Durch die Rotation wird die longitudinale Kontraktion unterstütz. Es ist möglich sowohl die longitudinale als auch die zirkuläre Verkürzung zu beurteilen. Die Dokumentation des Strains wird in Prozent angegeben. Da es sich um eine Verkürzung handelt ist dieser Wert negativ. Die Untersuchung ist bei Weitem Untersucher-unabhängig durchzuführen als die entsprechende Gewebedoppler, welche das Äquivalent in der klassischen Echokardiographie darstellen.

Unter „Speckles“ versteht man Musterungen des Myokards im Ultraschall, die durch unterschiedliche Reflexionen der myokardialen Strukturen entstehen. Das Speckle-Muster eines myokardialen Segmentes wird von einer Software erfasst und gespeichert und dessen Verlagerung während des Herzzyklus mitverfolgt. Dies ermöglicht die genaue Verkürzung einzelner Segmente und des gesamten Ventrikels zu beschreiben.

## 2.4 Definition der zirrhotische Kardiomyopathie

Die kardiale Veränderung im Laufe der Aggravierung der Leberzirrhose ist ein Phänomen, was bereits in den 1980er Jahren beschrieben wurde (Keller et al. 1988). Zu Beginn wurde jedoch nicht zwischen der alkoholbedingten direkten Schädigung des Myokards und der durch die Zirrhose bedingten Veränderungen unterschieden (Houda et al. 1983; Dancy et al. 1985). Ein Punkt, der aber auch bei späteren Veröffentlichungen immer wieder zu Kritik führte.

Auf der World Conference of Gastroenterology, 2005 in Montreal gelang es nun eine Definition zu erarbeiten. Es wurde festgelegt, dass die zirrhotische Kardiomyopathie als eine chronische kardiale Dysfunktion bei Patienten mit Zirrhose zu betrachten ist, welche sich durch eine diastolische oder systolische Dysfunktion äußern kann, oder

durch das Vorhandensein von elektrophysiologischen Anpassungstörungen äußern kann. Der wichtigste Punkt jedoch ist der Ausschluss andere struktureller Herzerkrankungen. Die Liste, welche dabei zu beachten ist, umfasst nicht nur die Ischämie bedingte oder valvuläre Dysfunktionen, sondern auch Bluthochdruck, Diabetes, chronische Anämie oder Medikamente.

Beschrieben ist die zirrhotische Kardiomyopathie unabhängig von der Ätiologie der Leberzirrhose. Das besondere an der zirrhotischen Kardiomyopathie ist, dass es sich oftmals um eine latente Störung handelt. Sie manifestiert sich zwar schon früh zu Beginn der Zirrhose, kommt aber erst im Rahmen von Traumata, Infekten und Operationen zum Tragen und führt zu Dekompensationen und Entwicklung eines ACLF.

### 2.4.1 Das Herz und die Leber

Viele Studien setzen sich mit den strukturellen Veränderungen des Herzens im Rahmen der fortschreitenden Leberzirrhose auseinander. Hierbei zeigte sich eine Zunahme der rechts- und linksventrikulären Myokardmasse und eine Septum Hypertrophie (Ruíz-del-Árbol et al. 2013; Günay et al. 2018; Pozzi et al. 1997; Merli et al. 2017). Autopsie-Studien bei Patienten mit Leberzirrhose konnten zeigen, dass eine Herzhypertrophie, Dilatation des rechten Ventrikels sowie das histologische Bild einer vermehrten Fibrose der Kardiomyozyten vorlag (Wehmeyer et al. 2015). Es ist davon auszugehen, dass diese Veränderungen des Myokards das Strukturelement der zirrhotischen Kardiomyopathie darstellt. Ein wichtiger Aspekt der Diskussion der strukturellen Veränderungen im Rahmen der Leberzirrhose ist, dass man von einer niedrigen Prävalenz von Atherosklerose bei Patienten mit Leberzirrhose ausging. Neuere Studien zeigen jedoch, dass die Koronarsklerose gleich, wenn nicht häufiger zu dokumentieren ist, als bei vergleichbaren Patienten ohne Zirrhose (Wehmeyer et al. 2015; Danielsen et al. 2018). Auch für künftige Studien zur zirrhotischen Kardiomyopathie wird der Ausschluss struktureller Herzerkrankungen daher sorgsam erfolgen müssen.

### 2.4.2 Systolische Dysfunktion

Um die systolische Funktionsstörung zu erkennen, ist es zunächst notwendig Normwerte zu definieren. Als pathologisch wird eine Erniedrigung des enddiastolischen Auswurffraktion kleiner 55% in Ruhe als auch eine fehlende Steigerung unter Belastung über 5% angesehen (Lancellotti et al. 2017). Hierbei ist anzumerken, dass diese Empfehlungen der European Association of Cardiovascular Imaging für alle nicht-ischämischer Herzkrankheit ausgesprochen wurde.

Die Anwendung in der Patientengruppe mit fortgeschrittener Leberzirrhose ist dabei nicht einfach umzusetzen. Oft besteht schon eine medikamentöse Therapie, wenn auch nur mit nicht selektiven Betablockern. Weiterhin ist aufgrund des Zustands der Hyperzirkulation die Nachlast verringert, was zu falsch nicht pathologischen Befund in der konventionellen Echocardiographie führt. Deshalb wird mehr und mehr der Global Longitudinal Strain als Messmethode in Studien eingeführt. Die Richtlinien der European Association of Cardiovascular Imaging definieren einen Global Longitudinal Strain, der eine Verkürzung des Myokards um weniger als –16 % aufweist, als pathologisch. Dies gilt aber wiederum für jeden Patienten und bezieht sich nicht alleinig auf die zirrhotische Kardiomyopathie (Yancy et al. 2013; Lang et al. 2015). Die Empfehlung des zirrhotische Kardiomyopathie-Konsortiums fasst die Kriterien daher wie folgt zusammen: Beträgt bei Patienten mit Zirrhose die enddiastolische Auswurffraktion weniger als 50 % oder der Global Longitudinal Strain ist kleiner 18%, ohne dass eine bekannte Herzerkrankung vorliegt, so sollte die Diagnose einer zirrhotischer Kardiomyopathie gestellt werden.

### 2.4.3 Diastolische Dysfunktion

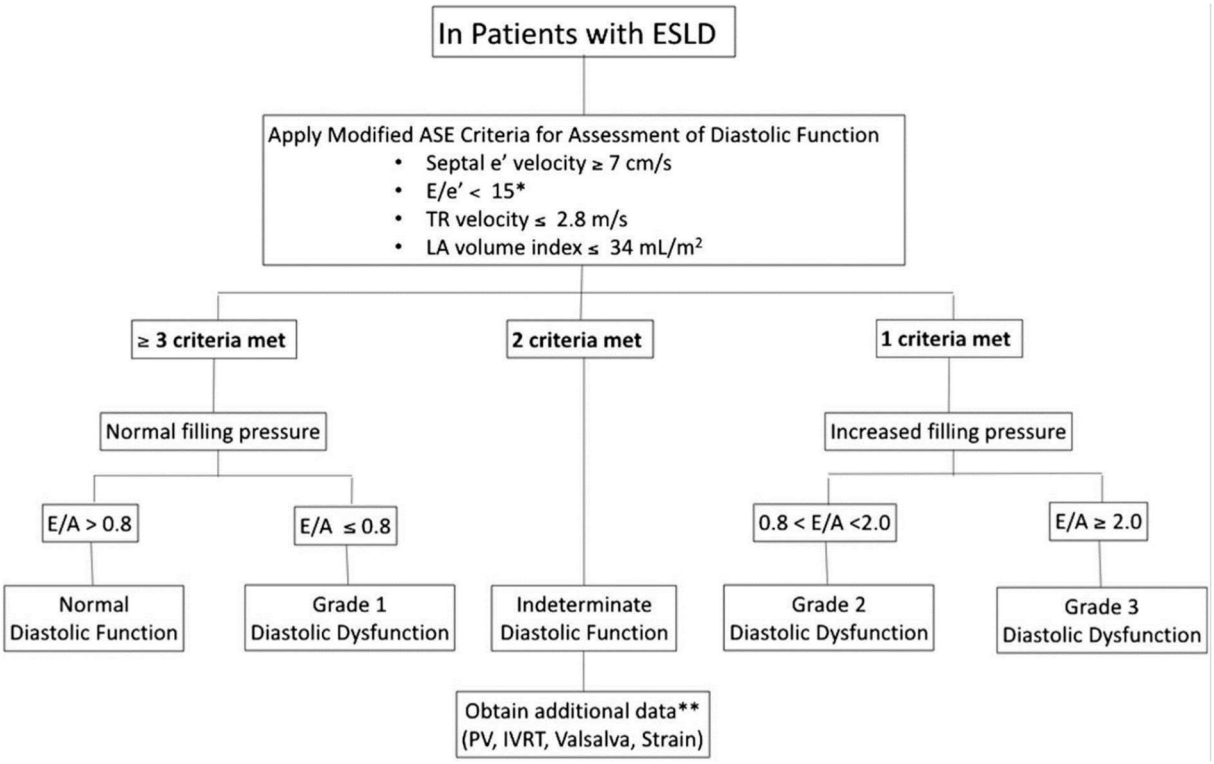
Gemäß den Richtlinien der American Society of Echocardiography aus dem Jahre 2016 ist die diastolische linksventrikuläre Funktion durch einen Algorithmus von vier Kriterien zu überprüfen.

Diese sind:

1. Septale mitralanuläre frühdiastolische Geschwindigkeit (e') durch Gewebedoppler <7 cm/Sekunde oder laterale e'-Geschwindigkeit <10 cm/Sekunde

2. Frühdiastolischer Mitraleinfluss Verhältnis von Geschwindigkeit (E) zu e' > 14 (unter Verwendung von durchschnittlichem e') oder 15 (unter Verwendung von medialem e')
3. Linksatrialer Volumenindex > 34 ml/$m^2$
4. Geschwindigkeit der Trikuspidalinsuffizienz > 2,8 m /Sekunde
   (Nagueh et al. 2016)

Dieser Algorithmus scheint schwierig und soll an dieser Stelle graphisch dargestellt werden.




(Izzy et al. 2020)

Dies ist für den nicht kardiologisch tätigen Arzt keine einfache Aufgabe. Die Empfehlung des Konsortiums für zirrhotische Kardiomyopathie lautet daher, eine interdisziplinäre Versorgung sicherzustellen.

### 2.4.4 Elektrophysiologische Störungen

Im Rahmen der zirrhotischen Kardiomyopathie werden neben verlängerten QT-Intervallen, elektromechanische Dyssynchronie und Chronotropie beobachtet. Es ist beschrieben, dass das QT-Intervall bei 30–50 % der Patienten mit bekannter Leberzirrhose verlängert ist und mit dem Schweregrad der Lebererkrankung, Ausmaß der portalen Hypertonie und Fläche der portosystemischen Shunts zunimmt (Bernardi

et al. 1998; Bernardi et al. 1991; BERNARDI et al. 2012). Ein interessanter Fakt, der die medikamentöse Therapie bei Patienten mit fortgeschrittener Leberzirrhose nicht einfach macht. Aus pathophysiologischer Sicht steht das verlängerte QT-Intervall im Zusammenhang mit einer Zunahme der autonomen Dysfunktion. Studien zeigten, dass nach Transplantation die Verlängerung des QT-Intervalls teilweise reversibel zu sein scheint (Liu et al. 2017; Torregrosa et al. 2005; Adigun et al. 2005). Weiterhin wurde gezeigt, dass die Gabe der nicht selektiven Betablocker das verlängerte QT-Intervall positiv beeinflusst. Ein weiterer wichtiger Aspekt der reduzierten kardiovaskulären Reaktionsfähigkeit erklärt sich in der verringerten Baroreflexempfindlichkeit. Dies führt zu chronotroper Inkompetenz, was das Unvermögen erklärt, die Herzfrequenz unter Belastung zu erhöhen (Møller et al. 2004; Krag et al. 2012; Møller et al. 2012).

### 2.4.5 Biomarker der kardialen Dysfunktion

Der bekannteste und wichtigste Biomarker zur Diagnosestellung einer Herzinsuffizienz sind das B-Typ-natriuretische Peptid und das Abspaltungsprodukt seiner Vorstufe - das NT-proBNP (Maisel et al. 2002). Dieses Hormon wird vom Ventrikelmyokard als Reaktion auf übermäßige Wandspannung ausgeschüttet. Die Ausschüttung von BNP führt zu einer Verschiebung des intravasalen Volumens in den Interzellularraum, sowie zu einer Inhibierung des Sympathikus (Kuwahara et al. 2010; Arjamaa 2014).

Ein weiters, relevantes Peptidhormon ist das Adrenomedullin, welches einen potenten Vasodilatator darstellt. Es wird in vielen Organen exprimiert. Am Herzen wirkt Adrenomedullin kontraktilitätsfördernd (Yu et al. 2001; Nishikimi et al. 1995). Durch seine kurze Halbwertszeit ist Adrenomedullin als Biomarker kaum nutzbar.

Ferner gibt es Fibrose-assoziierte Biomarker. Das Interleukin-1-Rezeptor-ähnliche Protein 1 ist ein Glykoprotein, welches von kardialen Fibroblasten als Reaktion auf Dehnungsreiz ausgeschüttet wird, und ein wichtiger Modulator für das kardiale Remodelling und Fibrosierung darstellt (Sanada et al. 2007).

In den letzten Jahren wurde die Rolle der MicroRNA als Biomarker in vielen Bereichen untersucht. MicroRNAs sind kurze RNA-Stränge, welche die Expression vieler Gene regulieren, aber nicht in Proteine transkribiert werden. Es konnte gezeigt werden, dass die Expression von miR-34a in Tiermodellen bei kardialem Stress erhöht ist (Bernardo

et al. 2014; Huang et al. 2014; Yang et al. 2015; Bernardo et al. 2016; Bernardo et al. 2012; Boon et al. 2013). Auch in klinischen Studien bei Menschen mit Herzinsuffizient konnte eine Erhöhung der miR-34a nachgewiesen werden. Daraus ließ sich ableiten, dass die therapeutische Hemmung von miR-34a einen positiven Effekt auf diverse Herzpathologien zeigt (Thum et al. 2007; Tabuchi et al. 2012).

### 2.4.6 Lebersteifigkeit

Zur Bestimmung der Lebersteifigkeit wird eine ultraschall-basierte Messung genutzt. Diese Messung wird als Elastographie bezeichnet und umfasst mehrere Verfahren. Das Verfahren, welches aufgrund der langen Verfügbarkeit, und der daraus entsprechend resultierenden guten Datenalge in der Klinik am häufigsten genutzt wird, ist die transiente dynamische Elastographie. Bei diesem Verfahren werden durch einen Transducer transthorakale Vibrationen appliziert. Aus der Geschwindigkeit der Scherwelle wird die Lebersteifigkeit abgeleitet und in kPa ausgegeben.

Das Konzept der Shear-Wave Elastographie ähnelt dem Prinzip der transienten dynamische Elastographie. Der Unterschied besteht darin, dass ein Impuls mit viel höherer Intensität gesendet wird. Die Scherwellen entstehen erst am Punkt der Messung. Die Shear-Wave Elastographie hat sich in mehreren Studien als effizientes Tool zur Beurteilung der Leberfibrose erwiesen (Deffieux et al. 2015; Ferraioli et al. 2012; Guibal et al. 2016; Leung et al. 2013; Zeng et al. 2014; Ozturk et al. 2022).

Die Lebersteifigkeit ist bedingt durch die Fibrose der Leber, sowie die Druckverhältnisse, die vor und nach der Leber herrschen. Es konnte nachgewiesen werden, dass bei der Herzinsuffizienz der rechtsseitige Füllungsdruck mit der Steifigkeit korreliert (Taniguchi et al. 2014). In dem untersuchten korrelierte die Steifigkeit aber nur schwach mit der Fibrose (Sugimoto et al. 2020).

### 2.4.7 Zirrhotische Kardiomyopathie und ACLF

Wie bereits beschrieben ist das ACLF mehr als das kumulative Auftreten einzelner Dekompensationen. In den Stadien der kompensierten Zirrhose sind die hämodynamischen Veränderungen moderat. Aber auch in diesem Stadium sind diese zu dokumentieren. Die systemische Vasodilatation führt zu einer Verringerung des

systemischen Gefäßwiderstands und damit zur Absenkung des arteriellen Blutdrucks als Resultat einer Reduktion des effektiven Blutvolumens (Møller und Bendtsen 2018; Fernandez 2015). Im Stadium der kompensierten Leberzirrhose sind die Auswirkungen der Vasodilatation durch die Erhöhung des Herzzeitvolumens auszugleichen.

Im Stadium der dekompensierten Zirrhose kommt es zu einer Vasodilatation, die nicht mehr kompensiert werden kann. Dies führt zu einer zentralen Hypovolämie und arteriellen Hypotonie. Die Aktivierung des sympathischen Systems, des Renin-Angiotensin-Aldosteron- und des Vasopressin-Systems stellen in der hyperdynamischen Kreislaufsituation keine adäquate Gegenregulation dar, da die Erhöhung der Herzfrequenz und Herzleistung nicht möglich ist. Dies führt unter Anderem zu einer Beeinträchtigung der Nierenfunktion. Ob es sich in diesem Fall um ein Hepatorenales- oder sekundär Kardiorenales-Syndrom handelt, ist schwierig zu differenzieren. Es wurde gezeigt, dass im Verlauf ein Anstieg der Zytokine wie TNF, IL-6, IL-8 zu dokumentieren ist (Arroyo 2017; Coenraad et al. 2018). Diese systemische Entzündung als treibende Kraft des ACLF induzieren direkte Zellschäden und Durchblutungsstörungen. Dies wiederum kann im Rahmen der dekompensierten Zirrhose zum Multiorganversagen führen (Turco et al. 2018).

## 2.5 Fragestellung

In den vorangegangenen Abschnitten wurde beschrieben, welche Komplikationen in der Entwicklung der Leberzirrhose zu erwarten sind und welche Auswirkungen sie auf die Morbidität und Mortalität in diesem Patientenkollektiv haben.

Weiterhin wurden mehrere Modelle und Scores vorgestellt, die eine Entscheidungshilfe in der Behandlung dieser Patienten darstellen. Ein besondere Punkt ist die Entwicklung des ACLF. Diese stellt nicht nur die Summe der einzelnen Dekompensationen dar, sondern ist in den letzten Jahren vielmehr als größter Risikofaktor für die Mortalität identifiziert worden. Ein Aspekt des ACLF ist die zirkulatorische Dysfunktion, die auch durch die zirrhotische Kardiomyopathie beeinflusst wird.

Bei der zirrhotische Kardiomyopathie handelt es sich um ein latentes Bild, welches erst im Rahmen der Dekompensation zum Tragen kommt. Zurzeit fehlt eine verlässliche Diagnostik, welche im klinischen Alltag die Identifizierung von Patienten mit hohem Risiko für die Entwicklung eines ACLF auf Grund einer zirrhotische Kardiomyopathie ermöglicht.

In dieser Habilitationsschrift werden nicht-invasive apparative Diagnostiken evaluiert, welche Patienten mit zirrhotischer Kardiomyopathie identifizieren können. Zum einen wurden Herzrhythmusanalysen, welche sich außerhalb der klinischen Routine bewegen gezeigt. In diesen wurden die Herzrhythmusvariabilität und Tubulenz bezüglich der prognostischen Wertigkeit auf das Outcome von Patienten mit Leberzirrhose untersucht. Zum anderen wurde untersucht, inwieweit Herzfunktionsstörungen, insbesondere die Kontraktilität, eine Rolle in der Bewertung der Prognose bei Patienten mit Zirrhose, die sich auf der Liste zu Lebertransplantation befanden, spielen, oder auf Grund von Dekompensationen einen transjugulären intrahepatischen portosystemischen Shunt erhielten. Zusätzlich wurden in diesem Kollektiv Biomarker untersucht, welche im Rahmen der Herzinsuffizienz bereits gut evaluiert wurden. Im Besonderen die geschlechtsspezifische Risikobewertung für Dekompensation auslösende Faktoren wie z. B. eine Blutung, sollte auch untersucht werden. Andere Studien zur kardialen Dekompensation lassen einen entsprechenden Rückschluss zu. Weiterhin wurde die Shear-Wave-Elastographie untersucht, um ein besseres Verständnis der bestimmenden Faktoren: Fibrose, portale Hypertension und

kardiale Stauung als Ausdruck der zirrhotischen Kardiomyopathie besser zu verstehen. Auch die kompensatorische Druckerhöhung des lymphatischen Systems, als weiterer Faktor der Volumenverschiebung wurden betrachtet.

# 3 Ergebnisse

## 3.1 Eine schwere pathologisch veränderte Herzfrequenzturbulenz ist mit einer Verschlechterung der Leberzirrhose verbunden.

**Jansen C***, Al-Kassou B*, Lehmann J, Pohlmann A, Chang J, Praktiknjo M, Nickenig G, Strassburg CP, Schrickel JW, Andrié R, Linhart M, Trebicka J. Severe abnormal Heart Rate Turbulence Onset is associated with deterioration of liver cirrhosis. PLoS One. 2018 Apr 10;13(4):e0195631. doi: 10.1371/journal.pone.0195631. PMID: 29634776; PMCID: PMC5892926.

Zielsetzung der Arbeit - Es sollten kardiale Risikofaktoren bei Patienten mit fortgeschrittener Leberzirrhose ermittelt werden, die in der Standard-24-Stunden Elektrokardiographie nicht erfasst, aber retrospektiv analysiert werden konnten. Die kardiale Dysfunktion bei Zirrhose scheint mit einer autonomen Dysfunktion verbunden zu sein. Die Herzfrequenzturbulenz wurde vor allem bei ischämisch bedingten Kardiomyopathien evaluiert. Ziel war es, die Zunahme der kardialen autonomen Dysfunktion im Verlauf der Leberzirrhose zu dokumentieren.

Methoden und Ergebnisse – In diese Studie wurden Patienten eingeschlossen, bei denen die Diagnose einer Leberzirrhose durch eine Bildgebung und/oder Biopsie gesichert wurde und die ein auswertbares Langzeit-Elektrokardiogramm erhalten hatten. Weiterhin mussten in den Aufzeichnungen mindestens drei vorzeitige ventrikuläre Kontraktionen auswertbar sein. Ausgeschlossen wurden Patienten, die strukturelle Herzerkrankungen oder kardiale Arrhythmien aufwiesen. Der Endpunkt wurde definiert als eine Verschlechterung der Leberzirrhose oder eine erneute Aufnahme zur stationären Behandlung bei Zeichen einer Dekompensation (z. B. hepatische Enzephalopathie, neu diagnostizierter Aszites, Varizenblutung). Es wurden konsekutive 82 Patienten mit einer Zirrhose identifiziert, die die Ein- und Ausschlusskriterien erfüllten. Alle Patienten erhielten ein 12-Kanal-Ruhe-EKG und unterzogen sich einer umfassenden 2D-transthorakalen Untersuchung. Das mediane Patientenalter betrug 61 Jahre (19 bis 89) und 52 Patienten waren Männer. Der mediane Child-Score war 6 (5 bis 12), der mediane MELD war 10 (6 bis 32). 20

Patienten präsentierten große Ösophagusvarizen, 21 Patienten präsentierten Aszites und 36 der Patienten nahmen Betablocker ein.

Schlussfolgerungen – Wir konnten zeigen, dass neben Geschlecht und Alter die QRS-Dauer und die Herzfrequenzturbulenz signifikant assoziiert sind mit Dekompensation und Verschlechterung der Lebererkrankung innerhalb von 6 Monaten. Leider konnte die Herzfrequenzturbulenz in der multivariaten Cox-Regressionsanalyse nicht als unabhängiger Faktor identifiziert werden.

PLOS ONE

RESEARCH ARTICLE

# Severe abnormal Heart Rate Turbulence Onset is associated with deterioration of liver cirrhosis

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**Citation:** Jansen C, Al-Kassou B, Lehmann J, Pohlmann A, Chang J, Praktiknjo M, et al. (2018) Severe abnormal Heart Rate Turbulence Onset is associated with deterioration of liver cirrhosis. PLoS ONE 13(4): e0195631. https://doi.org/10.1371/journal.pone.0195631

**Editor:** Pavel Strnad, Medizinische Fakultat der RWTH Aachen, GERMANY

**Received:** January 15, 2018

**Accepted:** March 25, 2018

**Published:** April 10, 2018



**Data Availability Statement:** The Ethikkommission der Medizinischen Fakultät der Universität Bonn (Ethics Committee of the Medical Faculty of the University of Bonn) has imposed ethical restrictions on making the data underlying this study publicly available because the data contains potentially sensitive information that identifies the patients. Due to these restrictions, it is not possible to provide a de-identified data set. Researchers can request access to this data from the University of Bonn Ethics Committee at Ethik@uni-bonn.de or from the corresponding author.

## Abstract

### Background

In patients with liver cirrhosis, cardiac dysfunction is frequent and is associated with increased morbidity and mortality. Cardiac dysfunction in cirrhosis seems to be linked to autonomic dysfunction. This study investigates the role of autonomic dysfunction assessed by Heart Rate Turbulence (HRT) analyses in patients with liver cirrhosis.

### Methods and patients

Inclusion criteria was (1) diagnosis of cirrhosis by clinical, imaging or biopsy and (2) evaluation by standard 12-lead-ECG and 24h holter monitoring and (3) at least 3 premature ventricular contractions. The exclusion criterion was presence of cardiac diseases, independent of liver cirrhosis. Biochemical parameters were analysed using standard methods. HRT was assessed using Turbulence onset (TO) and slope (TS). The endpoint was deterioration of liver cirrhosis defined as increased MELD and readmission for complications of liver cirrhosis.

### Results

Out of 122 cirrhotic patients, 82 patients (63% male) with median Child score of 6 (range 5–12) and median MELD score of 10 (range 6–32) were included. Increasing Child score, INR and decreasing albumin were correlated with TO. In addition, decompensated patients with ascites showed more abnormal TO and TS. During the observation period, patients with more abnormal TO showed significantly higher rate of rising MELD Score at 6 months ($p = 0.03$). Nevertheless, at least in our collective HRT-parameters were not independent predictors of deterioration of cirrhosis.

**Funding:** The authors were supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57) and Cellex Foundation. The funders had no influence on study design, data collection and analysis, decision to publish or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

**Abbreviations:** AD, autonomic dysfunction; CRP, C-reactive protein; Child score, Child-Turcotte-Pugh-score; ECG, electrocardiogram; Hb, Hemoglobin; HE, hepatic encephalopathy; HRS, hepatorenal syndrome; HRT, heart rate turbulence; HRV, heart rate variability; INR, International Normalized Ratio; MELD, Model for End-stage Liver Disease; PVC, premature ventricular contraction; QTc, QTc interval; TO, turbulence onset; TS, turbulence slope.

## Conclusion

Parameters of HRT are closely associated with deterioration of cirrhosis and might be helpful in its prediction.

## Introduction

In patients with liver cirrhosis, autonomic dysfunction (AD) is a frequent finding. Its presence aggravates morbidity and mortality and might play an important role in outcome [1]. AD comprises a broad spectrum of clinical signs and can be assessed by a variety of parameters such as heart rate or blood pressure changes after different maneuvers like Valsalva maneuver or isometric exercise [2]. Heart rate variability (HRV), QT-interval and other parameters have been extensively investigated in liver disease. HRT is a relatively novel HRV measure that describes baroreflex-mediated variations in sinus cycle length after the loss of cardiac output due to a premature ventricular contraction (PVC)[3]. HRT is characterized by two parameters, turbulence onset (TO) and slope (TS), that further describe the intensity of heart rate variation after a PVC. HRT is usually assessed from Holter recordings over at least 24 h[4]. HRT is a useful approach to assess AD [5]. It was initially evaluated for risk stratification in cardiac disease, especially in post-infarct patients. Since then, measures of HRT have been extensively tested in cardiac autonomic dysfunction and have been shown to be associated with prognosis of patients with cardiac diseases [6].

HRT in cirrhosis was described only in one case series of 18 patients and compared to healthy individuals [7]. This series demonstrated that HRT is abnormal in cirrhosis, but its role in the progression of cirrhosis is unclear. Nevertheless the clinical meaning of rate turbulence (HRT) has not been comprehensively investigated in cirrhosis to date.

Our study aimed to evaluate the association of HRT with severity and course of chronic liver disease.

## Patients and methods

### Study design

Inclusion criteria were (1) diagnosis of liver cirrhosis by unequivocal clinical signs, imaging or biopsy and (2) evaluation by 24h-holter monitoring and (3) at least 3 premature ventricular contractions. The exclusion criterion was presence of structural cardiac diseases or cardiac arrhythmias. The composite endpoint was deterioration of liver cirrhosis defined as increased MELD of more than 2 points or readmission in the hospital for complications of liver cirrhosis (e.g. hepatic encephalopathy, newly diagnosed ascites, variceal bleeding, etc.).

### Patients recruitment

122 consecutive cirrhotic patients, who were admitted for treatment of complications of liver cirrhosis at the Department of Internal Medicine I at the University Hospital Bonn, Germany, were screened in this study. Patients with permanent atrial fibrillation (n = 4), artificial cardiac pacemaker (n = 3), structural heart disease (n = 21) or less than 3 premature ventricular contractions (n = 12) were excluded. Finally, 82 patients (52 male) were included in the study and further analyzed. The study was approved by the local Ethics Committee of the University Clinic Bonn (No. 121/14). The patients signed a written inform consent for the procedures in the study. Baseline clinical and biochemical parameters were recorded.

All patients received a 12-lead resting ECG and underwent a comprehensive 2D-transthoracic echocardiography (Philips iE 33 ultrasound system, Amsterdam, The Netherlands). In

addition all patients underwent 24h holter ECG monitoring at baseline. For rate correction of QT interval the Bazett formula was applied[8].

The visits of patients were scheduled every 3 months, and the patients were followed for a median of 9 months. During the follow-up, development of CHILD and MELD Score, hepatorenal syndrome, SBP, other infection and death were recorded.

### Holter ECG

All patients underwent 24-h holter monitoring during hospitalization. The holter ECG recordings were archived with the SpiderView TM device (Ela medical Inc., CO, U.S.A.) Mean recording duration was 24 hours. Sample rate was 1000Hz. Standard holter ECG parameters were recorded including number of ventricular premature beat.

### Heart rate turbulence evaluation

All holter tracings were manually reviewed and artifacts were discarded by an operator blinded to the clinical data using the SyneScope$^{TM}$ Version 3.10 software (Ela medical Inc., CO, U.S.A.).

For calculation of heart rate turbulence, only patients that had at least 3 premature ventricular contractions (PVC) were included. The two parameters of HRT, i.e. turbulence onset (TO) and slope (TS), were calculated according to established protocols [9]. Three R-R intervals before and up to 21 intervals after each PVC were analyzed to calculate the sinus cycle length (mean of $R_{-3}R_{-2}$ and $R_{-2}R_{-1}$), the coupling interval of the PVC, the compensatory pause (first R-R after the PVC) and TO as well as TS. Turbulence onset was defined as the quotient of $((RR_1 + RR_2)-(RR_{-2} + RR_{-1}))$ and $(RR_{-2} + RR_{-1})$ multiplied by 100 (%). Turbulence slope was defined as the maximum positive regression slope assessed over any five consecutive sinus rhythm R-R intervals within the first 15 sinus rhythm R-R intervals after the PVC. To date, there are no established thresholds for differentiation between normal and pathologic values for TO and TS. Therefore, for analysis of association with outcomes, we created two patient cohorts, one with TO values below, one above the TO median of all patients. The analyzes of TO and TS have been shown to be reproducible[9–14], and in our study were performed blinded from the clinical data.

### Statistical analysis

Continuous variables are expressed as the median and range. Non-parametric Wilcoxon and Kruskal-Wallis-Test were used for unpaired comparisons. Correlations were analysed with the Spearman correlation coefficient. Kaplan-Meier curves were used to display deterioration of MELD Score in follow-up as assessed by the Log-rank test. Univariate and Cox regression multivariate analysis (forward step-wise likelihood quotient) was performed to predict decompensation rates. P-values $<0.05$ were considered statistically significant. Statistical analysis was performed by means of SPSS 22 for Windows (SPSS Inc. Chicago, IL, USA).

## Results

### General characteristics

82 patients were analysed in this study. The median patient age was 61 years (range 19 to 89) and 52 patients were men. The median Child score was 6 (range 5–12), the median MELD was 10 (range 6–32). 20 patients presented large oesophageal varices, 21 patients presented ascites and 36 of the patients were taking beta-blockers. Biochemical parameters were assessed during the study using standard methods and are displayed in Table 1.

## 12-channel-resting ECG

The median P duration was 90 ms (range 50–115), the median PQ interval was 135 ms (range 80–210), the median QRS duration was 100 ms (range 70–125) and the median QT interval was 390 ms (range 285–495). 41 patients (50%) had a QTc interval longer than 430 ms (range 321–519). QTc interval did not differ between Child classes. Patients with ascites had a significantly longer QTc interval than those without ascites ((median 469 ms (range 321–519) vs. 426 ms (range 348–515) resp; p = 0.011)). Furthermore, patients with history of bleeding had significant higher QTc intervals than patients without (462 ms (range 389–515) vs. 422 ms (range 321–519), p = 0.001).

**Table 1. Baseline parameters of the patients (n = 82).**

| Parameters | Values |
|---|---|
| **General characteristics** | |
| gender [female / male] | 30 /52 |
| age [years] | 61 (19–89) |
| etiology [alcohol / viral / other] | 52 / 20 / 10 |
| Child score | 6 (5–12) |
| MELD score | 10 (6–32) |
| oesophageal varices [absent / small / large] | 44/18/20 |
| ascites [absent / mild / severe] | 61/13/8 |
| beta-blocker treatment [yes / no] | 36/46 |
| **Laboratory values** | |
| sodium [mmol/L] | 140 (115–150) |
| potassium [mmol/L] | 4.15 (2.53–5.83) |
| serum creatinine [mg/dL] | 1.04 (0.6–7.89) |
| blood urea nitrogen [mg/dL] | 38.5 (13–207) |
| bilirubin [mg/dL] | 0.82 (0.18–27.62) |
| aspartate aminotransferase [U/L] | 29 (6–1208) |
| alkaline phosphatase [U/L] | 38 (8–424) |
| CRP [mg/L] | 6.9 (0.4–152) |
| albumin [g/L] | 35 (3.9–50) |
| INR | 1.1 (0.8–2.4) |
| total white blood cell count [G/L] | 5.75 (1.53–25.4) |
| haemoglobin [g/dL] | 11.6 (6.9–16.4) |
| haematocrit [%] | 34 (20–47) |
| platelet count [G/L] | 147 (22–737) |
| **ECG-Parameters** | |
| P duration [ms] | 90 (50–115) |
| PQ interval [ms] | 135 (80–210) |
| QRS duration [ms] | 100 (70–125) |
| QTc interval [ms] | 430 (321–519) |
| **Transthoracic echocardiography** | |
| LV end diastolic volume [mL] | 99.8 (41.4–213.7) |
| LV end systolic volume [mL] | 36.15 (12.5–101) |
| LV ejection fraction [%] | 60.35 (45–77.5) |

MELD, model for end-stage liver disease; CRP, C-reactive protein; INR, International Normalized Ratio; Hb, Hemoglobin; ECG, electrocardiogram; LV, left ventricular

https://doi.org/10.1371/journal.pone.0195631.t001

## Relationship between HRT, patient characteristics, severity and complications of liver cirrhosis

TO correlated significantly with CHILD score (rs = 0.513; p<0.001; Table 2), albumin (rs = -0.343; p = 0.016; Table 2), hemoglobin (rs = -0.395; p = 0.005; Table 2) and haematocrit (rs = -0.435; p = 0.002). Heart rate turbulence parameters TO and TS were significantly deteriorating (i.e. increase of TO and decrease of TS) with increasing severity of liver disease as indicated by increasing Child Class (Fig 1A and 1B). In addition, TO and TS were more pathological in patients with ascites (Fig 1C and 1D). Importantly, no association of HRT parameters was observed with etiology and the intake of non-selective beta-blockers.

When analysing HRT-parameters TO and TS with respect to deterioration of liver disease, severe abnormalities in TO as defined as values above the median TO were associated with higher probability of deterioration of liver disease, as shown by the Kaplan-Meier plot (Fig 2A). The graph demonstrates that more severe abnormalities in TO were significantly associated with deterioration of liver disease (Fig 2A) and showed a trend towards increased number of decompensating events within 6 months (Fig 2B).

Decompensations were developed in 7 patients in the first 6 months, 11 in the first year and 12 in the entire observation period. These were hepatic encephalopathy in 4 patients, newly diagnosed ascites in 2 patients, variceal bleeding in 3 patients, and hepatorenal syndrome in 3 patients. Moreover, we defined a MELD worsening that the baseline increased by more than 2 points. All patients with decompensation showed an increase of the MELD-score of more than 2 points. In total, there were 12 patients in the first 6 months, 23 in the first year and 40 in the entire observation period who met this composite endpoint. The patients who showed an increase of MELD more than 2 points, had at baseline a median MELD of 9 (6–22) and in the follow up a median MELD of 13 (9–31). By contrast, the patients with unchanged or decreasing MELD showed at baseline a median MELD of 13 (7–32) and in the follow up a median MELD of 9 (6–21).

Univariate time-to-event analysis was performed to evaluate the association of TO and other factors with decompensation and deterioration of liver disease after 6 months (Table 3). Besides gender and age, also total white blood count, QRS-duration and TO were significantly associated with decompensation and deterioration of liver disease within 6 months (Table 3). Nevertheless, multivariate cox-regression analysis did not reveal any significantly and independently associated factor with decompensation and deterioration of liver disease at 6 months (data not shown).

There was no significant association between any HRV parameter and overall survival (data not shown). This is might be explained by the fact that only 9 patients died during the observation period.

## Discussion

This study demonstrated a relationship between the HRT and deterioration of liver disease in the short-term follow up.

**Table 2. Correlation of heart rate turbulence (TO) with Child-score and biochemical parameters.**

| | Spearman correlation coefficient | p |
|---|---|---|
| Child score | 0.513 | p<0.001 |
| albumin | -0.343 | 0.016 |
| hemoglobin | -0.395 | 0.005 |
| hematocrit | -0.435 | 0.002 |

https://doi.org/10.1371/journal.pone.0195631.t002

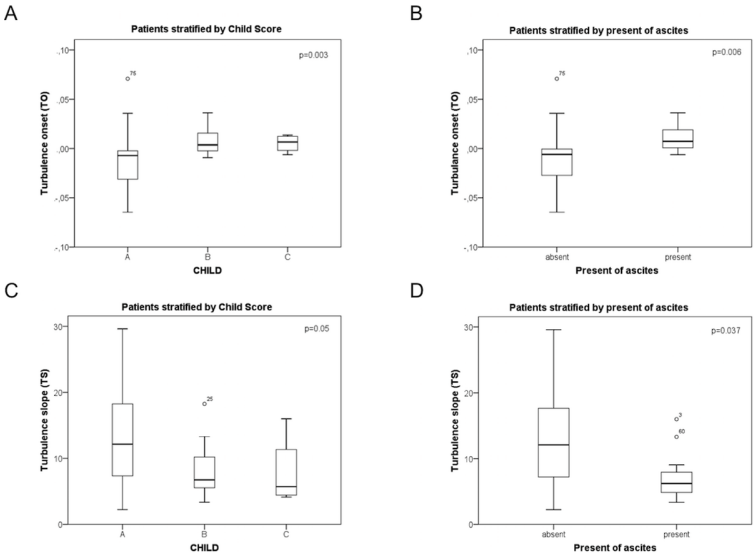


**Fig 1. Turbulence onset and turbulence slope differ with respect to the Child score and complications of liver cirrhosis.** Turbulence onset was stratified for Child score (A) and presence of ascites (B), as well as turbulence slope (C, D). Data were shown using boxplots and were analysed by Kruskal-Wallis test. Of note, data of two patients lay outside of the shown range, and were not shown to increase readability.

https://doi.org/10.1371/journal.pone.0195631.g001

HRV was the first non-invasive method to evaluate autonomic modulation of the sinus node in patients with different cardiac and non-cardiac diseases and to identify patients at risk for an increased cardiac mortality [15] and also has been extensively studied in liver cirrhosis [1, 16–20]. However, the role of HRT, a relatively novel approach to assessment of HRV, has not yet been explored in the outcome of patients with liver cirrhosis. To best of our knowledge,

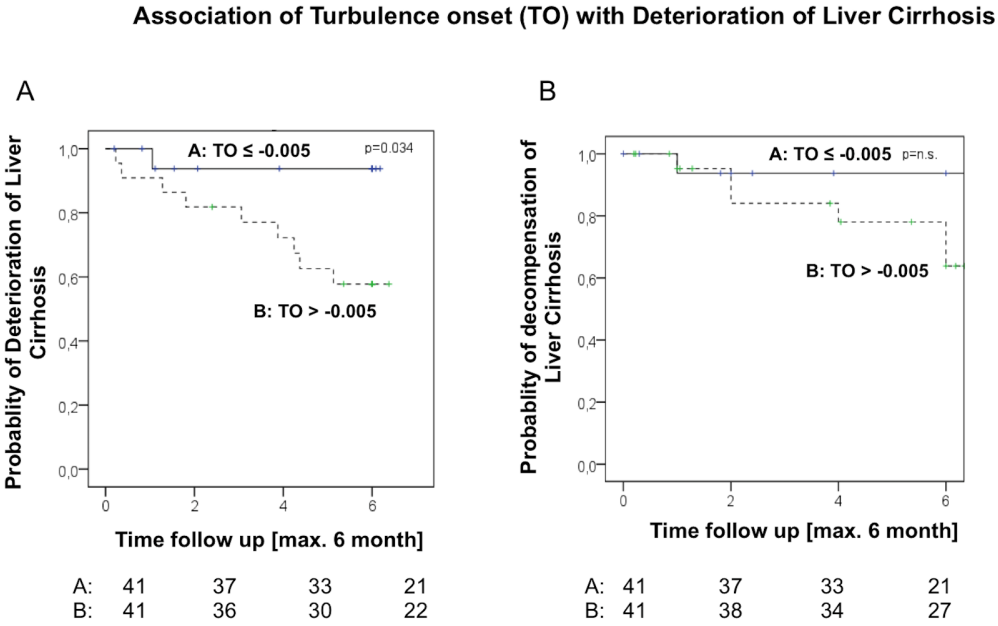


**Fig 2. Deterioration of hepatic disease (A) and the decompensation episodes (B) during follow-up stratified using the median of TO-values.** Rates are shown using Kaplan-Meier plots and analysed by log-rank test.

https://doi.org/10.1371/journal.pone.0195631.g002

**Table 3. Univariate time-to-event analysis (forward step-wise likelihood quotient) was performed to predict deterioration or decompensation in 6 months.**

| Deterioration or decompensation in 6 months | p-value | HR | 95% confidence interval | |
|---|---|---|---|---|
| **General characteristics** | | | | |
| gender | .035 | .266 | .078 | .909 |
| age | .019 | 1.070 | 1.011 | 1.132 |
| **Laboratory values** | | | | |
| total white blood cell count | .008 | 1.135 | 1.034 | 1.246 |
| **ECG** | | | | |
| QRS duration | .045 | 1.063 | 1.001 | 1.129 |
| **HRT** | | | | |
| TO | .032 | 7.400 | 4.700 | 11.600 |

https://doi.org/10.1371/journal.pone.0195631.t003

the only description of HRT in cirrhosis, is a small series of 18 patients, that demonstrated that HRT was abnormal when compared to healthy controls [7]. Our study is the first to demonstrate the impact as predictor in patients with liver cirrhosis.

HRV mainly reflects a long-term interaction between neural modulatory mechanism and heart frequency [15]. Briefly, vagal inhibition and sympathetic activation lead to an initial acceleration and thereby alter TO. This probably arises from an inhibition of baroreflex afferent input due to a hemodynamically inefficient ventricular contraction. Then, after the compensatory pause, the increased ventricular filling is responsible for the subsequent deceleration of heart rate [15]. These effects might be involved in the downregulation of myocardial beta-adrenoceptor density, which is has been described in patients with cirrhosis [21, 22]. The present study did not find any association of non-selective beta-blocker (NSBB) intake with HRT parameters, which suggest that HRT might influence beta-receptor density, vice-versa NSBB do not interact with HRT in cirrhotic patients.

The relationship between autonomic dysfunction and development of central hypovolemia is well established in cirrhosis [1]. Especially decompensated patients with ascites show central hypovolemia [1], which might prevent the above mentioned deceleration and therefore impairs HRT. The present study demonstrates the association of HRT parameters with presence of ascites, and substantiates these argumentations. Moreover, low albumin levels induce lower oncotic pressure and also are associated with central hypovolemia[23]. Indeed, there was a clear inverse correlation between albumin levels and TO, which again confirms this pathophysiological concept.

Limitations of this study are that the patients were majorly compensated with a low risk of decompensation and death and therefore lower rate of events, and that the response to stress was not tested to determine cirrhotic cardiomyopathy. The majority of the patients were followed in the out-patient clinic and therefore were rather stable patients. In these patients, HRT was correlated with hemoglobin level, which is a factor determining development of decompensation and acute-on-chronic liver failure in out-patients as recently described[24]. Even though ACLF patients are very severely ill patients, a recently published study demonstrated that these two parameters in stable out-patients determine the development of ACLF [24]. Our study confirms in this relatively compensated cohort of patients, that these two parameters are suitable to identify the patients with deterioration of disease. Moreover, similarly to the acutely decompensated patients in the CANONIC study, white blood count was a factor associated with deterioration of liver disease[25] in our study. These facts underline that the data collected in our study are in line with previously published data. Moreover, our findings support the association of HRT with deterioration of liver disease at least in the short-term follow up. Still our study was unable to show an independent role of HRT in deterioration of liver disease,

but offers HRT as a marker associated with deterioration of cirrhosis. It seems that in our study TO seem to be more important than TS, although no pathophysiological explanation can be offered for this finding.

In conclusion, cirrhosis revealed autonomic and cardiac dysfunction and abnormal cardiac distribution of sympathetic activity. Parameters of HRT indicate presence of abnormal sympathetic activity in patients with cirrhosis. Especially TO correlated with the degree and the complications of liver cirrhosis and might be useful to might predict outcome.

## Author Contributions

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**Writing – review & editing:** Christian Jansen, Jennifer Lehmann, Alessandra Pohlmann, Johannes Chang, Michael Praktiknjo, René Andrié, Markus Linhart, Jonel Trebicka.

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3.2 Eine signifikante Verringerung der Herzfrequenzvariabilität ist ein Merkmal der akuten Dekompensation der Zirrhose und gibt Auskunft über die 90-Tage-Mortalität.

**Jansen C**, Chatterjee DA, Thomsen KL, Al-Kassou B, Sawhney R, Jones H, Gallego-Leon A, Lehmann J, Pohlmann A, Nickenig G, Strassburg CP, Andrié R, Jalan R, Linhart M, Trebicka J, Mookerjee RP. Significant reduction in heart rate variability is a feature of acute decompensation of cirrhosis and predicts 90-day mortality. Aliment Pharmacol Ther. 2019 Sep;50(5):568-579. doi: 10.1111/apt.15365. Epub 2019 Jul 8. PMID: 31286545

Zielsetzung der Arbeit – Es sollte untersucht werden, inwieweit Veränderung der Herzfrequenzvariabilität bei Patienten mit Leberzirrhose im Verlauf der Erkrankung vorliegen. Weiterhin sollte untersucht werden, ob das Maß der Veränderung der Herzfrequenzvariabilität als ein Prädiktor für die Entwicklung eines akut-auf-chronischen Leberversagen (ACLF) dienen könnte.

Methoden und Ergebnisse – Es wurden 111 Patienten mit dem Risiko einer Zirrhose bedingten Dekompensation aus zwei klinischen Standorten bezüglich der Herzfrequenzvariabilität untersucht. Die Standardabweichung aller normalen Herzschlagintervalle, welche die Herzfrequenzvariabilität widerspiegeln, wurde mittels Fernüberwachung (Isansys Lifetouch) oder Langzeit-EKG-Aufzeichnung dokumentiert. Hierbei zeigte sich, dass eine reduzierte Herzfrequenzvariabilität mit der Wahrscheinlichkeit einer Dekompensation im Beobachtungszeitraum korrelierte. Darüber hinaus war die Herzfrequenzvariabilität signifikant bei den Patienten erniedrigt, bei denen sich ein ACLF zeigte. Eine Standardabweichung von weniger als 13,25 ms hatte einen 98 % negativen Vorhersagewert für das Auftreten eines ACLF.

Schlussfolgerungen – Eine pathologisch veränderte Herzfrequenzvariabilität kann Dekompensation und das Auftreten eines ACLF vorhersagen. Weiterhin lieferte diese Studie wichtige Erkenntnisse in Bezug auf das Verständnis der zirrhotischen Kardiomyopathie.

Received: 20 January 2019 | First decision: 18 February 2019 | Accepted: 17 May 2019

DOI: 10.1111/apt.15365

AP&T Alimentary Pharmacology & Therapeutics WILEY

# Significant reduction in heart rate variability is a feature of acute decompensation of cirrhosis and predicts 90-day mortality

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**Funding information**
This study was supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18), Cellex-Foundation and UCL Business award. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

## Summary

**Background:** Heart rate variability (HRV) is reduced in cirrhosis and in conditions of systemic inflammation. Whether HRV is associated with cirrhosis decompensation and development of acute-on-chronic liver failure (ACLF) is unknown.

**Aims:** To (a) validate wireless remote HRV monitoring in cirrhosis decompensation; (b) determine if severely reduced HRV is a surrogate for inflammation and progression of cirrhosis decompensation; (c) assess if measuring HRV determines prognosis in cirrhosis decompensation.

**Methods:** One hundred and eleven patients at risk of cirrhosis decompensation at two clinical sites were monitored for HRV. Standard deviation of all normal beat-beat intervals (SDNN) reflecting HRV was assessed using remote monitoring (Isansys Lifetouch) and/or Holter ECG recording. Clinical outcomes and major prognostic scores were recorded during 90-day follow-up.

**Results:** Reduced HRV denoted by lower baseline SDNN, correlated with severity of decompensation (median 14 (IQR 11-23) vs 33 (25-42); $P < 0.001$, decompensated patients vs stable outpatient cirrhosis). Furthermore, SDNN was significantly lower in patients developing ACLF compared to those with only decompensation (median 10 (IQR9-12) vs 16 (11-24); $P = 0.02$), and correlated inversely with MELD and Child-Pugh scores, and C-reactive protein (all $P < 0.0001$) and white cell count ($P < 0.001$). SDNN predicted disease progression on repeat measures and appeared an independent predictor of 90-day mortality (12 patients). An SDNN cut-off of 13.25 ms had a 98% negative predictive value.

Christian Jansen, Devnandan Amor Chatterjee, Karen Louise Thomsen, Baravan Al-Kassou, Jonel Trebicka and Rajeshwar P. Mookerjee contributed equally to this work.

The Handling Editor for this article was Professor Gideon Hirschfield, and it was accepted for publication after full peer-review.

Correction added on 19 July 2019, after first publication: The second author's name was previously misspelled and has been corrected to Devnandan Amor Chatterjee in this version.

**Conclusions:** This study demonstrates that remote wireless HRV monitoring identifies cirrhosis patients at high risk of developing ACLF and death, and suggests such monitoring might guide the need for early intervention in such patients. Clinical Trial number: NIHR clinical research network CPMS ID 4949.

## 1 | INTRODUCTION

Under normal physiological conditions, cardiac responses to physical activity and stress (increased cardiac output) are regulated largely by the autonomic nervous system. The interplay between sympathetic and parasympathetic autonomic nervous system activity is reflected in heart rate variability (HRV), the variation in normal heart beat-beat intervals, with greater parasympathetic modulation promoting increased HRV in a linear manner.[1] Loss of normal HRV is a feature of systemic inflammation and is associated with increased morbidity and mortality, particularly in conditions such as after cardiac ischaemia and in diabetes mellitus.[2,3]

Patients with cirrhosis are known to have numerous cardiac abnormalities including prolongation of the QTc interval[4,5] and altered baroreceptor responses.[6] Moreover, a reduction in HRV has been described in patients with cirrhosis and it has been suggested that a further loss occurs with increasing severity of cirrhosis, and in the presence of hepatic encephalopathy.[7,8] However, such observations have been largely based in stable cirrhosis outpatients with limited 5-minute electrocardiography (ECG) assessments. The change in HRV with acute decompensation of cirrhosis and development of acute-on-chronic liver failure (ACLF) and the prognostic significance of any such changes are unclear. Assessment of HRV in dynamic situations like cirrhosis, in which acute alterations in physiology and inflammation modify day-to-day changes in HRV, ideally requires continuous monitoring. The Isansys Lifetouch system (Isansys Limited, Oxford, UK) enables continuous, wireless collection of heart rate and cardiac performance data, sampling at a rate of 1000 Hz, using a simple non-invasive skin surface sensor, which attaches to the precordium and communicates with an accompanying wireless gateway. The heart beat-beat intervals recorded by the device can then be calculated through automatic QRS identification and extracted from the gateway following the recording period.

We hypothesized that in patients with cirrhosis hospitalized for acute decompensation (AD), the development of ACLF will be associated with a further reduction in HRV, reflecting the degree of hepatic decompensation and level of inflammatory response. This study aimed to validate wireless remote monitoring of HRV in AD patients and establish whether HRV measurement is a surrogate for progression and inflammation, and if its measurement can determine prognosis in AD.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and patient selection

Patients with an established diagnosis of cirrhosis based on clinical, ultrasound and/or laboratory/histological criteria of cirrhosis were included at two sites; the Liver and Transplantation Unit at the Royal Free Hospital (RFH), London, UK and the Department of Internal Medicine I, University of Bonn, Germany.

#### 2.1.1 | London patients

The study was approved by the local governing Research Ethics Committee (London – Harrow; REC Ref: 08/H0714/8) and all patients (or a family member if without capacity) provided informed consent in accordance with the 1975 Helsinki Declaration. Patients were recruited prospectively between March 2013 and July 2015 at the RFH, as part of an ongoing biomarker study of cirrhosis decompensation. This study screened patients presenting with acute decompensation of cirrhosis including those presenting with hepatic encephalopathy, infection (SBP and other), new onset jaundice, variceal bleeding, new renal impairment and increasing ascites. After a period of 48 hours during which the patient would receive specific treatment for their precipitating event, if there were no signs of clinical or biochemical improvement, and the patient had no study exclusion criteria and was deemed a candidate for intensive care support for organ failure should they require it, the patient was included into the parent biomarker study. Key exclusion criteria included: admission for reasons other than acute decompensation of cirrhosis (other co-morbid diseases, especially established cardiovascular or intrinsic renal disease); malignancy (extra-hepatic or a hepatocellular carcinoma); patients who had undergone major surgery or had unresolved surgical problems; pregnancy.

Only patients fulfilling the above criteria and willing to also undergo monitoring, were approached for the HRV sub-study protocol. In addition, patients were excluded from the HRV sub-study study if they had significant cardiac rhythm abnormalities including atrial fibrillation or/and cardiac pacemakers, or were taking negative chronotropic medications such as beta-blockers, adrenergic receptor blocking agents or anti-arrhythmics. Patients were recruited sequentially based on availability of the Isansys Gateways for monitoring.

#### 2.1.2 | Bonn patients

These comprised patients that were clinically stable, ambulant, outpatients, with either controlled ascites or previously treated decompensation events but who were now in a stable clinical state, to be maintained in the community. They were prospectively recruited as part of an observational study on cardiac function from February 2014 to January 2015, including Holter examination. The local ethics committee of the University of Bonn approved the study (No.

121/14). The main exclusion criteria were as per the patients from London. These patients provided a comparable stable outpatient cohort who were at risk of decompensation, to draw comparisons with those hospitalised for acute decompensation, and to determine if measurement of HRV could predict risk of poor outcome even in outpatients.

### 2.1.3 | Follow-up of the patients

All patients had clinical, demographic and laboratory parameters recorded at baseline with further serial measures during their period of follow-up till 90 days. All patients underwent a baseline ECG before initiating continuous HRV assessment as described below.

The Chronic Liver Failure (CLiF) Consortium criteria were used to define the development of ACLF during admission in AD patients.[9] Previously validated scores including Child-Pugh (CP) classification, Model for End Stage Liver Disease (MELD) and the CLiF Consortium Acute Decompensation (CLIF-C AD) score[9,10] were also applied to assess disease severity. During the follow-up, evolution of MELD score and development of organ failure, ACLF and death were recorded.

All patients underwent routine clinical care with management according to local hospital guidelines and directed by the treating clinicians. This included intensive care unit and specific organ support, such as renal filtration, when indicated in patients with acute decompensation. Such patients were observed until hospital discharge, with further follow-up data collected to 90 days.

## 2.2 | Heart rate variability evaluation

An initial pilot study was performed on 20 healthy adults without liver disease, and on no concomitant medication, nor with a significant past medical history, to test the feasibility of wireless remote monitoring of HRV using the Isansys Lifetouch system (Isansys Limited, Oxford, UK). Using systems integrated into the LifeTouch gateway and Kubios HRV Version 2.0 (Kubios Oy, Kuopio, Finland), three separate, 5 minutes, artefact-free, continuous R-R interval sessions, during the waking hours of 8AM and 7PM, were selected for analysis using Kubios HRV Version 2.0.[11] These sessions were used to establish standard deviation of all beat-to-beat (NN) intervals (SDNN), for a given subject for that corresponding day. Artefacts were identified through assessment of RR interval data, graphically and computationally represented in Kubios, and denoted if recordings deviated greatly from the average baseline RR interval during the selected time period.

Having demonstrated feasibility of measurement and reproducibility of HRV data with the Lifetouch system in controls, a further assessment was performed on the first 13 cirrhosis in-patients studied at the Royal Free site, to establish if there was good concordance between remote device measurement and standard ECG/Holter R-R interval recording. Once proven, all subsequent HRV data upon hospitalization were evaluated using the Isansys Lifetouch system through generation of SDNN values, as described above. In some patients, repeat measurements of HRV were availed during decompensation or development of ACLF, by leaving a device on the patient for up to 5 days. For stable outpatients, SDNN was derived from Holter devices at the time of review.

One individual (DAC) performed all the SDNN analysis and was blind to individual patient data and outcomes, and adopted the same filtering techniques for artefact and time window analysis across patients from both sites, for consistency. As we wished to assess the impact of ambulant patients and "real-life" activities that would reflect utility of monitoring in daily practice, we elected to study waking-hour time windows to assess differences in HRV that were then standardised across all patients.

## 2.3 | Plasma IL-6 and IL-8 measurement

IL-6 and IL-8 were measured using standard ELISA kits as per manufacturer's instruction (BD Biosciences, Oxford, UK).

## 2.4 | Statistical analysis

Given the concordance in SDNN availed from ECG and Lifetouch system data outputs and that we were seeking to explore the practical utility of HRV denoted by SDNN as a "real life" measure of risk of cirrhosis decompensation and mortality, we amalgamated the data sets from London and Bonn and considered the application of baseline SDNN across all included patients.

Regarding data analysis, continuous variables were expressed as median and interquartile range (IQR) and categorical variables as numbers and percentages. Uni-variate analysis was carried out to identify the baseline factors associated with occurrence of death. Differences in continuous variables between survivors and non-survivors were accessed using the Mann-Whitney test, whereas categorical variables were tested using Pearson $\chi^2$ test.

As this was an aetiological research study design, we used logistic regression to examine the impact of SDNN levels in predicting 90-day mortality, whilst adjusting for confounding variables by including all the relevant potential confounders of an association between SDNN and 90-day mortality, as elaborated in the results section. Correlations were analysed with the Spearman's rank correlation. $P < 0.05$ were considered statistically significant. Using ROC curve analysis to predict mortality, a cut-off for SDNN was found and the cohort was divided in two groups. Differences between mortality in each group were displayed by Kaplan-Meier curves and tested using the log-rank test. All statistical analysis was performed by means of Stata 14 (StataCorp, TX, USA).

The study is reported in line with STROBE guidance for Observational Cohorts.

# 3 | RESULTS

## 3.1 | Baseline characteristics of patients

Of the 145 patients assessed for eligibility for inclusion in the study, 111 patients (74 male) were included in London and Bonn and

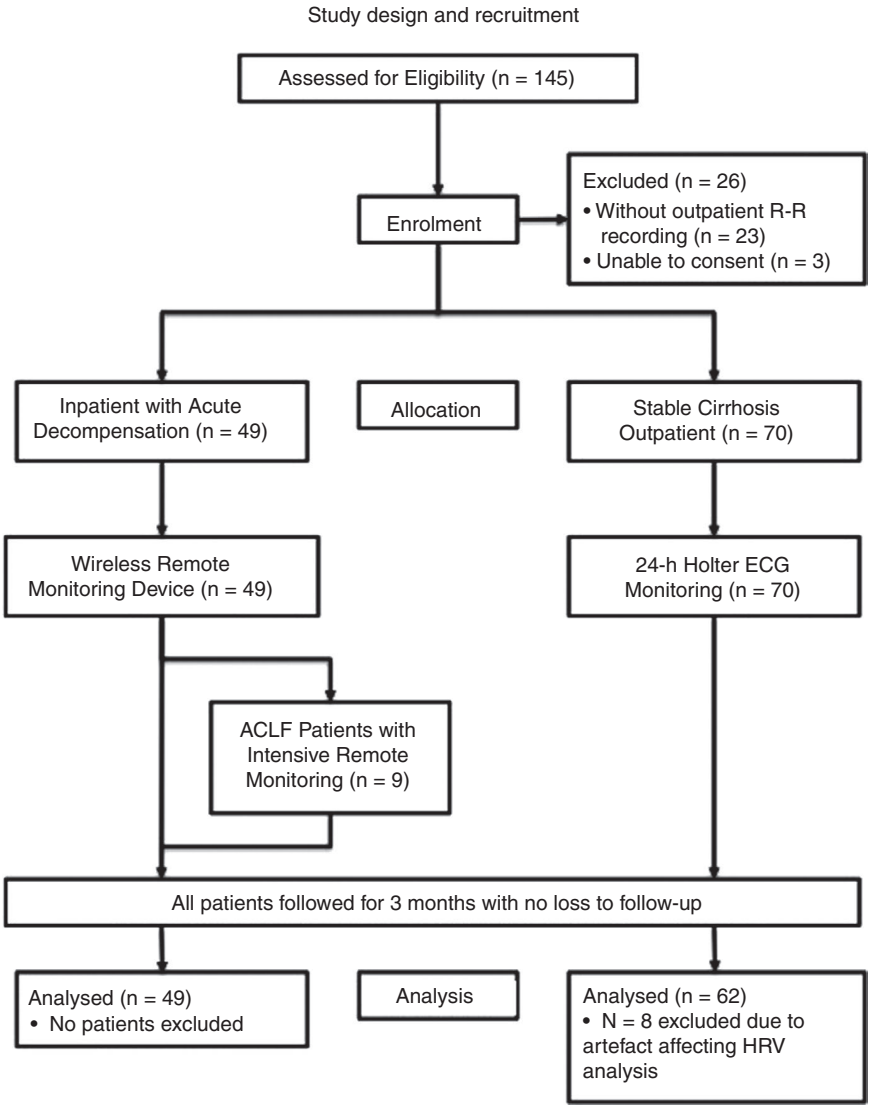


**FIGURE 1** Consort diagram showing study design, numbers of patients recruited and analysed

analysed for HRV as described in Figure 1. Amongst these, 49 had acute decompensation of cirrhosis based on development of hepatic encephalopathy, and/or renal dysfunction or increasing ascites and were admitted to the hospital. By comparison, the 62 cirrhosis outpatients without AD were clinically stable, under regular follow-up with no new changes in clinical condition or medication. There were no significant differences in gender between stable outpatients and AD patients with predominantly male subjects in both groups but AD patients were significantly younger (53 vs 59 years of age, $P < 0.02$). About 70% of patients in both groups were of alcoholic aetiology (actively consuming alcohol), the remainder split between viral, NAFLD and other causes, with no differences between the groups. Alcoholic hepatitis (AH) (as defined by a bilirubin > 5 mg/dL in an active drinker [>40 g alcohol/day], with AST > 50, AST > ALT ratio of 1.5, and/or histological criteria when present) was the precipitant for AD in 41% and infection in 27%. In 12% of AD patients both infection and AH were present, whilst in 20% of cases, no precipitant cause was noted. As anticipated, AD patients had significantly greater derangement in INR, were more hypoalbuminaemic, and had significantly higher plasma bilirubin compared to stable outpatients, along with higher aminotransferases (see Table 1). As a consequence, AD patients had significantly greater MELD scores (20 vs 9, $P < 0.001$). Moreover, AD patients had a median CLIF-C AD score of 55, suggesting an appreciable risk of mortality based on findings of the CANONIC study.[10] The principle organ dysfunction in AD patients was hepatic encephalopathy, in 41% of cases. As expected, amongst the 62 stable outpatients, most (84%) were Child A (5 [5-6]- median and IQR), whilst AD patients had significantly higher Child scores (9 [9-10]) split equally between Child B and C disease. AD patients also had significantly higher white blood cell counts (7.1 vs 5.7 $x10^9$/L; $P = 0.01$) and CRP levels (20 vs 5 mg/L; $P < 0.001$) compared to outpatients.

Nine patients who were admitted with AD met criteria for acute-on-chronic liver failure (ACLF) at the time of monitoring: two with ACLF1, five with ACLF2 and two with ACLF3.[9] Two patients required terlipressin whilst undergoing monitoring, one noradrenaline, and two who had been on beta-blockers, had these stopped upon admission.

**TABLE 1** Baseline patient characteristics

| | Stable outpatient cirrhosis (n = 62) | Acute decompensation (n = 49) | *P* value |
|---|---|---|---|
| Predisposition | | | |
| Male gender, n (%) | 40 (65%) | 34 (69%) | 0.59 |
| Age (y) (range) | 59 (19-89) | 53 (28-77) | 0.02 |
| Aetiology: Alcohol/viral/NASH/other (n) | 43/12/4/3 (69/19/7/5%) | 35/7/3/4 (72/14/6/8%) | 0.82 |
| Precipitant factor: AH/infection/both/unknown (n) | N/A | 20/13/6/10 (41/27/12/20%) | – |
| Laboratory Parameters | | | |
| ALT (U/L) | 35 (24-59) | 32 (20-56) | 0.34 |
| AST (U/L) | 36 (23-45) | 59 (43-108) | <0.001 |
| Bilirubin (mg/dL) | 0.8 (0.6-1.3) | 5.9 (1.5-16.1) | <0.001 |
| Albumin (g/dL) | 36 (33-43) | 32 (25-35) | <0.001 |
| INR | 1.1 (1.0-1.1) | 1.6 (1.3-2.2) | <0.001 |
| Creatinine (mg/dL) | 1.0 (0.8-1.4) | 1.0 (0.7-1.4) | 0.33 |
| Inflammatory response | | | |
| CRP (mg/L) | 5 (3-10) | 20 (11-52) | <0.001 |
| WBC ($x10^9$/L) | 5.7 (4.3-7.3) | 7.1 (5.1-12.4) | 0.01 |
| Severity and organ injury | | | |
| Child-Pugh A/B/C (n) | 52/10/0 (84/16/0%) | 2/24/23 (4/49/47%) | <0.001 |
| MELD | 9 (7-12) | 20 (14-26) | <0.001 |
| CCLIF-C AD score (n = 49) | N/A | 55 (46-60) | – |
| HE (+/-) | 2/60 (3/97%) | 20/29 (41/59%) | <0.001 |
| Renal dysfunction (+/-) | 13/49 (21/79%) | 9/40 (18/82%) | 0.73 |
| Heart rate (bpm) | 64 (52-88) | 82 (72-100) | 0.03 |
| MAP (mm Hg) | 87 (79-97) | 85 (77-92) | 0.087 |
| SDNN (ms) | 33 (25-42) | 14 (11-23) | <0.001 |

*Note*: Continuous variables are presented as median (IQR) and categorical variables as numbers and percentages.
Abbreviations: AH, alcoholic hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; both, refers to AH and infection; CLIF-C AD, CLIF Consortium Acute Decompensation; CRP, C-reactive protein; HE, hepatic encephalopathy; INR, international normalised ratio; MAP, mean arterial pressure; MELD, Model for End-Stage Liver Disease; NASH, non-alcoholic steatohepatitis; SDNN, standard deviation of all NN intervals; WBC, white blood cell count

### 3.2 | Validation of remote monitoring for HRV measurement in healthy volunteers and cirrhosis patients

Having established pilot HRV data collection in 20 healthy controls (40% male, mean age 33.5 ± 9.23 years), 13 consecutive enrolled cirrhosis patients had R-R intervals derived from ECG traces compared with the Isansys Lifetouch system to derive SDNN data. This showed good concordance with the SDNN values obtained through remote, continuous monitoring, as demonstrated by the Bland-Altman Plot in Figure S1. Subsequent data collection to assess HRV in all cirrhosis patients was evaluated through measuring SDNN.

Using the remote monitoring system, it was possible to analyse HRV in all monitored decompensated inpatients. Monitoring was performed continuously and there were no statistically significant differences between day and night measures of SDNN (median 10.3 (IQR 6.7-15.0) vs 13.9 (5.0 - 16.7); P=0.63).

Amongst the outpatients with Holter ECG monitoring, HRV was interpreted in 89% of patients. Due to the difficulty in obtaining an artefact-free, 5-minute period at the predetermined analysis time set out in the blinded analysis protocol, and the resulting computational errors in calculating HRV parameters, eight outpatients were excluded at the time of HRV analysis. However, importantly, there were no statistically significant differences in SDNN evaluated between the sites as exemplified by patients with MELD > 16: Bonn SDNN median 14 (IQR 10-16) vs RFH 12 (10-15); P=0.60 and similarly, Child C patients: Bonn SDNN 11 (10-16) vs RFH 11 (9-13); $P$ = 0.48. Also worthy of note there was no significant correlation noted between QT intervals and SDNN: Spearman's $\rho$ = 0.02; $P$ = 0.85.

### 3.3 | Association between HRV and acute decompensation and progression to ACLF

All cirrhosis patients had considerably lower HRV as assessed by SDNN, compared to healthy subjects in whom median SDNN was 55 ms. Baseline SDNN was noted to be significantly lower in AD patients compared to cirrhosis outpatients (median 14 (11-23) vs 33

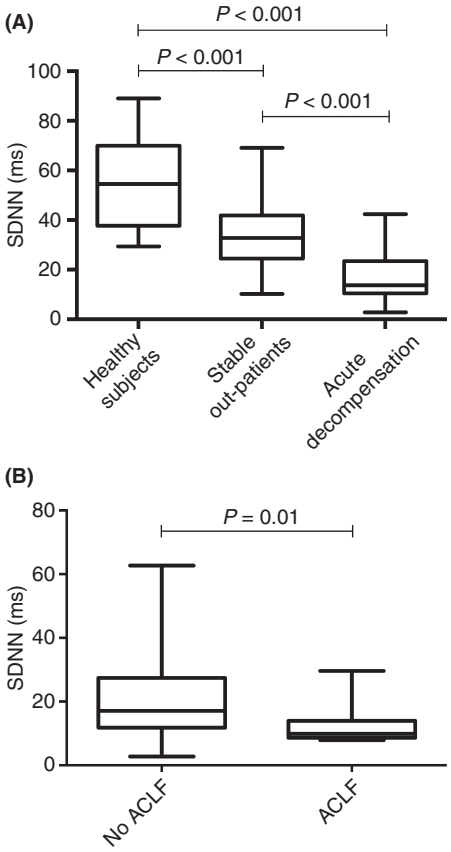


**FIGURE 2** A, SDNN in healthy subjects (n = 20), stable outpatient cirrhosis (n = 62) and acute decompensated cirrhosis patients (n = 49). The solid horizontal lines indicate the median values, the boxes the IQR and the error bars min-max. As cirrhosis progresses to acute decompensation, there is a clear reduction in HRV as noted by the statistically significant reduction in SDNN. B, SDNN in acute decompensated cirrhotic patients with no ACLF (n = 40) and with ACLF (n = 9). The solid horizontal lines indicate the median values, the boxes the IQR and the error bars min-max

(25-42) ms; $P < 0.001$). By contrast the baseline heart rate was significantly elevated in AD patients (median 82 (72-100) vs 64 (52-88) bpm; $P = 0.03$). There were no differences in mean arterial pressure between cirrhosis outpatients and AD patients.

As can be seen in Figure 2A, there is a clear relationship between reduction in HRV as denoted by lower SDNN, and progression of liver disease from stable outpatients to AD patients in hospital. Moreover, as patients progress to ACLF, there is a further significant reduction in median SDNN [IQR] (AD vs ACLF: 16 [11-24] vs 10 [9-12] ms; $P = 0.02$), as shown in Figure 2B. This marked reduction in SDNN with AD progression and ACLF, reflecting further impairment of HRV, is demonstrated by the examples of Poincaré plots shown in Figure S2A-D. This representative data show that there is significantly reduced HRV compared to healthy subjects when liver disease progresses, most evident, from stable outpatients to AD patients. The magnitude of reduction in HRV is best exemplified in Figure S2D, reflecting a case of a patient who developed ACLF, with multi-organ failure and succumbed in the intensive care unit, with

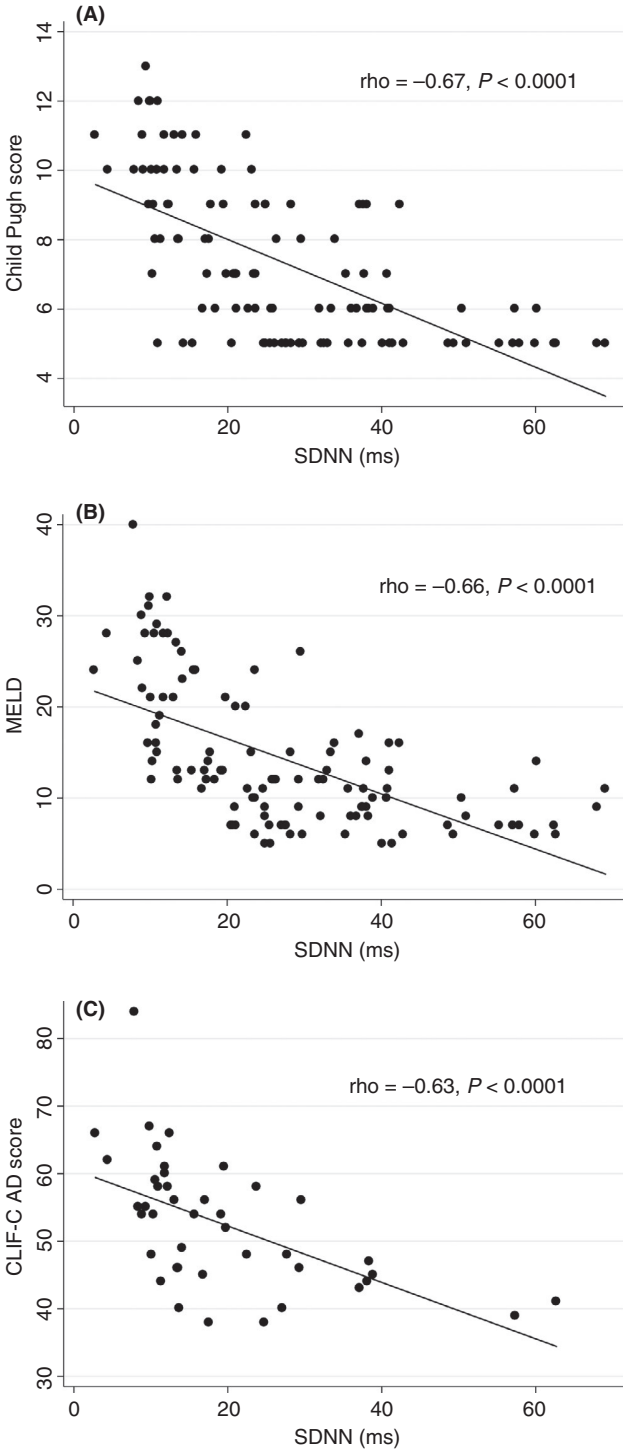


**FIGURE 3** Correlation between SDNN and Child-Pugh (CP) score (ρ= −0.67; $P < 0.0001$) (A), Model for End-stage Liver Disease(MELD) score (ρ = −0.66; $P < 0.0001$) (B) and CLIF-C AD score (ρ = −0.63; $P < 0.0001$) (C). The linear regression line shows the correlation

almost negligible HRV, despite the high physiological stress and use of inotropes in this setting.

Across all the studied patients (Figure 3A-C), SDNN correlated strongly and inversely with CP score (ρ = −0.67; $P < 0.0001$), MELD

**TABLE 2** Patient characteristics in accordance with 90-day survival

| | 90-day survival | | |
|---|---|---|---|
| | Yes (n = 99) | No (n = 12) | *P* value |
| Predisposition | | | |
| Male gender, n (%) | 67 (68%) | 7 (58%) | 0.52 |
| Age (y) | 58 (47-65) | 58 (49-68) | 0.91 |
| Aetiology: Alcohol/viral/NASH/other (n) | 70/17/6/6 (71/17/6/6%) | 8/2/1/1 (67/17/8/8%) | 0.98 |
| Laboratory parameters | | | |
| ALT (U/L) | 32 (23-53) | 50 (22-76) | 0.33 |
| AST (U/L) | 40 (32-68) | 61 (35-114) | 0.18 |
| Bilirubin (mg/dL) | 1.2 (0.7-2.7) | 6.6 (1.2-10.9) | 0.06 |
| Albumin (g/dL) | 35 (30-41) | 31 (25-35) | 0.02 |
| INR | 1.1 (1.0-1.5) | 1.6 (1.3-2.2) | <0.01 |
| Creatinine (mg/dL) | 1.0 (0.8-1.4) | 1.0 (0.7-1.4) | 0.99 |
| Inflammatory response | | | |
| CRP (mg/L) | 8 (4-20) | 40 (26-65) | <0.001 |
| WBC ($x10^9$/L) | 5.8 (4.3-8.5) | 9.8 (5.7-16.5) | 0.02 |
| Severity and organ injury | | | |
| Child-Pugh A/B/C (n) | 54/29/16 (55/29/16%) | 0/5/7 (0/42/58%) | <0.001 |
| MELD | 12 (8-16) | 21 (16-24) | <0.01 |
| CLIF-C AD score (n = 49) | 49 (45-58) | 57 (55-60) | 0.11 |
| HE (+/-) | 17/82 (17/83%) | 5/7 (42/58%) | 0.04 |
| Renal dysfunction (+/-) | 20/79 (20/80%) | 2/10 (17/83%) | 0.77 |
| Heart rate (bpm) | 79 (68-98) | 87 (68-110) | 0.28 |
| MAP (mm Hg) | 85 (77-92) | 90 (82-93) | 0.32 |
| SDNN (ms) | 26 (17-38) | 11 (10-12) | <0.0001 |

*Note*: Continuous variables are presented as median (IQR) and categorical variables as numbers and percentages.
Abbreviations: AH, alcoholic hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIF-C AD, CLIF Consortium Acute Decompensation; CRP, C-reactive protein; HE, hepatic encephalopathy; INR, international normalised ratio; MAP, mean arterial pressure; MELD, Model for End-Stage Liver Disease; NASH, non-alcoholic steatohepatitis; SDNN, standard deviation of all NN intervals; WBC, white blood cell count.

score ($\rho = -0.66$; $P < 0.0001$), and in AD patients, CLIF-C AD score ($\rho = -0.63$; $P < 0.0001$). SDNN also correlated inversely and significantly with heart rate (Figure S3), suggesting a potential relationship between the hyperdynamic circulation and reduced HRV.

### 3.4 | Evaluation of factors that predicted mortality on uni- and multi-variate analysis

All patients had data collected on 90-day mortality, status of liver disease severity and development of organ dysfunction. There were 12 deaths (7 male) that occurred within 90 days. No differences in age, gender distribution, nor aetiology were noted between survivors and nonsurvivors. Nonsurvivors had higher bilirubin (median 6.6 vs 1.2 mg/dl; $P = 0.06$), significantly higher INR (1.6 vs 1.1; $P < 0.01$) and lower albumin levels (31 vs 35 g/L; $P = 0.02$) compared with those alive at 90 days (Table 2). Similarly, WBC (9.8 vs 5.8; $P < 0.02$) and CRP (40 vs 8 mg/L; $P < 0.001$) were significantly elevated in nonsurvivors. As anticipated, nonsurvivors had higher MELD scores and were predominantly Child C disease. In relation to hepatic encephalopathy, there was a greater number of nonsurviving patients presenting with hepatic encephalopathy compared to survivors. In patients that died, infection was the main injurious trigger in at least 50% of cases, often associated with subsequent multi-organ failure. However, there were no statistically significant differences in renal dysfunction (defined as increase in serum creatinine by more than 0.3mg/dl within 48 hours or to more than 1.5-fold at the time of monitoring, compared to baseline levels at admission) between the groups.

SDNN was significantly lower in nonsurvivors than survivors (11 vs 26 ms; $P < 0.0001$). The repeat measurement of SDNN over a mean period of 5 days demonstrated a 55% decrease in SDNN from baseline in AD patients that died within 90 days, whilst there was no significant change from baseline in survivors. However, in the subgroup who developed ACLF, a 25% decrease from baseline SDNN was observed in those patients that died, as opposed to a 30% increase in SDNN in patients that survived their ACLF, despite all patients being managed according to the same standard protocols.

By using logistic regression analysis, the crude odds ratio (OR) for SDNN in predicting 90-day mortality was 0.83 (95% confidence

interval (CI): 0.74 - 0.93; $P$ = 0.002). We then performed multivariate analyses and introduced potential confounders for analysis in a logistic regression model. We adjusted for confounding by patients' gender, age, presence of HE and bilirubin, INR, albumin, sodium, creatinine, CRP and WBC levels and chose to introduce the actual variables, instead of the composite scores. The impression that SDNN was associated with 90-day mortality persisted after confounder adjustment; the adjusted OR for SDNN was 0.79 (95% CI: 0.65 - 0.97; $P$ = 0.02) and the analyses disclosed baseline SDNN as the only independent predictor of 90-day mortality (Table S1).

### 3.5 | Utility of SDNN in predicting 90-day mortality

The predictive utility of baseline SDNN in determining 90-day mortality was evaluated using Area under Receiver Operating Characteristic (AUROC) curve analysis. Given the clinical use of other scoring systems and their strengths on univariate analysis, we also compared performance of SDNN to CP score (AUC: 0.85; 95%-CI: 0.76-0.93), MELD score (AUC: 0.77; 95%-CI:0.64-0.90), MELD-Na score (AUC: 0.76; 95%-CI:0.61-0.90), CRP-values (AUC: 0.85; 95%-CI:0.76-0.93) and WBC (AUC:0.0.70; 95%-CI:0.53-0.88). Interestingly, baseline SDNN had the highest AUROC for 90-day mortality (AUC: 0.87; 95%-CI:0.79-0.96), as shown in Figure 4A.

Using an optimal sensitivity and specificity from the ROC curve, a baseline SDNN cut-off value of 13.25 ms had a 98% negative predictive value (sensitivity 83%, specificity 86%) for predicting 90-day mortality. This cut-off could distinguish between survivors and non-survivors at 90 days, as shown in the Kaplan-Meier analysis (Figure 4B).

When patients were compared applying an SDNN cut-off above and below 13.25 ms, the principle variables that differed between

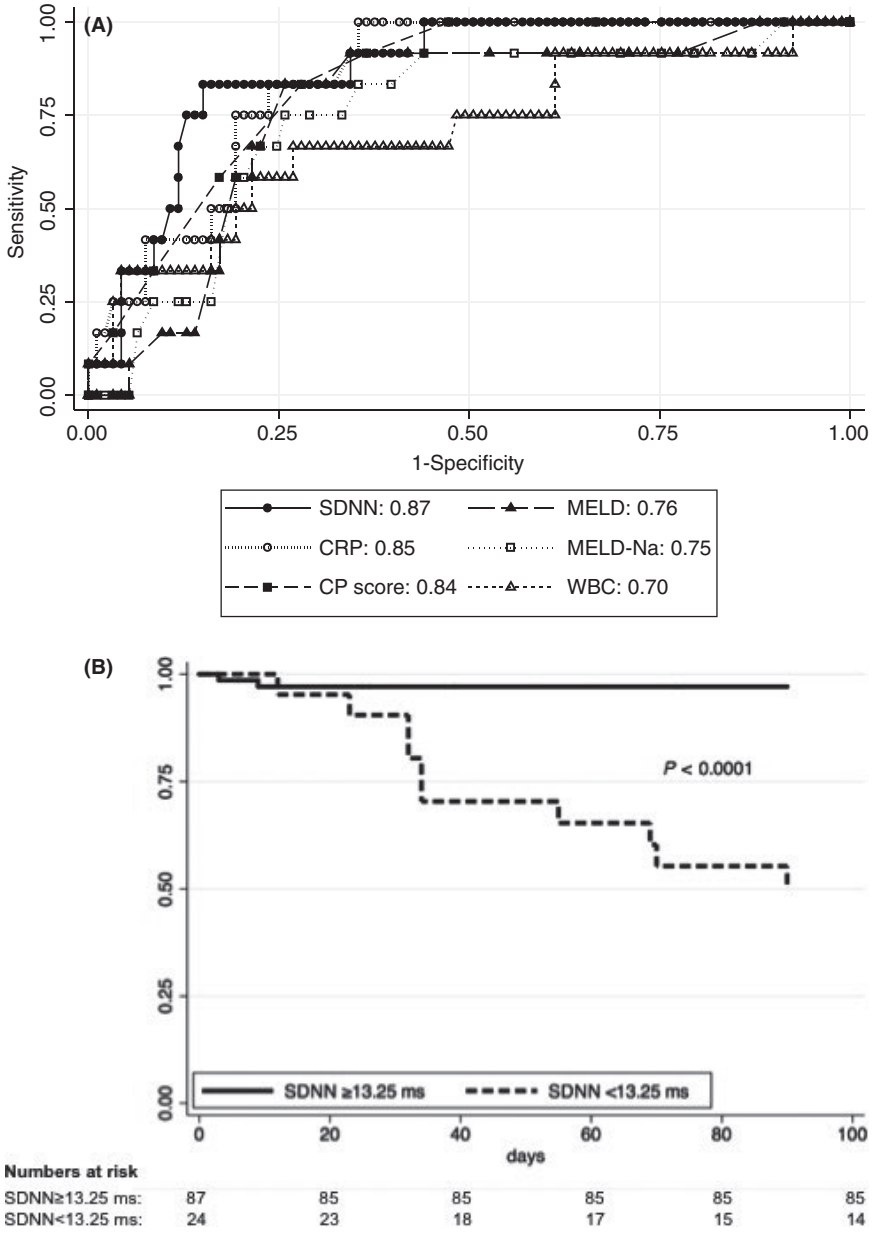


**FIGURE 4** A, Receiver operating characteristic (ROC) curve analyses of SDNN, C-reactive protein (CRP), Child-Pugh (CP) score, Model for End-stage Liver Disease (MELD) score, MELD-sodium (Na) score and white blood cell count (WBC) as predictors for 3 months mortality. B, Survival curves for patients according to SDNN. Three months survival curves for patients with SDNN ≥ 13.25 ms (n = 87, solid line) and < 13.25 ms (n = 24, dashed line) ($P$ < 0.0001)

**TABLE 3** Patient characteristics in accordance with baseline SDNN above or below the threshold of 13.25 ms

| | SDNN < 13.25 ms (n = 24) | SDNN ≥ 13.25 ms (n = 87) | *P* value |
|---|---|---|---|
| Predisposition | | | |
| Male gender, n (%) | 15 (63%) | 59 (68%) | 0.63 |
| Age (y) | 53 (43-62) | 59 (49-67) | 0.07 |
| Aetiology: Alcohol/viral/NASH/other (n) | 15/5/2/2 (63/21/8/8%) | 63/14/5/5 (72/16/6/6%) | 0.82 |
| Laboratory parameters | | | |
| ALT (U/L) | 34 (24-66) | 32 (23-53) | 0.50 |
| AST (U/L) | 76 (44-125) | 38 (28-56) | <0.001 |
| Bilirubin (mg/dL) | 11.1 (4.3-26.3) | 1.0 (0.7-1.8) | <0.0001 |
| Albumin (g/dL) | 33 (27-35) | 35 (30-41) | 0.03 |
| INR | 2.1 (1.4-2.4) | 1.1 (1.0-1.3) | <0.0001 |
| Creatinine (mg/dL) | 1.1 (0.7-1.8) | 1.0 (0.8-1.4) | 0.86 |
| Inflammatory response | | | |
| CRP (mg/L) | 40 (19-59) | 7 (3-16) | <0.0001 |
| WBC ($x10^9$/L) | 10.5 (5.7-16.3) | 5.8 (4.4-7.8) | <0.001 |
| IL-6 (pg/ml) | 21 (14-58) | 11 (7-24) | 0.06 |
| IL-8 (pg/ml) | 65 (34-96) | 20 (12-55) | 0.02 |
| Severity and organ injury | | | |
| Child-Pugh A/B/C (n) | 1/7/16 (4/29/67%) | 53/27/7 (61/31/8%) | <0.0001 |
| MELD | 25 (19-29) | 11 (7-13) | <0.0001 |
| CLIF-C AD score (n = 49) | 59 (55-64) | 46 (42-53) | <0.0001 |
| HE (+/-) | 6/18 (25/75%) | 16/71 (18/82%) | 0.47 |
| Renal dysfunction (+/-) | 7/17 (29/71%) | 15/72 (17/83%) | 0.19 |
| Heart rate (bpm) | 87 (80-100) | 72 (64-85) | 0.02 |
| MAP (mm Hg) | 88 (78-93) | 85 (78-90) | 0.37 |

*Note*:: Continuous variables are presented as median (IQR) and categorical variables as numbers and percentages.
Abbreviations: AH, alcoholic hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIF-C AD, CLIF Consortium Acute Decompensation; CRP, C-reactive protein; HE, hepatic encephalopathy; IL, interleukin; INR, international normalised ratio; MAP, mean arterial pressure; MELD, Model for End-Stage Liver Disease; NASH, non-alcoholic steatohepatitis; WBC, white blood cell count.

the groups were that patients with an SDNN < 13.25 ms had significantly elevated bilirubin (11.1 vs 1 mg/dl, $P < 0.0001$); INR (2.1 vs 1.1, $P < 0.0001$); MELD score (25 vs 11, $P < 0.0001$); and CLIF-C AD score (59 vs 46, $P < 0.0001$), as shown in Table 3. Moreover, those with an SDNN < 13.25 ms had significantly higher heart rates compared to those with SDNN > 13.25 ms: [87 (80-100) vs 72 (64-85), $P = 0.02$]. Of note, there were no differences in renal function or encephalopathy, nor aetiological factors for liver disease, nor age in patients above and below the SDNN cut-off of 13.25 ms. However, infection was more commonly noted in 67% of cases as a factor during decompensation, in those with SDNN < 13.25 ms.

### 3.6 | Systemic inflammation and its association with HRV

As might be expected, stable outpatients had considerably lower CRP values compared to patients with AD (10 vs 5 mg/L, $P < 0.001$), as was their WBC. Of interest, SDNN was found to strongly and inversely correlate with CRP (Spearman $\rho = -0.56$; $P < 0.0001$). SDNN also correlated inversely with WBC ($\rho = -0.34$; $P < 0.001$), Figure S4A and B. Moreover, in patients with SDNN < 13.25 ms, WBC (10.5 vs 5.8, $P < 0.001$) and CRP (40 vs 7, $P < 0.0001$) were markedly elevated, compared to patients with SDNN values above this threshold (Table 3). Assessment of the pro-inflammatory cytokines IL-6 and IL-8, also showed these to be markedly increased in patients with an SDNN cut-off < 13.25 ms compared to those ≥ 13.25 ms (Table 3; Figure S4C and D).

## 4 | DISCUSSION

Reduction in HRV, as a manifestation of autonomic system dysfunction, has been described in many conditions including cardiac ischaemia, diabetes mellitus, sepsis [2,3,12] and chronic liver disease.[7]

The key findings reported in this study are: (a) continuous, wireless remote monitoring of HRV is feasible in patients with cirrhosis,

and measuring SDNN reflects changes in HRV in this population. (b) SDNN is reduced significantly with progression of cirrhosis to decompensation and is further reduced in patients with ACLF. (c) Reduction in SDNN, reflecting loss of HRV, negatively correlates with disease severity scores such as MELD and CP score and importantly, also with indicators of inflammation (CRP and WBC). (d) Measurement of SDNN shows utility in predicting 90-day mortality and a SDNN cut-off of < 13.25 ms we show has a negative predictive value of 98% in determining outcome.

Numerous factors affect HRV measurement including respiration, intrinsic cardiac abnormalities, circadian rhythm, age and genetic factors. Moreover, given significant inter- and intra-individual differences in HRV, reliable measurement of HRV has historically been deemed to be complex and requisite of specific conditions and necessitating special equipment. In this study, we describe a continuous, ambulatory, remote measurement of HRV in patients with established cirrhosis, in whom autonomic dysfunction is common (up to 80%).[13,14] Traditional ECG measurement of HRV encompasses short-term 5 minutes ECG segments being interpreted separately as reflecting HRV in that specific time window, under stable physiological conditions. The Lifetouch system by contrast, not only facilitates continuous monitoring irrespective of the individuals' daily activity or physical ill-health but also helps negate the short coming of limited ECG time capture, where artefact and premature beats caused by these factors over the 5 minutes of analysed R-R interval, make further interpretation difficult. This is particularly the case in patients with cirrhosis, in whom high respiratory rates, inflammation and impaired baroreceptor responses interfere considerably with standard ECG R-R interpretation.[4,15-17] Using the remote monitoring device, we were able to interpret HRV in all monitored inpatients with acute decompensation.

HRV data can be described in both time domain and frequency domain variables. SDNN is the most commonly described HRV time domain measurement in part, through its simple evaluation without the need for complex analytical systems, unlike frequency domain variables.[18] In addition, many frequency domain measures are influenced by high respiratory rate and gender, and necessitate significant short-term data filtering.[19,20] As a consequence, we elected to focus on SDNN in this study given the increased respiratory rate and systemic inflammation in our patient cohort, and in order to reduce the filtering requirements on each data set.

A key finding in this study was the demonstration that HRV as assessed by SDNN was significantly lower in patients hospitalised with acute decompensation as compared to stable outpatients. Moreover, HRV was further reduced in patients with ACLF. SDNN levels in decompensated cirrhosis patients fell to approximately 25% of the median level in controls, and to half the value seen in compensated patients. There was also a strong inverse correlation between SDNN and prognostic scores such as MELD and CP and also importantly, the CLIF-C AD score. The latter has been shown to most accurately prognosticate in those patients with cirrhosis decompensation at risk of progression to ACLF and mortality.[10] It is therefore noteworthy that in this study, SDNN was the only independent predictor of 3-month mortality. Moreover, repeat measurement of SDNN during a patient's journey through hospital admission, demonstrated that in those who manifest a significant increase in SDNN, there was a potential for recovery, whilst a further decrease in SDNN was more commonly associated with mortality. This suggests a future potential for continuous monitoring whereby an increase in SDNN following intervention, might inform of favourable response to therapy, whilst conversely, failure to recover SDNN might indicate patients with a higher risk of death.

A further interesting observation was that HRV as assessed by SDNN demonstrated a good inverse correlation with CRP values and also with WBC. Moreover, there was also a weak but statistically significant association between low SDNN and increased concentrations of the pro-inflammatory cytokine IL-6 but only a trend of an association with IL-8. This is notable given that 50% of our studied patients that died did so from infection, and also the relationship between low SDNN and the development of the inflammatory syndrome of ACLF. The loss of HRV as indicated by reduction in SDNN may, therefore, reflect a response to inflammation, whether driven by infection or a sterile inflammatory response, as often seen in ACLF.[21,22] This is supported by the wider literature on HRV, where for example after unstable angina pectoris, high levels of CRP correlate inversely with loss of HRV.[23] Similarly, following traumatic brain injury, there is a reduction in HRV and this is associated with a change in immune responses,[24,25] whilst in patients treated for sepsis, reduced SDNN has been linked to poor outcome.[12]

The recent literature in decompensated cirrhosis patients has highlighted the pathophysiologic significance of systemic inflammatory response, as demonstrated by raised leucocyte counts and more recently elevated CRP, as being associated with poor outcome.[10,26] Of interest, in this study, only SDNN was an independent predictor of mortality. However, the measurement of SDNN may potentially serve as a surrogate of inflammatory activity that can be availed remotely in patients at risk of acute decompensation. If SDNN decreases further, this may indicate a need for closer clinical review of the patient to look for development of ACLF or new infection, before organ failure ensues, and when early intervention with antibiotics or organ support may have most optimal effect. Clearly, this assertion requires further evaluation in a controlled study and also further mechanistic evaluation, to determine whether reduction in HRV reflecting impaired autonomic function, is causally related to poor outcome.

ROC curve analysis demonstrated SDNN has good predictive utility for 90-day mortality (AUC of 0.87) and the SDNN cut-off of >13.25 ms had a negative predictive value of 98% in determining outcome. Patients with an SDNN <13.25 ms invariably had worse liver function (MELD), state of liver decompensation (CLIF-C AD score) and inflammatory indices (CRP and WBC) than patients with SDNN >13.25 ms. This is aligned with a study demonstrating that septic patients on the intensive care unit had an increased risk of death in lower SDNN ranges, even after adjusting for other organ failure severity scores.[12] The need for a larger validation cohort for the SDNN cut-off of 13.25 ms for 90-day mortality is one limitation of our study.

There are few other limitations to consider when interpreting the data presented in this study: first, the patients were recruited

at two different sites with a mix of compensated and decompensated patients. Whilst there was heterogeneity in the population, we feel this reflects the "real-life" situation of patients presenting to the hospital who are at risk of decompensation or/and have had prior decompensation. Furthermore, there were no clear differences noted in liver disease aetiology, renal function or blood pressure between compensated and decompensated patients at baseline. The only clear differences observed between these groups were in liver prognostic scores and SDNN, which was the focus of the study.

A second point of note is the constraint placed by limited availability of the Lifetouch monitors such that it was not possible to accrue repeat measures over long periods, during every patient's treatment journey. As such, only a limited number of patients had repeated measurements beyond baseline SDNN, and from this we were able to learn that changes in SDNN may indicate a trajectory of improvement or deterioration. However, given the limited sample size and that only 12 patients died within the 90-day period, there is statistical under-powering for this aspect of the data analysis, and clearly a dedicated study of long-term SDNN monitoring is required. This would help determine its value in predicting recovery or progression from a decompensation episode.

In conclusion, we describe the wireless, remote monitoring of HRV and demonstrate that a significant reduction in SDNN (reflecting changes in HRV), in patients with cirrhosis, correlates with severity of liver decompensation and inflammation, deteriorates further in those with ACLF, and acts as an independent prognostic factor for mortality. Our data suggest that using such tools to measure HRV remotely may facilitate a means of monitoring cirrhosis patients at risk of decompensation and high mortality. This will help guide appropriate early intervention if SDNN drops below a determined threshold, and to assess response to therapy, with potential for health economic benefit. The data presented support the need for further controlled studies of remote HRV monitoring in cirrhosis patients at risk of decompensation.

## ACKNOWLEDGEMENTS

We thank Isansys Limited for their technical support in delivering this study. This study was supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18), Cellex-Foundation and UCL Business award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

*Declaration of personal interests*: UCL Business has filed a patent application on the use of Heart Rate Variability in cirrhosis decompensation.

## AUTHORSHIP

*Guarantor of the article*: RPM.

*Authors' contributions*: RPM, JT, study concept and design; CJ, DC, RS, B Al-K, RPM, ML, acquisition of data; DC, RPM, KLT, RS, CJ, HJ, JT, analysis and interpretation of data; RPM, KLT, JT, RJ, DC, CJ, drafting of the manuscript; KLT, RS, DC, RPM, CJ, JT, HJ, statistical analysis; DC, RS, HJ, AG-L, administrative, technical and material support. All authors read and approved the final manuscript.

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## SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Jansen C, Chatterjee DA, Thomsen KL, et al. Significant reduction in heart rate variability is a feature of acute decompensation of cirrhosis and predicts 90-day mortality. *Aliment Pharmacol Ther*. 2019;50:568–579. https://doi.org/10.1111/apt.15365

## 3.3 Erhöhte myokardiale Kontraktilität identifiziert Patienten mit dekompensierter Zirrhose, die eine Lebertransplantation benötigen.

**Jansen C***, Cox A*, Schueler R, Schneider M, Lehmann J, Praktiknjo M, Pohlmann A, Chang J, Manekeller S, Nickenig G, Berlakovich G, Strassburg CP, Hammerstingl C, Staufer K, Trebicka J. Increased myocardial contractility identifies patients with decompensated cirrhosis requiring liver transplantation. Liver Transpl. 2018 Jan;24(1):15-25. doi: 10.1002/lt.24846. Epub 2017 Dec 1. PMID: 28834154

Zielsetzung der Arbeit – Es sollte untersucht werden, inwieweit Herzfunktionsstörungen eine Rolle in der Bewertung der Prognose bei Patienten mit Leberzirrhose spielen. Der Fokus lag dabei auf der myokardialen Kontraktilität, die in diesem Kollektiv noch nicht hinreichend untersucht wurde. Die Kontraktilität wurde mit Hilfe der Speckle-Tracking-Echokardiographie bei Patienten ermittelt, die sich auf der Warteliste zur Lebertransplantation befanden.

Methoden und Ergebnisse – Insgesamt wurden 168 Patienten mit Zirrhose (Trainings-Kohorte, 111; Validierungskohorte, 57) in Erwartung einer Lebertransplantation in zwei Zentren retrospektiv in die Studie eingeschlossen. 51 Patienten der Trainings- Kohorte und alle Patienten aus der Validierungskohorte wurden transplantiert. 36 Patienten aus der Trainings-Kohorte und 38 aus der Validierungskohorte lebten nach Ablauf des Follow-ups von 21 Monaten und waren nicht transplantiert. Wir sahen, dass die Kontraktilität des linken Ventrikels mit der Schwere der Lebererkrankung (Child-Pugh Score) zunahm. Interessanterweise war eine höhere Linksventrikuläre-Kontraktilität bei den Patienten der Trainingskohorte, insbesondere bei Patienten mit Child-Pugh C, ein unabhängiger Prädiktor für das reduzierte transplantationsfreie Überleben. Bei männlichen Patienten sind die Auswirkungen bei weitem stärker zu beobachten. Insbesondere zeigten die konkurrierende Risikoanalysen, dass eine erhöhte Kontraktilität damit verbunden ist, früher eine Transplantation zu benötigen. Wichtig ist, dass die Kontraktilität des Myokards keinen Einfluss auf das Überleben nach Lebertransplantation hatte. Dies unterstützt die Vermutung, dass die zirrhotische Kardiomyopathie reversibel zu sein scheint.

Schlussfolgerungen – Diese Studie zeigt zum ersten Mal, dass mittels Speckle-Tracking-Echokardiographie Patienten identifizieren werden können, die von einer frühen Lebertransplantation profitieren würden.

HEPATOLOGY

HEPATOLOGY, VOL. 67, NO. 4, 2018

# Increase in Liver Stiffness After Transjugular Intrahepatic Portosystemic Shunt Is Associated With Inflammation and Predicts Mortality

Christian Jansen,[1*] Philipp Möller,[1*] Carsten Meyer,[2] Carl Christian Kolbe,[3] Christopher Bogs,[1] Alessandra Pohlmann,[1] Robert Schierwagen,[1] Michael Praktiknjo,[1] Zeinab Abdullah,[4] Jennifer Lehmann,[1] Daniel Thomas,[2] Christian P. Strassburg,[1] Eicke Latz,[3,5] Sebastian Mueller,[6] Martin Rössle,[7] and Jonel Trebicka [1,8-10]

**Transjugular intrahepatic portosystemic shunt (TIPS) efficiently treats complications of portal hypertension. Liver and spleen stiffness might predict clinically significant portal hypertension. This prospective study investigated liver stiffness in patients receiving TIPS regardless of indication. Of 83 included patients, 16 underwent transient elastography immediately before and 30 minutes after TIPS (acute group), while 67 received shear wave elastography of liver and spleen 1 day before and 7 days after TIPS (chronic group) and were followed further. In blood samples obtained before TIPS from cubital, portal, and hepatic veins, levels of several interleukins (IL1b, IL6, IL8, IL10, IL18) and interferon-gamma were analyzed. In 27 patients (5 acute, 22 chronic), it resulted in an increase in liver stiffness of >10%. In 56 patients, liver stiffness decreased or remained unchanged (<10%). Importantly, spleen stiffness measured by shear wave elastography decreased in all patients (chronic group). None of the clinical or laboratory parameters differed between patients with increase in liver stiffness and those without. Of note, patients with increased liver stiffness showed higher overall and/or hepatic venous levels of proinflammatory cytokines at TIPS and higher incidence of organ failure and worse survival after TIPS. C-reactive protein values and increase of >10% in liver stiffness after TIPS were the only independent predictors of mortality in these patients. *Conclusion:* This study demonstrates that the presence of systemic inflammation predisposes patients to develop increased liver stiffness after TIPS, a predictor of organ failure and death. (NCT03072615) (HEPATOLOGY 2018;67:1472-1484).**

Decompensated liver cirrhosis, often due to complications of clinically significant portal hypertension,[1] might end in acute-on-chronic liver failure (ACLF), which is associated with elevated systemic inflammation[2] and high mortality.[3] Complications of portal hypertension can be successfully treated by implantation of a transjugular intrahepatic portosystemic shunt (TIPS).[4-6] TIPS leads to immediate decompression in the portal venous system and subsequent increase of effective blood volume. It stops variceal rebleeding and improves renal function.[7] The clinical stratification of patients

*Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; CI, confidence interval; CLIF-C, Chronic Liver Failure Consortium; CRP, C-reactive protein; HR, hazard ratio; IL, interleukin; MELD, Model for End-Stage Liver Disease; SWE, shear wave elastography; TE, transient elastography; TIPS, transjugular intrahepatic portosystemic shunt; TWBC, total white blood cell count.*

*Received June 30, 2017; accepted October 18, 2017.*

*Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29612/suppinfo.*

**These authors contributed equally to this work.*

*Supported by the Deutsche Forschungsgemeinschaft (SFB TRR57), the European Union's Horizon 2020 Research and Innovation Programme (668031), and the Cellex Foundation. The funders had no influence on study design, data collection and analysis, decision to publish, or preparation of the manuscript.*



*View this article online at wileyonlinelibrary.com.*

*DOI 10.1002/hep.29612*

*Potential conflict of interest: Dr. Latz consults for IFM.*

receiving TIPS has been investigated in several studies, selecting either high-risk patients receiving TIPS for variceal bleeding[4] or patients with a lower risk receiving TIPS for refractory ascites and hepatorenal syndrome.[7] However, while the indication for TIPS might be one of the above, many patients present with other complications of portal hypertension and acute decompensation (AD) of liver cirrhosis.[8] Therefore, other predictors at TIPS insertion and at short-term clinical follow-up are needed to improve the disease management in these patients.[7]

Liver stiffness, measured by either transient elastography (TE)—recently approved by the Food and Drug Administration in the United States—or shear wave elastography (SWE), is a useful parameter in the diagnosis of clinically significant portal hypertension and varices requiring treatment.[9-15] While it has been shown that liver and spleen stiffness might detect changes in hepatic venous pressure gradient after nonselective beta-blockers, the data were not as conclusive after TIPS.[16-19] Importantly, a larger recent study could not sufficiently explain the reason behind the change of liver stiffness after TIPS.[20] This is probably due to the fact that liver stiffness is a result of various different factors, especially blood (arterial and venous) pressure, inflammation, and fibrosis.[21-23]

The aim of this prospective observational cohort study, carried out in two centers, was to evaluate the change in liver and spleen stiffness measurement after TIPS as a parameter to stratify and follow up patients.

# Patients and Methods

## PATIENTS

This prospective observational cohort study was carried out in two centers and included patients with the following criteria: (1) reliable SWE or TE measurement of the liver before TIPS insertion and (2) feasible TIPS insertion. Exclusion criteria were (1) contraindication for TIPS (including diastolic dysfunction), (2) no reliable SWE/TE measurement, and (3) patient refusal or noncompliance.

Between April 2013 and November 2015, 128 patients were screened at the Department of Internal Medicine I of the University Hospital Bonn, Germany, and at the Department of Gastroenterology, University Hospital Freiburg, Freiburg, Germany. Of these, 83 patients were enrolled, while 45 patients did not fulfill the inclusion criteria (Fig. 1). In 16 patients (acute group), liver stiffness was measured by TE immediately before and 30 minutes after TIPS insertion to detect acute changes. In 67 patients (chronic group), liver and spleen SWE was performed 1 day before and 7 days after TIPS insertion to detect chronic changes. Blood samples from portal, liver, and cubital veins were collected at TIPS insertion in 45 patients of the chronic group. The chronic group was followed in detail regarding short-term (median 7 days) and long-term follow-up. The study was registered after completion at clinicaltrials.gov (NCT03072615). All authors had access and were able to review and approve the final manuscript.

Within the context of another study, an additional 7 patients received TE and SWE of the liver before, 30 minutes after, and 7 days after TIPS.

## ETHICS CONSIDERATION

Patients gave signed written informed consent for the procedures of the study. The local ethics committees of the Universities of Bonn (no. 121/14) and Freiburg (S-384/2009) approved the study in accordance with the Declaration of Helsinki.

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## TE PROCEDURE

Liver stiffness measurements using TE were performed immediately before and 30 minutes after TIPS insertion after fasting for at least 8 hours. TE measurement was performed as described.[24-26] In this study, only procedures with at least 10 valid shots, a success rate of at least 80%, and ratio of interquartile range and median <0.3 were considered reliable and used for statistical analysis.[24-26] The XL probe was used in patients with ascites according to earlier reports.[27] Nevertheless, all patients received paracentesis before TIPS and usually a drain catheter during hospitalization. Therefore, TE or SWE measurements were not influenced by ascites.

## SWE PROCEDURE

Only accurate SWE measurements were performed after overnight fasting and considered and performed as described.[13,14] An accurate SWE measurement showed (1) stability of the selected liver area for at least 3 seconds before measurement, (2) two-dimensional quality confirmed by homogenous color in the region of interest, and (3) a measurement region of at least 10 mm. Up to three separate measurements were performed, and results are reported as the mean of the total number of valid measurements. An increase or decrease in stiffness was regarded only if SWE measurement changed >10% because this was the variance within measurements applied in the same patients in previous studies.[13,14]

## MEASUREMENTS OF PROINFLAMMATORY PROTEINS

During the TIPS procedure, to determine levels of different inflammatory proteins, first blood from cubital, then hepatic veins were collected, finally as soon as the portal vein branch was cannulated the portal venous sample was collected as well, as described.[28-32] In these patients and in 42 healthy controls, we measured interleukin 1beta (IL1$\beta$), IL6, and IL8 in the cubital vein using an enzyme-linked immunosorbent assay according to the manufacturer's guidelines (R&D Systems Inc., Minneapolis, MN).

Additionally, in 20 patients selected regarding their evolution of liver stiffness, samples taken from the cubital, liver, and portal veins of IL1$\beta$, IL6, IL8, IL10, IL18, and interferon-gamma were measured using the Luminex MAGPIX system (EMD Millipore, Darmstadt, Germany) with ProcartaPlex Mix&Match Human 15-plex (eBiosciences, Carlsbad, CA) according to the manufacturer's instructions.

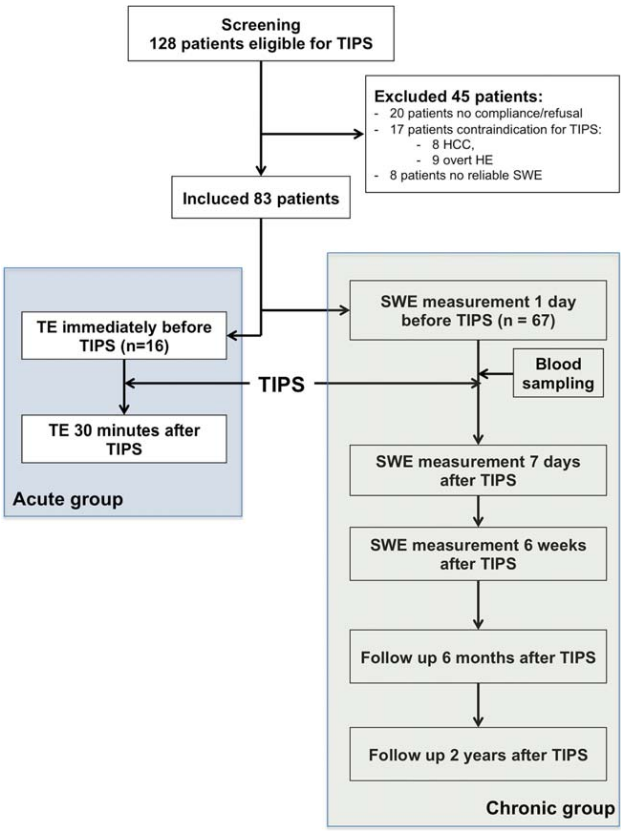


**FIG. 1.** Recruitment and study design. The flowchart illustrates screening and recruitment of the patients. In the acute group (recruitment in Freiburg), patients received liver stiffness measurements using TE immediately before TIPS and 30 minutes after TIPS. In the chronic group of patients (recruitment in Bonn), liver and spleen SWE was performed 1 day before TIPS and 7 days, 6 weeks, and 3 months after TIPS. Patients were followed for a maximum of 2 years. Abbreviations: HCC, hepatocellular carcinoma; HE, hepatic encephalopathy.

## TIPS INSERTION AND PRESSURE MEASUREMENT

TIPS (8 mm and 10 mm, Viatorr; W. L. Gore & Associates, Inc., AZ) insertion was performed as described.[6,28-33] Portal and inferior cava venous pressures were measured invasively using a multichannel monitor (Sirecust; Siemens, Germany). The difference between these pressures was defined as the pressure gradient.

**TABLE 1. General Characteristics of Patients at Baseline**

| Parameters | All | Acute | Chronic | *P* |
|---|---|---|---|---|
| Sex (male/female) | 51/32 | 12/4 | 39/28 | 0.22 |
| Age (years) | 60 (18-81) | 63 (43-77) | 59 (18-81) | 0.19 |
| Etiology (alcohol/viral hepatitis/other) | 53/11/19 | 15/1 | 38/10/19 | 0.06 |
| Indication of TIPS (bleeding/ascites/both) | 33/42/8 | 6/6/4 | 27/36/4 | 0.34 |
| Child-Pugh score | 8 (5-12) | 8 (5-9) | 8 (5-12) | 0.37 |
| MELD score | 9 (6-26) | 12 (8-22) | 9 (6-26) | 0.03 |
| Creatinine (mg/dL) | 1 (0.48-7.61) | 1.1 (0.66-4) | 1 (0.48-7.61) | 0.11 |
| Bilirubin (mg/dL) | 1 (0.16-6.3) | 1.1 (0.4-2.8) | 0.98 (0.16-6.3) | 0.64 |
| INR | 1.1 (0.9-1.63) | 1.2 (1-1.63) | 1.1 (0.9-1.5) | 0.07 |
| γGT (IU/L) | 134.5 (10-779) | 145 (33-593) | 128 (10-779) | 0.09 |
| ALT (IU/L) | 26 (4-121) | 22 (4-65) | 28 (6-121) | 0.08 |
| AST (IU/L) | 39 (13-95) | 43.5 (26-85) | 38 (13-95) | 0.08 |
| TWBC (g/L) | 5.5 (2.18-15.61) | 5 (3-11) | 5.5 (2.18-15.6) | 0.48 |
| Platelets (g/L) | 118 (39-509) | 107 (52-279) | 124 (39-509) | 0.72 |

Data are medians and (ranges). The Wilcoxon test results are shown with *P* value.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, gamma-glutamyltransferase; INR, international normalized ratio.

## STATISTICAL ANALYSIS

A nonparametric Wilcoxon test was used to compare two sets of paired data. In addition, a Friedman test was used for more than two sets of paired data. A Mann-Whitney test was used for unpaired comparisons and a Kruskal-Wallis test if more than two groups were compared. Correlations were analyzed with Spearman's correlation coefficient. Univariate time-to-event analysis was performed to identify parameters which might significantly predict survival. Cox regression analysis (forward stepwise likelihood quotient) using the significant predictors in the univariate analysis was performed to identify independent predictors of survival. Kaplan-Meier curves were used to analyze the survival rates of patients using a log-rank test. Data are presented as median and ranges unless otherwise declared. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL) and Prism 4.0 to plot a part of the graphs.

# Results

## RECRUITMENT AND GENERAL CHARACTERISTICS OF THE COHORT

In total, 128 patients were screened, of whom 45 were excluded. Seventeen patients had contraindications for TIPS: in eight cases, this was due to hepatocellular carcinoma and in nine cases, to severe overt hepatic encephalopathy. Twenty patients were excluded for noncompliance to the protocol, and in eight patients, no reliable SWE could be obtained due to obesity (Fig. 1). Thus, 83 patients were included in the study, 16 patients received in Freiburg the acute protocol using TE and 67 patients received in Bonn the chronic protocol using SWE. Of these, 16 patients were included in the acute group receiving TE measurement of the liver immediately before and 30 minutes after TIPS and 67 patients were included in the chronic group receiving SWE of the liver and spleen 1 day before and 7 days after TIPS (Fig. 1). The latter group was followed for a maximum of 24 months.

The general characteristics of the cohort and of the acute and the chronic groups are outlined in Table 1 and Supporting Tables S1 and S2. Most of the included patients were male (61.4%), with a median age of 60 years. Of these patients, 64% suffered from alcoholic liver cirrhosis, 13% from chronic viral hepatitis, and 23% from other etiologies. In the whole cohort, median Child score was 8 and median Model for End-Stage Liver Disease (MELD) score was 9 (Table 1). Median Chronic Liver Failure Consortium (CLIF-C) AD score was 45.6, while median CLIF-C ACLF score was 35 (Supporting Table S2). No major differences were found between the acute and the chronic groups, except for a higher MELD score in the acute group (Table 1).

## ACUTE EFFECT OF TIPS ON LIVER STIFFNESS

In the acute group, liver stiffness was measured using the XL probe, which is validated for patients with ascites. In this group, TIPS decreased overall liver

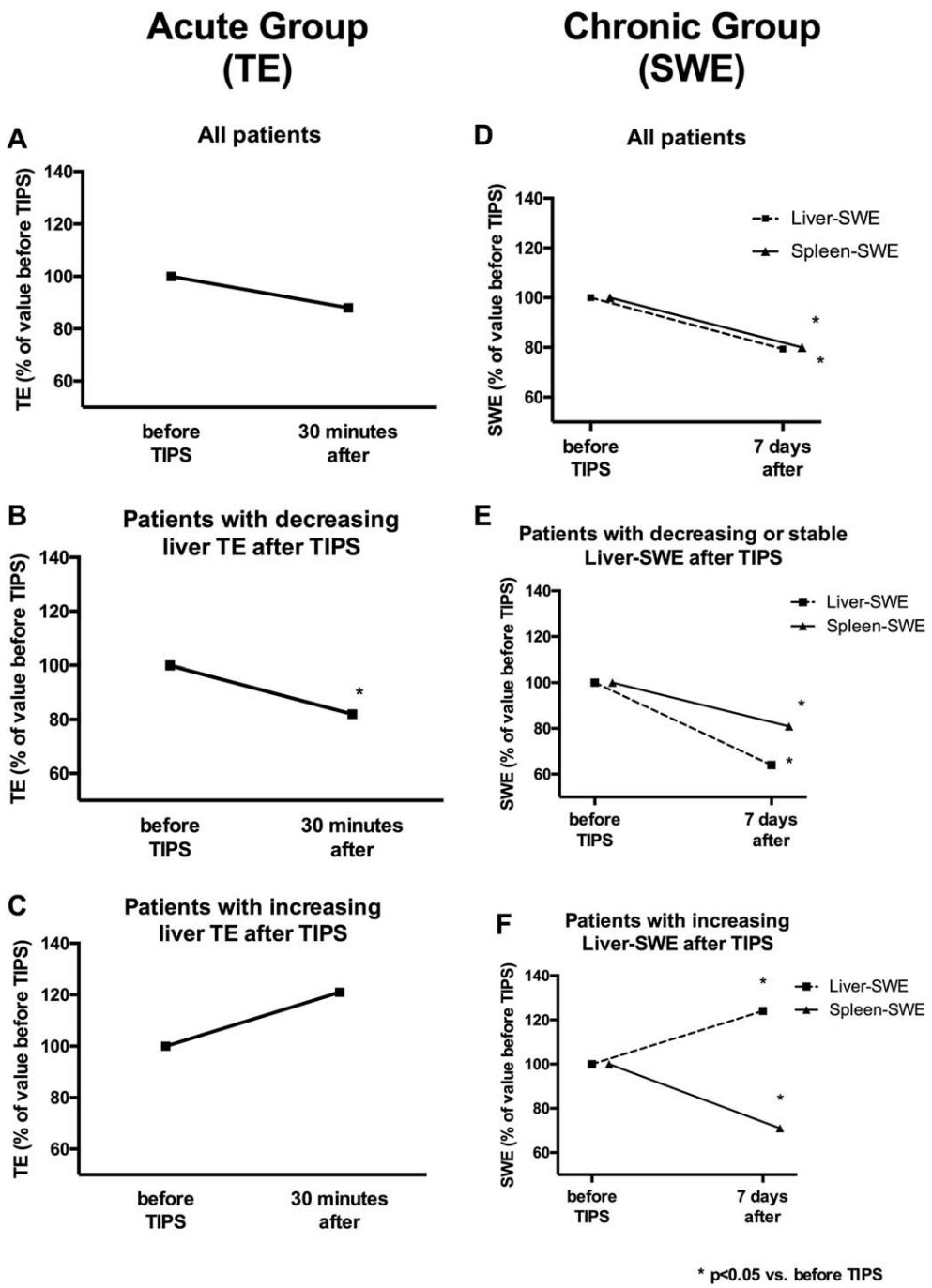


**FIG. 2.** Effect of TIPS on acute and chronic changes of liver stiffness and chronic changes in spleen stiffness. In the acute group, liver TE showed a nonsignificant trend toward decrease in liver stiffness 30 minutes after TIPS (A). In two thirds of the patients from the acute group, liver stiffness significantly decreased (B), while in one third of the patients, liver TE increased without reaching significance (C). In the chronic group, liver and spleen SWE decreased 7 days after TIPS (D). Similarly, two groups of patients could be identified: patients with a decrease in liver SWE and spleen SWE after TIPS (E) and patients with an increase in liver SWE while spleen SWE decreased (F). Data are shown as mean and were analyzed using paired nonparametric comparisons with the Wilcoxon test. Mean values are available in the Supporting Table S3, and individual data are available upon request. *$P < 0.05$ versus before TIPS.

**TABLE 2. Clinical, Biochemical, and Hemodynamic Parameters of Patients in the Chronic Group Stratified by Change of Liver SWE 7 Days After TIPS**

| | Before TIPS | | | After TIPS | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameters | Decreased or unchanged liver SWE | Increased liver SWE | *P* | Decreased or unchanged liver SWE | Increased liver SWE | *P* | *P** | *P*† |
| Child-Pugh score | 8 (5-12) | 7 (5-11) | 0.5 | 7 (5-10) | 7 (5-9) | 0.98 | 0.019 | 0.22 |
| MELD score | 9.4 (6-26) | 8.7 (6-17) | 0.22 | 11 (6-26) | 9.4 (6-21) | 0.45 | 0.13 | 0.04 |
| CLIF-C AD score | 45 (23.3-72) | 47 (31-57) | 0.47 | 47 (30-71) | 49 (37-62) | 0.12 | 0.97 | 0.025 |
| CLIF-C ACLF score | 35 (13-50) | 36 (22-44) | 0.29 | 35 (17-48) | 37 (25-46) | 0.06 | 0.84 | 0.013 |
| Creatinine (mg/dL) | 0.97 (0.48-7.61) | 0.99 (0.56-1.91) | 0.71 | 0.92 (0.46-6.36) | 1.01 (0.66-2.97) | 0.29 | <0.001 | 0.29 |
| Bilirubin (mg/dL) | 1.08 (0.16-6.31) | 0.82 (0.29-4.1) | 0.06 | 1.44 (0.23-7.88) | 1.27 (0.44-3.86) | 0.33 | 0.024 | 0.1 |
| INR | 1.1 (0.9-1.5) | 1.1 (1-1.4) | 0.73 | 1.2 (1-2.2) | 1.2 (1-1.4) | 0.63 | 0.001 | 0.21 |
| ALT (IU/L) | 24.5 (9-121) | 33 (6-90) | 0.12 | 42 (19-781) | 53 (8-1317) | 0.46 | <0.001 | 0.003 |
| AST (IU/L) | 38.5 (15-95) | 34 (13-95) | 0.42 | 51 (24-247) | 59 (12-627) | 0.9 | 0.002 | 0.016 |
| γGT (IU/L) | 131 (10-779) | 108 (53-501) | 0.8 | 172 (32-663) | 118 (61-656) | 0.16 | 0.01 | 0.65 |
| CRP (mg/L) | 10.3 (0-153) | 11.7 (0.3-106) | 0.6 | 11 (1.3-62) | 18 (1-99) | 0.13 | 0.18 | 0.022 |
| TWBC (G/L) | 5.95 (2.18-15.61) | 5.21 (2.24-8.07) | 0.1 | 5.94 (3.12-15.92) | 6.42 (3.13-12.09) | 0.9 | 0.72 | 0.04 |
| Pressure gradient (mm Hg) | 21 (10-42) | 18 (12-33) | 0.02 | 8.5 (1-36) | 7 (4-13) | 0.38 | <0.001 | <0.001 |
| Portal pressure (mm Hg) | 27 (15-46) | 26 (14-35) | 0.53 | 18 (8-43) | 19 (9-25) | 0.62 | <0.001 | <0.001 |
| Central venous pressure (mm Hg) | 6 (0-17) | 8 (2-22) | 0.09 | 10 (2-22) | 11 (1-19) | 0.19 | <0.001 | <0.001 |

Data are shown as medians and (ranges). The Wilcoxon test results are shown with *P* value.
*Intragroup comparison of patients with decreased or unchanged liver SWE after TIPS.
†Intragroup comparison of patients with increased liver SWE after TIPS.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, gamma-glutamyltransferase; INR, international normalized ratio.

stiffness measured by TE, although without statistical significance (Fig. 2A; Supporting Table S3A). Interestingly, in 11 patients a significant decrease of TE (Fig. 2B; Supporting Table S3A) and in 5 patients an increase of TE (Fig. 2C; Supporting Table S3A) was found.

In these two different groups of patients, no significant changes were observed at baseline, except for a tendency to higher bilirubin, international normalized ratio, alanine aminotransferase, aspartate aminotransferase, and total white blood cell count (TWBC) in the group with increase in liver stiffness after TIPS (Supporting Table S1).

A recent series of 7 patients received TE and SWE of the liver before, 30 minutes after, and 7 days after TIPS within a context of another study. Importantly, 30 minutes after TIPS, liver SWE and TE values remained unchanged on day 7 after TIPS (data not shown).

## EFFECT OF TIPS ON LIVER AND SPLEEN STIFFNESS AFTER 7 DAYS

In the chronic group, reliable liver SWE measurements were obtained in 67 patients and reliable spleen SWE measurements could be documented in 62 patients.

Liver and spleen stiffness measured by SWE decreased significantly after 7 days (Fig. 2D; Supporting Table S3B). Similar to the acute group, in these patients, two different groups could be distinguished. There were 45 patients with a decrease or a change in liver SWE of <10% (Fig. 2E; Supporting Table S3B) because this was the variance within measurements in the same patients in previous studies.[13,14] These patients also showed a significant decrease in spleen SWE (Fig. 2E; Supporting Table S3B), whereas 22 patients showed an increase of >10% in liver SWE 7 days after TIPS and at the same time a significant decrease in spleen SWE (Fig. 2F; Supporting Table S3B).

Comparing the baseline data of these two groups receiving SWE, no difference was found except for a tendency toward higher CLIF-C AD score, CLIF-C ACLF score, alanine aminotransferase, and C-reactive protein (CRP) but lower Child-Pugh score, MELD score, bilirubin, aspartate aminotransferase, gamma-glutamyltransferase, TWBC, and pressure gradient (Table 2). However, none of these parameters were statistically significant, except for pressure gradient

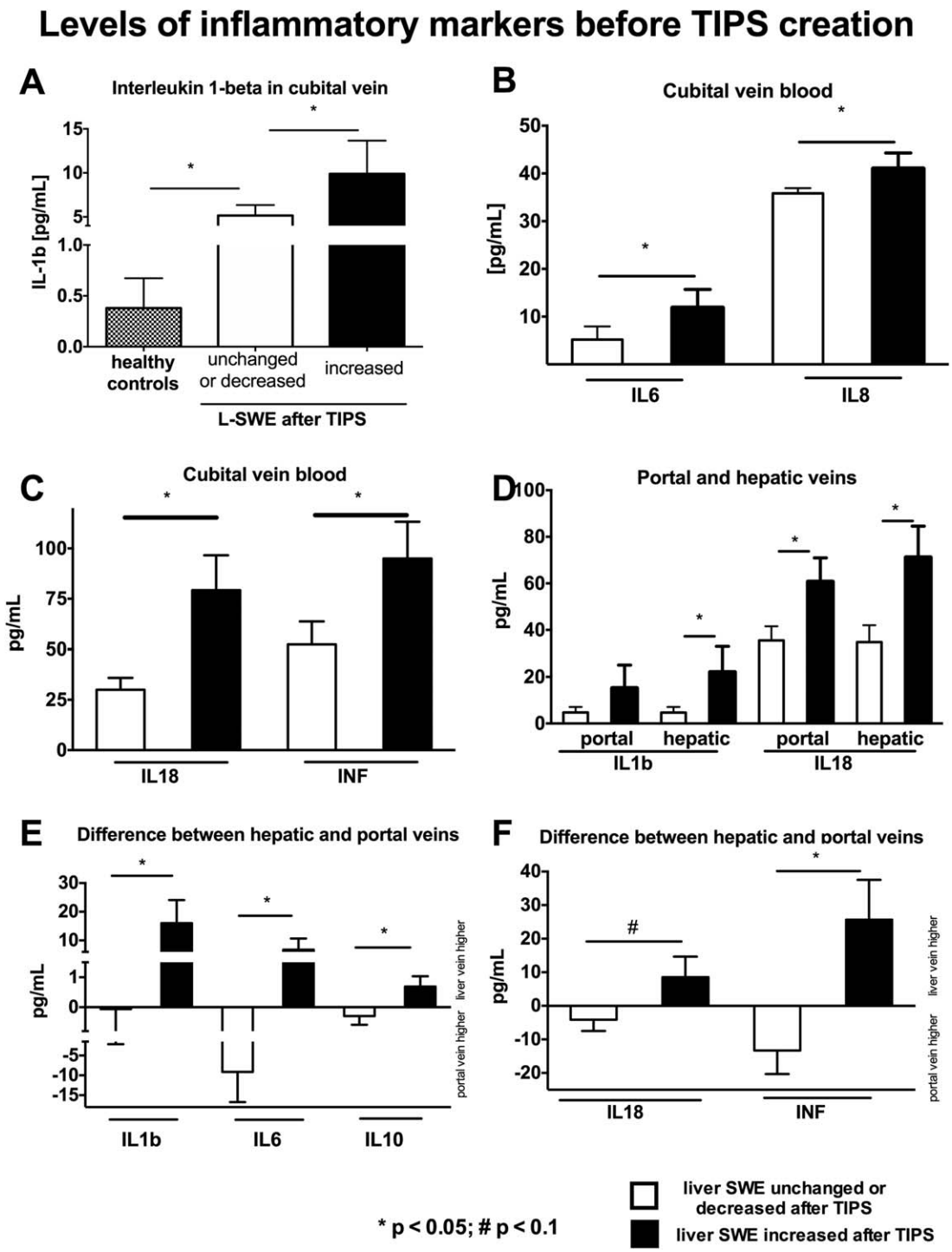


**FIG. 3.** Levels of inflammatory cytokines in samples of cubital, portal, and hepatic vein blood collected at TIPS. IL1$\beta$ levels in the cubital vein were higher in the group with increased liver SWE after TIPS (n = 15) compared to the rest of the patients (n = 39) and healthy controls (n = 42), while all patients with cirrhosis showed higher levels than healthy controls (A). Similarly, IL6 and IL8 in (B) as well as IL18 and interferon-gamma in (C) showed higher levels in the group with increased liver SWE after TIPS (n = 9) compared to the rest of the patients (n = 11). Comparing levels of IL1$\beta$ and IL18 in the portal and hepatic veins reveals similar results to findings from peripheral blood (D). Interestingly, intraindividual differences of IL1$\beta$, IL6, and IL10 between the levels found in the hepatic and portal veins were higher in the group with increased liver SWE after TIPS (n = 9) compared to the rest of the patients (n = 11) (E). Similarly, the difference between hepatic and portal venous levels of IL18 and interferon-gamma was also higher in the high-risk group (F). Data are shown as mean and standard error of mean, and comparison between groups was performed using the nonparametric Mann-Whitney U test. $^{*}P < 0.05$, $^{\#}P < 0.1$. Abbreviation: INF, interferon-gamma.

before TIPS. In summary, the baseline clinical and laboratory parameters of the entire cohort revealed no statistically significant differences (Supporting Table S2). Also, the stent diameter had no effect on development of liver SWE. Importantly, development of liver SWE was similar for the different indications for TIPS. Therefore, the cohorts of different indications were analyzed together.

## INFLAMMATORY MARKERS BEFORE TIPS AND RESPONSE OF LIVER SWE TO TIPS

Yet, it remains unclear as to why liver stiffness might increase in around one third of the patients after TIPS, despite portal pressure decreases of >50%, which is reflected by a decrease in spleen SWE (Fig. 2F; Supporting Table S3B).

To address this question, levels of proinflammatory cytokines were measured in peripheral blood samples of these patients. Interestingly, IL1$\beta$ was significantly higher in the group with increased liver SWE when compared to the other patients. As expected, patients with cirrhosis showed higher levels of IL1$\beta$ compared to controls (Fig. 3A). Similarly, peripheral blood levels of IL6, IL8, IL18, and interferon-gamma were also higher in patients with an increase in liver SWE after TIPS (Fig. 3B,C). These data suggest that systemic inflammation is already increased at TIPS insertion in patients who develop an increased liver SWE after TIPS.

However, the source of this systemic proinflammatory situation remains unclear. To address this question, cytokine levels were measured in the portal and hepatic veins. Interestingly, only IL1$\beta$ and IL18 showed higher levels in the portal and hepatic veins in the group of patients with an increase in liver SWE after TIPS (Fig. 3D). However, comparison of the difference in the levels of each cytokine between the hepatic and portal veins revealed a significant difference between the groups (Fig. 3E,F). The gradient of proinflammatory cytokines IL1$\beta$, IL6, IL10, IL18, and interferon-gamma across the liver (levels in the hepatic veins were higher than in the portal veins) was significantly higher in patients with increased liver SWE after TIPS (Fig. 3E,F). These data suggest that in these patients the liver has already reached a strong proinflammatory situation, as reflected in the higher levels of systemic inflammation measured in the peripheral blood samples (Fig. 3A-C), but without, as yet, evidence in clinical and laboratory values (Table 2).

## SHORT-TERM CLINICAL EVOLUTION AFTER TIPS AND RESPONSE OF LIVER SWE TO TIPS

Although all patients experienced a similar decrease in portal pressure gradient after TIPS (Table 2), patients with an increase of liver SWE after TIPS seem to develop increased systemic and hepatic inflammation at TIPS insertion. Therefore, the outcome after TIPS was observed in more detail (Table 2).

Patients with decreased or unchanged liver SWE after TIPS showed a significant improvement in Child-Pugh score and creatinine, while liver function tests deteriorated significantly and MELD, CLIF-C AD, and CLIF-C ACLF scores, as well as TWBC and CRP, remained unchanged (Table 2). By contrast, patients with an increase in liver SWE after TIPS showed significantly higher MELD, CLIF-C AD, and CLIF-C ACLF scores, as well as higher levels of alanine aminotransferase, aspartate aminotransferase, CRP, and TWBC (Table 2). The latter patients showed no signs of congestions (heart failure) or hepatic ischemia. Moreover, while ascites disappeared in a considerable proportion of the patients with decreased or unchanged liver SWE after TIPS, the response to TIPS was significantly less in patients with an increased liver SWE after TIPS (Fig. 4A). These data suggest that increased liver SWE after TIPS is associated with an inflammatory response mainly of hepatic origin, preventing clinical improvement in these patients after TIPS.

## DEVELOPMENT OF ORGAN FAILURE AND LONG-TERM SURVIVAL AFTER TIPS

Because systemic inflammation is a hallmark of the development of organ failure and ultimately ACLF in liver cirrhosis, the incidence of organ failure was assessed 6 months after TIPS (Fig. 4B). Indeed, there was a significantly higher incidence of organ failure in patients with an increase in liver SWE after TIPS (Fig. 4B). Moreover, 2-year survival was significantly worse in patients with an increase in liver SWE after TIPS (Fig. 4C). None of the patients has been transplanted.

When analyzing the parameters associated with survival in these patients, decreased or unchanged liver SWE 7 days after TIPS correlated significantly (hazard ratio [HR], 0.064; 95% confidence interval [CI],

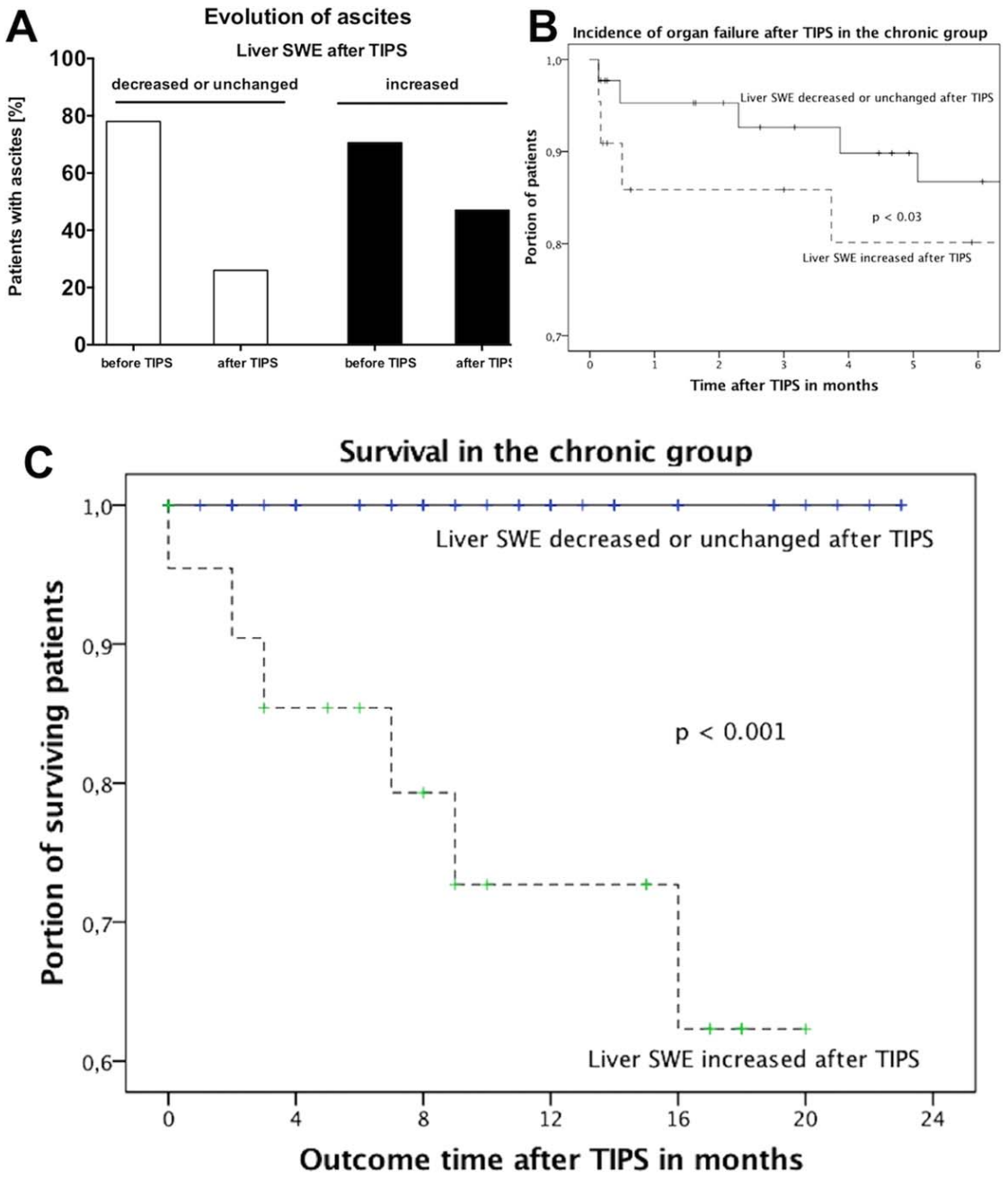


**FIG. 4.** Outcome of the patients in the follow-up. Among patients with increased liver SWE after TIPS, response of ascites to TIPS occurred in only 20% of patients compared to 60% of patients in the group with decreased or unchanged liver SWE after TIPS (A). Incidence of organ failure after 6 months was significantly higher in the high-risk group, as shown by Kaplan-Meier plot and analyzed by log-rank test (B). Importantly, the overall survival rate was unimpaired in patients with decreased or unchanged liver SWE after TIPS, while mortality was increased in patients with increased liver SWE after TIPS, as shown by Kaplan-Meier plot and analyzed by log-rank test (C).

0.007-0.547; $P = 0.012$) with survival (Table 3). Further parameters, such as age, CLIF-C AD score, creatinine 7 days after TIPS, as well as CRP 7 days after TIPS were identified as predictors of survival in univariate time-to-event analysis (Table 3), while small liver size, low platelet count, and low hemoglobin levels were not significantly associated with survival in this cohort. Importantly, the MELD score showed an

**TABLE 3. Univariate and Multivariate Time-to-Event Analysis and Cox Regression Analysis (Forward Stepwise Likelihood Quotient) in the Chronic Group to Predict Survival With Death as Endpoint**

| Parameters | *P* | HR | 95% CI for HR |
|---|---|---|---|
| *Univariate time-to-event analysis* | | | |
| Age at time of insertion | 0.033 | 1.099 | 1.008-1.199 |
| CLIF C AD score before TIPS | 0.042 | 0.103 | 0.012-0.925 |
| CRP after TIPS | 0.008 | 1.051 | 1.013-1.09 |
| Creatinine after TIPS | 0.044 | 0.019 | 0.001-0.906 |
| Liver SWE decreased/unchanged | 0.012 | 0.064 | 0.007-0.547 |
| *Forward stepwise likelihood quotient* | | | |
| CRP after TIPS | 0.03 | 1.044 | 1.004-1.084 |
| Liver SWE decreased/unchanged | 0.022 | 0.079 | 0.009-0.690 |

HR of 1.001 with a 95% CI of 0.83-1.19 and a *P* value of 0.58. Therefore, the MELD score was also not included in the multivariate analysis.

Multivariable Cox regression analysis of these significant variables identified CRP (HR, 1.044; 95% CI, 1.004-1.084; $P = 0.03$) and decreased or unchanged liver SWE (HR, 0.079; 95% CI, 0.009-0.690; $P = 0.022$), both 7 days after TIPS, as independent predictors of survival (Table 3).

# Discussion

This prospective observational cohort study demonstrates that liver stiffness after TIPS is a stratification tool for high-risk patients. Moreover, this study possibly explains the increase of liver stiffness after TIPS in some patients, while it also confirms the important role of hepatically derived systemic inflammation in the outcome of patients with decompensated cirrhosis.

Liver stiffness has been introduced in the field of digestive diseases to identify significant liver fibrosis.[26] Liver stiffness assessed by TE[9-11,24,26,34,35] or SWE[12,36] might identify clinically significant portal hypertension. However, liver TE and SWE very much depend on other factors, such as alcohol and meal ingestion, right heart function, volume changes, arterial pressure, cholestasis, and inflammation.[22,23,37-42] While TIPS decreased portal pressure by >50%, liver stiffness was not proportionally decreased, as assessed by two different techniques of measuring liver stiffness, at different time points after TIPS and in different centers. In a similar portion of patients, liver stiffness increased after TIPS, regardless of TIPS indication or diameter.

Moreover, the present study defines the prognostic impact of an increase in liver stiffness after TIPS. These patients could only be identified by the increase of liver stiffness after TIPS and not by any other clinical or routine laboratory parameters. The increase occurred very early after TIPS insertion, as shown in the acute cohort, and continued up to the next follow-up visit. This finding outperforms the MELD score for overall survival, which was designed for outcome assessment 3 months after TIPS.[43]

The pathophysiological processes responsible for the deterioration of clinical outcome and increase in liver stiffness in this subgroup of patients receiving TIPS are insufficiently explored. Frequently, the opening of the route for portal venous blood into the systemic circulation by TIPS has been blamed for increased systemic inflammation in TIPS patients. The present study clearly demonstrates that systemic inflammation had already increased in these patients before TIPS, as shown by higher levels of IL1$\beta$, IL6, IL8, IL18, as well as interferon-gamma in peripheral blood. Moreover, levels of IL1$\beta$ and IL18 were higher in the hepatic and portal veins of patients receiving TIPS. Interestingly, in patients who developed increased liver stiffness after TIPS and who had poor outcome, much higher inflammatory markers were found in the hepatic veins than in the portal veins. This suggests that the source of systemic inflammation in these patients is the diseased liver, while in patients who recovered after TIPS and who had a good prognosis, levels of inflammatory cytokines in the hepatic veins were lower than in the portal veins. Apart from the acute testing of liver stiffness in these patients, the difference between portal and hepatic levels of cytokines appears also to be fitting to distinguish between patients with better or worse prognosis. Especially the increase of IL6, which is a marker of systemic inflammation, seems to derive from the injury the liver had sustained already before TIPS insertion. IL6 has been shown to predict variceal bleeding as well as mortality in patients with ACLF.[2,44] In this study, we suggest that it might derive from the immune cells of the injured liver. Moreover, at the next follow-up, systemic inflammation aggravated and was clinically evident, as shown by increases in CRP and

TWBC. This was also mirrored by AD and ACLF scores, which predict the incidence of ACLF and survival.[45,46] Recently, the close relationship between systemic inflammation, organ failure, ACLF, and mortality has been shown in the CANONIC cohort.[2] Indeed, the increase of liver stiffness after TIPS identified patients developing organ failure and independently predicted mortality in patients receiving TIPS, which is also supported by previous observations of our group ascribing an important prognostic value to inflammatory cytokines.[28-30,32]

However, it is difficult to predict whether patients with increased liver stiffness after TIPS would not have developed organ failure or ACLF and would not have shown a higher mortality without TIPS, a question to be addressed in future studies. Turning the argument around, TIPS might prevent ACLF in patients with decreased stiffness because portal hypertension is a predisposing factor for ACLF.[47] TIPS might prevent ACLF in several situations[7]; e.g., early TIPS prevents the inflammatory response induced by further bleeding and improves survival.[4] Apart from these open questions, the present study demonstrates and offers an interesting and easy approach for the clinical routine to identify high-risk patients after TIPS.

Our data suggest that liver stiffness increases immediately after TIPS. Interestingly, these patients do not show higher stiffness before TIPS. Although liver stiffness might change after alcohol withdrawal[37] and correlates with liver damage,[48] the rather prompt change of liver stiffness only minutes after the TIPS procedure suggests pressure changes as the underlying cause. In general, liver stiffness measurement is an integrative measure of fibrosis, inflammation, and sinusoidal pressure.[21-23] In this system, TIPS might decrease the portal pressure and portal perfusion of the sinusoids but increase arterial perfusion of the sinusoids,[49] while in our patients, fibrosis and arterial pressures remained unchanged. The only difference between the groups of patients in whom liver stiffness increased after TIPS was the presence of hepatic and systemic inflammation. More importantly, it has recently been shown that systemic inflammation increases intrahepatic sinusoidal resistance.[50] Thus, to speculate, one is tempted to assume that challenge of the sinusoids with twice the hepatic arterial perfusion[49] might be the reason for the acute increase in stiffness after TIPS.

Another important finding of this study is that liver SWE is unsuitable to reflect changes in the portal pressure gradient in decompensated patients. Previous data have shown that liver TE or SWE possibly reflects the effect of nonselective beta-blockers[16,17] and that SWE also excludes or includes with high accuracy the presence of clinically significant portal hypertension,[12,17,36] a prerequisite for decompensation, complications, and, ultimately, ACLF.[1,7,47] Recent studies have suggested that spleen SWE is very important for the diagnosis of portal hypertension and changes in portal pressure.[13,14,19,20] Indeed, our study might provide an explanation for these findings.

Despite the fact that our study is a prospective observational study carried out in two centers, with two different well-characterized patient cohorts at two different time points, several limitations must be mentioned. First, this is an observational study, and due to its exploratory nature, it lacks a clear hypothesis and sample size calculation. Although we used the XL probe for TE, which has been validated for ascites, this may be cause for some criticism regarding reliability. Although in the patients of this study, we could not provide data of TE and SWE from the same patients at the same time points, within the context of another study, we recently performed a small series of 7 patients with SWE and TE of the liver at the same time, before, 30 minutes after, and 7 days after TIPS. Another limitation is that in the smaller TE group, no follow-up data were collected because this was an acute study. However, recently, together with others, we found very similar results in the TE and the SWE response to different challenges to the portal venous system.[38] Therefore, we assume that this is also the case in the present study. A further limitation is the rather small size of the cohort. However, this is the largest cohort in patients with TIPS. Despite the rather small group of patients, our study demonstrated high examiner independency because at two different sites, using two different techniques at different time points to measure liver stiffness, the results were very similar. Finally, because post-TIPS SWE change is a marker of poor prognosis after TIPS, it is not helpful to select patients before TIPS. However, it might be helpful for risk stratification in patients receiving TIPS because they will receive TIPS for a clear indication unrelated to SWE. Moreover, the measurement of systemic inflammation, as demonstrated in our cohort, might be useful to stratify patients before TIPS insertion.

In conclusion, this study demonstrates that it is mainly liver-derived systemic inflammation that predisposes patients receiving TIPS to develop organ failure and ACLF. These patients show an increased liver stiffness after TIPS immediately and at follow-up.

Therefore, inflammation at TIPS insertion and increase of liver stiffness after TIPS insertion predict organ failure and death in these patients, who require closer management.

*Acknowledgment:* We thank Gudrun Hack, Silke Bellinghausen, Nadine Köstlmeier, Julia Groffy, Teresija Boscnic, and Kristin Gehrmann for excellent technical assistance and Sabine Dentler for critical reading.

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# Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29612/suppinfo.

3.4 Die linksventrikuläre Kontraktilität kann Patienten identifizieren, die nach Anlage eines transjugulären intrahepatischen portosystemischen Shunts ein erhöhtes Risiko für die Entwicklung eines akut-auf-chronisches Leberversagen und Tod aufweisen.

**Jansen C***, Schröder A*, Schueler R, Lehmann J, Praktiknjo M, Uschner FE, Schierwagen R, Thomas D, Monteiro S, Nickenig G, Strassburg CP, Meyer C, Arroyo V, Hammerstingl C, Trebicka J. Left Ventricular Longitudinal Contractility Predicts Acute-on-Chronic Liver Failure Development and Mortality After Transjugular Intrahepatic Portosystemic Shunt. Hepatol Commun. 2019 Jan 22;3(3):340-347. doi: 10.1002/hep4.1308. PMID: 30984902; PMCID: PMC6444053.

Zielsetzung der Arbeit – Ziel dieser Studie war es, die Rolle der Kontraktilität, unter Nutzung der Speckle-Tracking-Echokardiographie nach transjugulärer intrahepatischer portosystemischer Shunt-Anlage in Hinblick auf dessen prognostischen Wert zu untersuchen.

Methoden und Ergebnisse – Es wurden 114 Patienten (48 weibliche Patientinnen) aus dem Evaluierungsprogramm für einen transjugulären intrahepatischen portosystemischen Shunt wurden in diese Studie eingeschlossen. Wir analysierten die Assoziation der kardialen Kontraktilität mit der Gesamtsterblichkeit und der Entwicklung eines akut-auf-chronischen Leberversages in dieser Kohorte. Die kardiale Kontraktilität konnte als unabhängiger Prädiktor für die Mortalität identifiziert werden.

Schlussfolgerungen – Die kardiale Kontraktilität ist ein Parameter, um Risikopatienten zu identifizieren, welche eine hohe Wahrscheinlichkeit aufweisen, ein akut-auf-chronisches Leberversages nach transjugulärer intrahepatischer portosystemischer Shunt-Anlage zu entwickeln.

BRIEF REPORTS | HEPATOLOGY COMMUNICATIONS, VOL. 3, NO. 3, 2019

# Left Ventricular Longitudinal Contractility Predicts Acute-on-Chronic Liver Failure Development and Mortality After Transjugular Intrahepatic Portosystemic Shunt

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**Acute deterioration of liver cirrhosis (e.g., infections, acute-on-chronic liver failure [ACLF]) requires an increase in cardiac contractility. The insufficiency to respond to these situations could be deleterious. Left ventricular global longitudinal strain (LV-GLS) has been shown to reflect left cardiac contractility in cirrhosis better than other parameters and might bear prognostic value. Therefore, this retrospective study investigated the role of LV-GLS in the outcome after transjugular intrahepatic portosystemic shunt (TIPS) and the development of ACLF. We included 114 patients (48 female patients) from the Noninvasive Evaluation Program for TIPS and Their Follow-Up Network (NEPTUN) cohort. This number provided sufficient quality and structured follow-up with the possibility of calculating major scores (Child, Model for End-Stage Liver Disease [MELD], Chronic Liver Failure Consortium acute decompensation [CLIF-C AD] scores) and recording of the events (development of decompensation episode and ACLF). We analyzed the association of LV-GLS with overall mortality and development of ACLF in patients with TIPS. LV-GLS was independently associated with overall mortality (hazard ratio [HR], 1.123; 95% confidence interval [CI],1.010-1.250) together with aspartate aminotransferase (HR, 1.009; 95% CI, 1.004-1.014) and CLIF-C AD score (HR, 1.080; 95% CI, 1.018-1.137). Area under the receiver operating characteristic curve (AUROC) analysis for LV-GLS for overall survival showed higher area under the curve (AUC) than MELD and CLIF-C AD scores (AUC, 0.688 versus 0.646 and 0.573, respectively). The best AUROC-determined LV-GLS cutoff was –16.6% to identify patients with a significantly worse outcome after TIPS at 3 months, 6 months, and overall. LV-GLS was independently associated with development of ACLF (HR, 1.613; 95% CI, 1.025-2.540) together with a MELD score above 15 (HR, 2.222; 95% CI, 1.400-3.528). *Conclusion:* LV-GLS is useful for identifying patients at risk of developing ACLF and a worse outcome after TIPS. Although validation is required, this tool might help to stratify risk in patients receiving TIPS. (*Hepatology Communications* 2019;3:340-347).**

Portal hypertension in liver cirrhosis is frequent, causes serious complications, and predisposes for the development of acute-on-chronic liver failure (ACLF).[1] Insertion of a transjugular intrahepatic portosystemic shunt (TIPS) induces a prompt decompression of the portal venous system and

*Abbreviations: A, peak late diastolic atrial filling velocity; ACLF, acute-on-chronic liver failure; AST, aspartate aminotransferase; AUC, area under the curve; CLIF-C AD, Chronic Liver Failure Consortium acute decompensation; E, peak early diastolic filling velocity; E', peak early diastolic mitral annular velocity; GLS, global longitudinal strain; HR, hazard ratio; LV, left ventricular; MELD, Model for End-Stage Liver Disease; NEPTUN, Noninvasive Evaluation Program for TIPS and Their Follow-Up Network; STE, speckle tracking echocardiography; TIPS, transjugular intrahepatic portosystemic shunt; TTE, transthoracic echocardiography.*

*Received July 12, 2018; accepted December 20, 2018.*

*Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1308/suppinfo.*

*Supported by the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18 to J.T.), the European Union's Horizon 2020 Research and Innovation Program (No. 668031 to J.T.) and Societal Challenges (Health, demographic change, and well-being, No. 731875 to J.T.), and the Cellex Foundation (PREDICT to V.A., J.T.).*

effectively treats complications of portal hypertension; however, careful selection of patients is required.[2,3] TIPS insertion increases central blood volume despite a severe aggravation of preload.[4] These changes increase cardiac workload and encroach on cardiac reserves, which are especially decreased in patients with decompensated cirrhosis.[5-7] In addition to TIPS, other physical stress situations (e.g., infections) could require a further increase in cardiac output, which might not be possible in an impaired hemodynamic situation.[7] Therefore, assessment of the left ventricular function might help in the selection of patients for TIPS and enable stratification of management after TIPS.

In the development of ACLF, circulatory dysfunction has been defined only by arterial pressure and/or use of vasopressors[8,9]; this describes a severely deranged hemodynamic situation but may not be sensitive enough in earlier but still relevant stages. Diastolic dysfunction (assessed by the early peak diastolic filling velocity to the peak late diastolic mitral annular velocity ratio [E:A] and the early peak diastolic filling velocity to the peak early diastolic mitral annular velocity [E:E′] ratio) could be associated with outcome.[10,11] However, its prognostic value is still debated.[12] Moreover, the assessment of this parameter is angle dependent and limited by frame rate. Systolic function, usually assessed by ejection fraction, is easily misinterpreted in hyperdynamic circulation, e.g., in decompensated cirrhosis. Two-dimensional speckle tracking echocardiography (STE) is an objective surrogate of cardiac contractility.[13,14] Left ventricular global longitudinal strain (LV-GLS) is associated with outcome in patients with cirrhosis.[4,15] In the present study, we evaluated for the first time the prognostic value of LV-GLS for the development of ACLF and mortality in patients with cirrhosis treated with TIPS.

# Patients and Methods

## STUDY DESIGN AND PATIENTS

We included patients with sufficient quality of STE and structured follow-up with the possibility of calculating major prognostic scores. A total of 110 patients were excluded from the study for the following reasons: poor quality of transthoracic echocardiography (TTE) before TIPS in 32 patients, prior history of ischemic heart disease in 31 patients, other chronic heart failure in 23 patients, severe valvular diseases in

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View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep4.1308

Potential conflict of interest: Nothing to report.

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18 patients, and atrial fibrillation in 6 patients. The main endpoints of the study were development of ACLF, as defined by the CANONIC study,[8,9] and overall mortality. This analysis included 114 patients (66 male adults) from the Noninvasive Evaluation Program for TIPS and Their Follow-Up Network (NEPTUN) cohort. NEPTUN consists of patients with liver cirrhosis receiving TIPS at the Department of Internal Medicine I, University of Bonn, Germany, and receiving a structured routine evaluation and follow-up. The patients included in this analysis received TIPS between June 2008 and June 2015. The diagnosis of cirrhosis was based on clinical, hemodynamic, and biochemical parameters as well as ultrasound and/or biopsy criteria. The local ethics committee of the University of Bonn (No. 121/14) approved the study in accordance with the Declaration of Helsinki, and the patients gave their signed written informed consent.

## CONVENTIONAL ECHOCARDIOGRAPHY

TTE examination was performed using commercially available equipment (Vivid 7 [General Electric Medical Health, Waukesha, WI]; iE33 [Philips Medical Systems, Koninklijke N.V., Heerlen, the Netherlands]) with a 2.5-MHz phased-array transducer as described.[15] Briefly, the Simpson method was used to calculate the LV ejection fraction from the four-chamber view. To assess LV diastolic dysfunction, conventional pulsed-wave Doppler techniques were used with the sample volume positioned at the tip of the mitral valve leaflets to measure mitral inflow velocities. E, A, and the E:A ratio were calculated. A sample volume was taken at the septal wall on a level with the mitral annulus to measure E′ and the E:E′ ratio to differentiate between normal and grade 2 impaired LV diastolic function as described.[15]

## STE

STE was performed as described.[15] Briefly, four-chamber views were recorded during routine TTE in the context of evaluation for TIPS insertion. Image Arena 4.3 (TomTec Imaging Systems GmbH, 2001-2010, Unterschleissheim, Germany) automatically measures frame by frame myocardial shortening of a distance between two points on the myocardium. Longitudinal and radial segmental two-dimensional strains of the left ventricle were analyzed in the apical four-chamber view. For this purpose, the endocardial boundary in the left ventricle was set manually. Each ventricle was automatically divided into six segments: basal lateral/septal, mid lateral/septal, and apical lateral/septal. LV-GLS was calculated.

## STATISTICAL ANALYSIS

We used the nonparametric Mann-Whitney test for unpaired comparisons. Correlations were analyzed with Spearman's correlation coefficient. Univariate time-to-event analysis was performed to identify parameters that significantly predict survival. Multivariate Cox regression analysis (forward stepwise likelihood quotient) using the significant predictors in the univariate analysis was performed to identify independent predictors of survival. For the selection of cut-off values, receiver operating characteristics (ROC) analysis with survival as endpoint was calculated. Kaplan-Meier curves were used to compare the survival rates of patients using the log-rank test. Statistical analysis was performed using SPSS 22 for Windows (SPSS, Inc., Chicago, IL).

# Results

## GENERAL PATIENT CHARACTERISTICS

Clinical characteristics of the patients are listed in Table 1. There were 48 female and 66 male patients with a median age of 59 years (range, 18-80 years). The etiology of cirrhosis was alcohol in 74 patients, chronic viral hepatitis in 14 patients, and other chronic liver disease in 26 patients. Twenty-six patients presented with Child A, 76 with Child B, and 12 with Child C liver cirrhosis. The median Model for End-Stage Liver Disease (MELD) score was 11. Forty patients showed no ascites at the time of TIPS insertion, 29 patients had a history of at least one episode of hepatorenal syndrome, and 22 patients had experienced at least one episode of hepatic encephalopathy (Table 1).

The more frequent indication for TIPS was refractory ascites (62%, 71 patients). The median portosystemic pressure gradient was 19 mm Hg. The median Chronic Liver Failure Consortium acute

**TABLE 1. GENERAL PATIENT CHARACTERISTICS OF THE COHORT**

| | Parameters | Values* |
|---|---|---|
| General Characteristics | Sex (male/female) | 66/48 |
| | Indication (bleeding/ascites/both) | 40/71/3 |
| | Age median (range) | 59 (18-80) |
| | Etiology (alcohol/viral/other) | 74/14/26 |
| | Child category (A/B/C) | 26/76/12 |
| | MELD score median (range) | 11 (6-40) |
| | Ascites (absent/present) | 40/74 |
| | Previous HRS (no/yes) | 85/29 |
| | Previous HE (0/I-II/III-IV) | 92/21/1 |
| | Central venous pressure (mm Hg) | 8 (0-23) |
| | Portal pressure (mm Hg) | 28 (10-49) |
| | Pressure gradient (mm Hg) | 19 (7-42) |
| | CLIF-C AD score | 21 (7-27) |
| Biochemical Characteristics | Sodium (mmol/L) | 138 (114-146) |
| | Creatinine (mg/dL) | 1.3 (0.5-8.5) |
| | Bilirubin (mg/dL) | 1.0 (0.2-30.3) |
| | CRP (mg/L) | 10.3 (0.3-107.0) |
| | Albumin (g/L) | 31.1 (14.6-43.9) |
| | INR | 1.1 (0.9-2.3) |
| | TWC (G/L) | 6.0 (1.7-20.7) |
| | Hb (g/dL) | 10.3 (6.5-15.7) |
| | Platelets (G/L) | 120 (28-679) |
| | GGT (U/L) | 126 (23-788) |
| | ALT (U/L) | 24 (8-120) |
| | AST (U/L) | 39 (11-361) |
| Cardiac Parameters | EDV (mL) | 97 (39-195) |
| | ESV (mL) | 34 (12-85) |
| | EF (%) | 67 (51-85) |
| | Diastolic dysfunction present (no/yes) | 61/53 |
| | LV-GLS (%) | –16.6 (–7.0 to –26.7) |

*Unless otherwise specified, values represent median (range). Abbreviations: ALT, alanine aminotransferase; CRP, C-reactive protein; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; GGT, gamma-glutamyltransferase; Hb, hemoglobin; HE, hepatic encephalopathy; HRS, hepatorenal syndrome; INR, international normalized ratio; TWC, total white blood cell count.

decompensation (CLIF-C AD) score was low at 21, indicating that these decompensated patients were stabilized at the time of TIPS insertion (Table 1). The CLIF-C AD score includes surrogate parameters of systemic inflammation, which is missing in the MELD score.

In TTE and STE, diastolic dysfunction was present in 53 patients (46%), and none of the patients had a compromised ejection fraction (Table 1). LV-GLS ranged from –7% to –26.7%, with a median of –16.6%. More detailed information on the TTE data of the cohort is shown in Supporting Table S1. Importantly, no correlation between the baseline parameters and LV-GLS could be assessed (data not shown).

## THE ASSOCIATION OF LV-GLS WITH DEVELOPMENT OF ACLF AND MORTALITY

During the 24 months of structured per protocol follow-up, 54 patients developed ACLF according to European Association for the Study of the Liver-CLIF criteria. ACLF grade I was present in 17 patients, grade II in 19 patients, and grade III in 18 patients. The median time span from insertion of TIPS to occurrence of ACLF was 65 days. More detailed information regarding the different ACLF grades is provided in Supporting Table S3.

The most often occurring type of organ failure was kidney injury. In 28 out of 54 patients developing ACLF, ACLF was the cause of death. LV-GLS of patients developing ACLF after TIPS was similar compared to LV-GLS of patients who did not develop the syndrome. Interestingly, LV-GLS, together with a MELD score above 15, was independently associated with development of ACLF (Table 2). This showed the time association between LV-GLS and ACLF.

In univariate time-to-event analysis, a number of parameters were identified to be associated with overall survival. In addition to a MELD score above 15, serum bilirubin, albumin, aspartate aminotransferase (AST), CLIF-C AD score, and LV-GLS were also significantly associated with overall survival (Table 3). In multivariable Cox regression analysis, only AST, CLIF-C AD score, and LV-GLS were independently associated with overall mortality (Table 3). These effects might be due to the strong effect in the subgroup of patients receiving TIPS for ascites (Supporting Table S2). Still, the overall result remains when controlling for the indication of TIPS.

ROC analysis with mortality as the endpoint was performed to define a cutoff for LV-GLS. The best cutoff for LV-GLS was –16.6%. Kaplan-Meier analysis stratified for median LV-GLS showed that patients with low contractility (higher LV-GLS) developed ACLF significantly earlier than patients with higher cardiac contractility (lower LV-GLS) (Fig. 1). Kaplan-Meier analysis stratified for LV-GLS showed higher

**TABLE 2. UNIVARIATE TIME-TO-EVENT ANALYSIS OF COLLECTED DATA TO PREDICT ACLF OCCURRENCE AND MULTIVARIABLE COX REGRESSION ANALYSIS (FORWARD STEPWISE LIKELIHOOD QUOTIENT) USING THE VARIABLE FROM UNIVARIATE ANALYSIS TO PREDICT ACLF OCCURRENCE**

| | Univariate Analysis | | | | Multivariable Analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 95% CI for HR | | | | 95% CI for HR | |
| Parameters | *P* | HR | Lower | Upper | *P* | HR | Lower | Upper |
| Sex | n.s. | 1.1 | 0.632 | 1.9 | | | | |
| Age | n.s. | 1 | 0.986 | 1.03 | | | | |
| Bilirubin | n.s. | 1 | 0.997 | 1.0 | | | | |
| Albumin | n.s. | 1 | 0.937 | 1.1 | | | | |
| AST | n.s. | 1 | 0.97 | 1.1 | | | | |
| MELD score above 15 | <0.001 | 2.348 | 1.482 | 3.719 | 0.001 | 2.222 | 1.400 | 3.528 |
| LV-GLS | 0.016 | 1.740 | 1.109 | 2.731 | 0.039 | 1.613 | 1.025 | 2.540 |

Abbreviation: n.s., nonsignificant.

**TABLE 3. UNIVARIATE TIME-TO-EVENT ANALYSIS OF COLLECTED DATA TO PREDICT SURVIVAL AND MULTIVARIABLE COX REGRESSION ANALYSIS (FORWARD STEPWISE LIKELIHOOD QUOTIENT) USING THE VARIABLE FROM UNIVARIATE ANALYSIS TO PREDICT SURVIVAL**

| | Univariate Analysis | | | | Multivariable Analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 95% CI for HR | | | | 95% CI for HR | |
| Parameters | *P* | HR | Lower | Upper | *P* | HR | Lower | Upper |
| Sex | n.s. | 1.071 | 0.549 | 2.092 | | | | |
| Age | 0.035 | 1.032 | 1.002 | 1.064 | | | | |
| MELD score | 0.025 | 1.474 | 1.247 | 1.909 | | | | |
| Bilirubin | 0.002 | 1.131 | 1.045 | 1.224 | | | | |
| Albumin | 0.051 | 0.952 | 0.906 | 1.000 | | | | |
| AST | 0.002 | 1.008 | 1.003 | 1.014 | <0.001 | 1.009 | 1.004 | 1.014 |
| LV-GLS | 0.017 | 1.441 | 1.224 | 1.866 | 0.03 | 1.123 | 1.010 | 1.250 |
| CLIF-C AD score | 0.014 | 1.136 | 1.030 | 1.260 | 0.01 | 1.080 | 1.018 | 1.137 |

Abbreviation: n.s., nonsignificant.

mortality in patients with high LV-GLS (Fig. 2). Interestingly, LV-GLS (area under the curve [AUC], 0.688; $P = 0.03$) showed a higher AUC compared to the MELD score (AUC, 0.646; $P = 0.08$) and CLIF-C AD score (AUC, 0.573; $P = 0.39$).

# Discussion

We show for the first time that LV-GLS is independently associated with the development of ACLF and overall survival of patients with cirrhosis after TIPS. Therefore, LV-GLS allows a better characterization of the circulatory and hemodynamic dysfunction in decompensated patients and thus enables stratification of patients at risk.

The CANONIC study has shown that circulatory failure, defined as use of vasopressors, is detrimental in patients with ACLF.[8] However, there are no good parameters for early identification of patients at risk to develop circulatory failure. Moreover, the reduced effective arterial volume, which occurs particularly in patients who are acutely decompensated, possibly predisposes or even induces organ failure, especially renal failure, which is highly dependent on organ perfusion.[3] STE parameters of the LV function have been shown in other large studies to be clearly related to the development of renal dysfunction.[13]

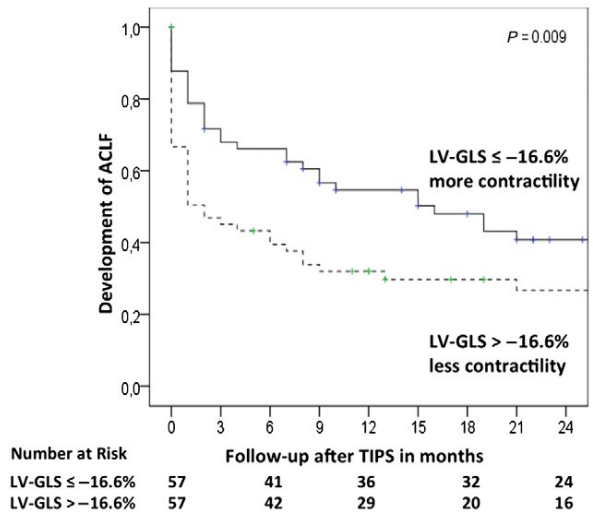


**FIG. 1.** Time association of LV-GLS and ACLF development. Kaplan-Meier analysis shows development of ACLF in patients stratified by LV-GLS (cutoff, -16.6%). Lower LV-GLS levels mean better cardiac contractility, whereas higher LV-GLS levels reflect worse cardiac contractility. Rates of ACLF development are shown using Kaplan-Meier plots and are analyzed by the log-rank test.

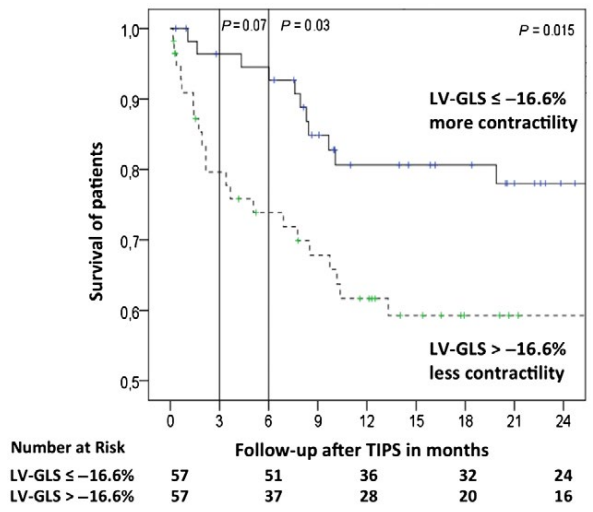


**FIG. 2.** Survival after TIPS stratified by their cardiac contractility, assessed using LV-GLS. Kaplan-Meier analysis shows survival of patients stratified by LV-GLS (cutoff, -16.6%). Lower LV-GLS levels mean better cardiac contractility, whereas higher LV-GLS levels reflect worse cardiac contractility. Survival rates are shown using Kaplan-Meier plots and are analyzed by the log-rank test.

This possibly also explains the prediction of the need for liver transplantation by STE,[15] which is MELD based and renal function dependent. Therefore, it is not surprising that STE parameters, and specifically LV-GLS, independently predict development of ACLF in these patients who are decompensated and receiving TIPS mainly for refractory ascites.

ACLF is defined by the development of organ failures after precipitating events, which in many cases are infections.[8,9] Impaired organ perfusion, in particular, might be the reason for the development of organ failure, especially of kidney and brain.[13,16] Impaired organ perfusion may be at least partly a consequence of decreased cardiac contractility and less responsive myocardium as assessed by STE.[13] Infections, the most frequent cause of ACLF, are known to require a higher cardiac reserve to maintain adequate circulation. Therefore, latent cardiac dysfunction could lead to the development of organ failure, ACLF, and death.[17] The present study supports this hypothesis and further suggests that impaired cardiac contractility, reflected by higher LV-GLS, predisposes to the development of ACLF and death in cirrhosis.

As TIPS insertion itself requires sufficient cardiac reserve, it is contraindicated in patients with severe cardiac pathologies, such as severe tricuspid regurgitation and severe pulmonary hypertension. However, the role of other cardiac parameters in patient selection has been a matter of debate.[3,18] Previous studies, which evaluated conventional TTE, reported TIPS insertion resulting in a significant increase of left atrial diameter, LV end-diastolic diameter, and pulmonary arterial systolic pressure,[5,19,20] parameters associated with overall survival of patients with cirrhosis.[21] Moreover, diastolic dysfunction is frequent in advanced cirrhosis and is associated with failure to resolve ascites and increased mortality after TIPS.[11] However, no correlation between diastolic dysfunction and severity of liver disease was shown.[12,13] In previous animal and human studies on cirrhosis, unimpaired resting systolic function decompensated after several precipitating factors, such as physical stress and exercise.[22] These apparently contradictive findings can explain whether TIPS is regarded as the factor that unmasks a preexisting subclinical cardiomyopathy in patients with cirrhosis. Our findings underline this hypothesis and show that patients with better cardiac contractility develop less ACLF and death after TIPS. This effect was mainly observed in the patients with ascites of our cohort, but when all patients were analyzed, the effect was still statistically significant.

The question remains as to what technique should be used for the detection of circulatory dysfunction or predisposition to it. Conventional echocardiography shows several limitations, as outlined above, whereas TTE could assess early and subclinical myocardial dysfunction when enhanced by speckle tracking.[23-26] Speckle tracking is independent of volume status, hyperdynamic situation, and as shown by us previously,[15] may give a more objective view on the estimation of potential subclinical cardiomyopathy in patients with cirrhosis. As already mentioned, LV contractility appears to be decreased in patients with cirrhosis,[14] whereas the lack of response of LV contractility to hemodynamic changes might be the reason for development of ascites and decompensation.[13] In one elaborate, invasive, pathophysiologic, but small study in patients with TIPS, profound changes were induced in cardiac contractility after TIPS, which could only be reflected noninvasively by speckle tracking.[4] Our present study confirms that the most suitable, noninvasive, and less biased technique seems to be STE, while it describes for the first time LV-GLS as the best predictor of ACLF development and mortality.

Although the patients were well characterized, the conclusions of the present study are limited due to its retrospective nature. A selection bias cannot be excluded because only patients with good quality TTE were included. Moreover, the subgroup of patients with ascites in our cohort was predominant, while patients receiving TIPS for bleeding and hepatorenal syndrome were fewer; therefore this might have introduced another bias. However, the results were similar when controlling for the indication of TIPS. Independent validation of these findings and prospective stratification of management based on LV-GLS are tasks for future studies.

Summarizing, LV-GLS may identify patients with latent cardiac dysfunction, who, following stress, such as TIPS insertion, are then more predisposed to deterioration, development of ACLF, and death. Our results suggest a more meticulous patient selection for TIPS and a more personalized management approach to react to decompensating events. Finally, this study suggests a cutoff for LV-GLS to identify patients at risk of developing ACLF and death and thereby to stratify management of patients receiving TIPS.

*Acknowledgment:* We thank Kristin Gehrmann, Nadine Koestlmeier, Gudrun Hack, and Silke Bellinghausen for excellent technical support.

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# Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1308/suppinfo.

## 3.5 Die Veränderung der linksventrikulären myokardialen Kontraktilität in der Speckle-Tracking-Echokardiographie nach transjugulärem intrahepatischem portosystemischem Shunt ist ein Marker für die Mortalität.

**5: Jansen C***, Nordmann P*, Cremonese C*, Praktiknjo M, Chang J, Lehmann J, Thomas D, Nickenig G, Weber M, Stöhr E, Öztürk C, Zachoval C, Hammerstingl C, Strassburg C, Meyer* C, Trebicka J*. Change of Left Ventricular Myocardial Contractility in Speckle Tracking Echocardiography After Transjugular Intrahepatic Portosystemic Shunt Predicts Survival, Front. Gastroenterol., 12 April 2022 Sec.Hepatology https://doi.org/10.3389/fgstr.2022.860800

Zielsetzung der Arbeit – Das Ziel dieser Studie war es, die Änderung der Kontraktilität des Myokards nach Platzierung eines intrahepatischen portosystemischen Shunts zu untersuchen. Dazu wurde die Speckle-Tracking-Echokardiographie genutzt.

Methoden und Ergebnisse – In diese Studie wurden 206 Patienten (126 Männer) mit Leberzirrhose eingeschlossen, die einen intrahepatischen portosystemischen Shunt erhielten. Bei allen Studienpatienten wurde eine konventionelle transthorakale Echokardiographie durchgeführt. Diese erfolgte vor und in den ersten Wochen nach der intrahepatischen portosystemischen Shunt Platzierung. Weiterhin erfolgte die Erfassung planarer und funktioneller Parameter aus der Standardechokardiographie. Hämodynamische und klinische Parameter wurden vor intrahepatischer portosystemischer Shunt Anlage und während der Nachsorge beurteilt. Wie erwartet, zeigten die meisten Parameter der konventionellen transthorakalen Echokardiographie eine signifikante Veränderung nach intrahepatischer portosystemischer Shunt-Anlage. Allerdings zeigten weder die absoluten Werte noch die Änderungen von konventionellen Echokardiographie Parametern eine Assoziation mit dem Überleben. Dagegen zeigte sich die Steigerung der Kontraktilität um mehr als 20 % nach Shunt-Anlage als unabhängiger Prädiktor für die Mortalität.

Schlussfolgerungen – Diese Ergebnisse zeigen, dass eine Erhöhung der linksventrikulären Kontraktilität von mehr als 20 % nach der intrahepatischen portosystemischen Shunt-Anlage einen unabhängigen Prädiktor für das Überleben darstellt. Diese Patienten erhalten nun eine engere Nachsorge in unserem Zentrum.

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ORIGINAL RESEARCH
published: 12 April 2022
doi: 10.3389/fgstr.2022.860800

# Change of Left Ventricular Myocardial Contractility in Speckle Tracking Echocardiography After Transjugular Intrahepatic Portosystemic Shunt Predicts Survival

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**Background:** Left ventricular global longitudinal strain (LV-GLS) has been shown to better reflect the left cardiac contractility in cirrhosis than other investigations and might bear prognostic value. The aim of this study was to investigate the evolution of myocardial contractility assessed by speckle tracking echocardiography (STE) after transjugular intrahepatic portosystemic shunt (TIPS) placement and its prognostic value in outcome.

**Methods:** In this study, 206 (126 males) patients with liver cirrhosis receiving TIPS were included. In all study patients, conventional transthoracic echocardiography (TTE) was performed before and in the first weeks after TIPS placement to assess left and right ventricular volume, planar and functional parameters. Also, LV-GLS was measured by STE to assess left ventricular contractility as surrogate for myocardial dysfunction. Hemodynamic and clinical parameters were assessed before TIPS and during follow-up.

**Results:** As expected, most conventional parameters of TTE showed a significant change after TIPS placement. However, neither the absolute values, nor the changes of conventional cardiac parameters of TTE before and after TIPS insertion were associated with survival. By contrast, an increase in contractility of more than 20% using STE after TIPS was an independent predictor of mortality.

**Conclusion:** These results demonstrate that an increase of left ventricular contractility of more than 20% after TIPS insertion is an independent predictor of survival and this may identify patients at risk and in need of closer follow-up care.

**Keywords: STE, TIPS (transjugular intrahepatic portosystemic shunt), cirrhosis, transthoracic echocardiography (TTE), decompensation**

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***Edited by:***
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***Specialty section:***
*This article was submitted to Hepatology, a section of the journal Frontiers in Gastroenterology*

***Received:*** *23 January 2022*
***Accepted:*** *18 March 2022*
***Published:*** *12 April 2022*

***Citation:***
*Jansen C, Nordmann P, Cremonese C, Praktiknjo M, Chang J, Lehmann J, Thomas D, Nickenig G, Weber M, Stöhr E, Öztürk C, Zachoval C, Hammerstingl C, Strassburg CP, Meyer C and Trebicka J (2022) Change of Left Ventricular Myocardial Contractility in Speckle Tracking Echocardiography After Transjugular Intrahepatic Portosystemic Shunt Predicts Survival. Front. Gastroenterol. 1:860800. doi: 10.3389/fgstr.2022.860800*

## INTRODUCTION

During the course of cirrhosis, portal hypertension will lead to various complications, such as variceal hemorrhage, refractory ascites and hepatorenal syndrome (1). In recent decades, transjugular intrahepatic portosystemic shunt (TIPS) insertion has been proven to successfully attenuate portal hypertension in patients with advanced cirrhosis. In well selected patients, TIPS can prevent bleeding and hydropic decompensation by shunting portal blood volume into the post-hepatic venous circulation (2, 3). Increased cardiac preload caused by TIPS may unmask impaired left ventricular contractility (4) as one feature of cirrhotic cardiomyopathy (CCM). However, the pathophysiological effects after TIPS remain unclear and to date, have not been investigated using speckle tracking (STE) in a large cohort with long follow-up.

CCM is a latent cardiac dysfunction related to cirrhotic liver disease. CCM occurs when the patient is exposed to physical, pharmacological or hemodynamic stress, such as TIPS insertion or liver transplantation (4, 5). Left ventricular contractility is a major aspect of myocardial performance and is associated with renal function (6). Recent studies have mainly focused on conventional echocardiography to detect early signs of CCM and deterioration of cardiac function after TIPS (4–6). The STE is able to measure myocardial fiber shortening expressed as change in the absolute value. In order to simplify understanding, we shall therefore use in the following the terminology of more or less contractility. Also in cirrhosis, ventricular global longitudinal strain (LV-GLS) assessed by STE measures contractility of the left ventricle as surrogate for myocardial dysfunction. In this study, we analyzed whether the change in LV-GLS after TIPS can be identified as a prognostic marker.

## PATIENTS AND METHODS

### Patients and Data Collection

In this retrospective study, we enrolled 588 consecutive patients suffering from liver cirrhosis who were admitted to the Department of Internal Medicine I, University Clinic Bonn, Bonn, Germany, to receive TIPS between 2006 and 2017. The main inclusion criterion was the performance of standardized two-dimensional (2D) transthoracic echocardiography (TTE) before and after TIPS. All patients received a covered stent without controlled expansion.

Of the enrolled patients, 382 were excluded as no TTE was performed before or after TIPS or no echocardiographic standard values could be measured due to bad image quality of TTE or no images were stored in the system. In total, we included 206 patients. The recruitment of patients is listed in **Figure 1**. Primary endpoints of the study were death (n=72) and liver transplantation (n=7) as a sign of liver failure.

Clinical data of all patients were collected during hospital visits and included general clinical data, medical history, medication, laboratory parameters, and data from conventional transthoracic echocardiography. Invasive hemodynamic parameters were assessed during TIPS insertion as previously described. Most stents were dilated to a size of 8mm. The analysis of the TTE data using STE was carried out retrospectively. The diagnosis of liver cirrhosis was based on ultrasound procedures and/or liver biopsy. Patients were evaluated before TIPS placement, seven days after TIPS placement and six weeks after TIPS placement within a 24-month period of follow-up. The median follow up was 15 month.

We followed 40 patients for 36 months, 66 for 24 months and 100 for 12 months, while 60 died. The ethical committee of the University of Bonn approved the study in accordance with the Declaration of Helsinki (No. 121/14).

### Measurement of the Portal Systemic Pressure Gradient

Portosystemic pressure gradient (PSPG) data were acquired from all patients during TIPS placement as previously described (7). After puncturing of the right internal jugular vein, a catheter was ultrasound-guided into place. Portal venous and hepatic venous pressure were measured invasively. The difference between these two pressures was defined as the portosystemic pressure gradient.

### Conventional Transthoracic Echocardiography

All patients underwent standardized 2D transthoracic echocardiography (TTE) for the determination of left ventricular (LV) and right ventricular (RV) function and dimensions as previously described (8). Apical four- and two-chamber views as well as parasternal long- and short-axis views were acquired with the patient lying in the left lateral decubitus position. Images were obtained in 2D greyscale, color Doppler and tissue Doppler modes. Left ventricular ejection fraction (LVEF) was calculated using the biplane Simpson method after manually tracing the endocardial border in apical 4-chamber-view in end-diastole as well as end-systole. Mitral flow velocities were measured using standard pulsed-wave Doppler mode at the tips of the mitral valve leaflets in apical 4-chamber-view. Systolic pulmonary artery pressure (sPAP) was measured by analyzing the peak systolic tricuspid regurgitate velocity flow in continuous wave Doppler mode. Relevant pulmonary hypertension was defined as estimated sPAP 30 mmHg. For 2D estimation of right ventricular function, tricuspid annular plane systolic excursion (TAPSE) was

**Abbreviations:** ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CCM, cirrhotic cardiomyopathy; CRP, C-reactive protein; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; Hb, hemoglobin; HE, hepatic encephalopathy; Hr, heart rate; HR, hazard ratio; HRS, hepatorenal syndrome; PSPG, portosystemic pressure gradient; GGT, gamma-glutamyltransferase; INR, international normalized ratio; LV, left ventricle; LVEF, left ventricular ejection fraction; LV-GLS, left ventricular global longitudinal strain; MELD, Model for End-Stage Liver Disease; P, p-value; RV, right ventricle; RVFAC, right ventricular fractional area change; RAA, right atrial area; RVD, right ventricular diameter; RVV, right ventricular volume; sPAP, systolic pulmonary arterial pressure; STE, speckle tracking echocardiography; TAPSE, tricuspid annular plane systolic excursion; TIPS, transjugular intrahepatic portosystemic shunt; TTE, transthoracic echocardiography; TWBC, total white blood cell count.

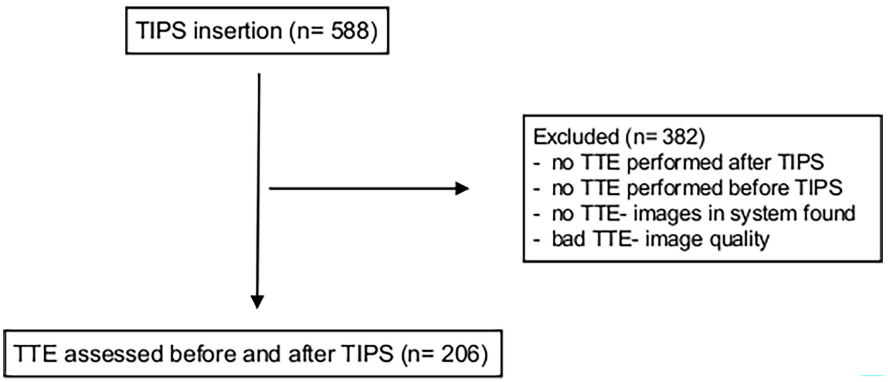


**FIGURE 1** | Recruitment Table. In total, 206 patients with TIPS insertion were included retrospectively. Main inclusion criteria were conventional transthoracic echocardiography before and after TIPS placement. TIPS, transjugular intrahepatic portosystemic shunt; TTE, transthoracic echocardiography.

measured in 4-chamber-view using M-mode. Impaired RV function was defined as TAPSE <15 mm. The median follow up was 2 months after TIPS insertion.

## Speckle Tracking Echocardiography

Several previous studies have used LVEF as an indicator for left ventricular systolic function. However, more recent studies have suggested the left ventricular myocardial strain to be a less load-dependent indicator for myocardial dysfunction. Furthermore, it takes into account the directions of contraction of the different myocardial layers. In the course of evaluating for TIPS placement, apical 4-chamber views of each study patient were assessed by conventional transthoracic echocardiography. STE was performed as described. In short, Image Arena 4.3 (TomTec Imaging Systems GmbH, 2001-2010, Unterschleissheim, Germany) was used for frame-by-frame measuring of the shortening between two manually set points in the endocardium. In our study, only the left ventricle was analyzed. It was automatically divided into six segments: basal lateral/septal, mid lateral/septal and apical lateral/septal. Longitudinal and radial segmental 2D strains of the left ventricle were analyzed. Longitudinal strain of the left ventricle (LV-GLS) was calculated.

## Statistical Analysis

Clinical data were collected retrospectively and evaluated by means of SPSS statistical analysis software (IBM SPSS Statistics for Windows, version 22.0, released 2013. Armonk, NY: IBM Corp.). P-values <0.05 were considered to be statistically significant. Data of all patients were assessed using descriptive statistics and are presented as means ± standard deviation or standard error of the mean and ranges, unless labeled otherwise. The Mann-Whitney-U test was chosen to compare unpaired data. The correlations were analyzed with Spearman´s correlation coefficient. Univariate time-to-event-analysis was performed to identify parameters that significantly predict survival. Using the significant predictors of the univariate analysis, multivariate Cox regression analysis (forward stepwise likelihood quotient) was performed to identify independent predictors of survival. To compare the survival rates of patients by using the log-rank test, Kaplan-Meier plots were used. The cut-off value was determined using an auc-roc analysis.

# RESULTS

## General Characteristics of Patients at Baseline and During Follow-Up

The clinical characteristics at baseline (before TIPS placement) are presented in **Table 1A**. Overall, 126 male and 80 female patients suffering from liver cirrhosis were included. Mean age was 59.6 years (**Table 1A**). The main etiology of cirrhosis was alcohol (130 patients). In most cases, TIPS was placed due to refractory ascites (120 patients) and bleeding (56 patients). More than half of the patients presented with Child B (113 patients), 56 patients presented with Child A and only 37 patients with Child C (**Table 1A**). Mean MELD (Model for End-Stage Liver Disease) score was 11. The most frequent complication of cirrhosis at baseline was ascites (145 patients). A smaller number of patients (n=49) presented with hepatorenal syndrome (HRS). The mean follow up period was 24 months.

Portosystemic pressure gradient (PSPG) data was acquired invasively in all patients during TIPS placement. As expected, a significant increase in central venous pressure (6 to 10 mmHg) and a decreased portal venous pressure (27 to 18 mmHg) after TIPS insertion could be assessed. The portal systemic pressure gradient was reduced to 50 percent of baseline value (19 to 8 mmHg) (**Table 1B**).

Parameters assessed by standardized 2D transthoracic echocardiography (TTE) and speckle tracking (STE) values before and after TIPS measurements are listed in **Table 1C**. Most of the conventional echocardiographic parameters (EDV, ESV, TAPSE, sPAP, RVV, RVD, RAA) showed a significant change after TIPS insertion (**Figure 2**).

**TABLE 1 |** A. Clinical parameters of cirrhotic patients before and after TIPS insertion. B. Invasive measurement of portal systemic pressure gradient during TIPS insertion. C. Standardized 2-dimensional transthoracic echocardiography (TTE) parameters and LV-GLS of cirrhotic patients before and after TIPS insertion.

| A. | Clinical parameters | Before TIPS | After TIPS |
|---|---|---|---|
| | Gender (male/female) | 126/80 | |
| | Age | 59.6 (20 - 87) | |
| | 0BMI | 24.5 (15 - 43) | |
| | Etiology (alcohol/viral/other) | 130/25/51 | |
| | Indication (bleeding/ascites/both/other) | 56/120/13/17 | |
| | Child category (A/B/C) | 56/113/37 | |
| | MELD score | 11.3 (6 - 30) | |
| | Ascites (absent/present) | 61/145 | |
| | Previous HRS (no/yes) | 157/49 | |
| | Previous HE (0/I-II/III-IV) | 158/39/8 | |
| B. | Portal- and systemic pressure parameters | | |
| | Central venous pressure (mmHg) | 6 (0 - 24) | 10 (0 - 31) |
| | Portal venous pressure (mmHg) | 27 (8- 50) | 18 (3 - 39) |
| | Portal systemic pressure gradient (mmHg) | 19 (15 - 39) | 8 (0 - 23) |
| C. | TTE and STE parameters | | |
| | LV-GLS (%) | -13.65 (-24.9 - 1) | -13.42 (-27.75 - 1) |
| | EDV (mL) | 89.7 (25.8 - 197.5) | 97.8 (29.7 - 241.8) |
| | ESV (mL) | 30.65 (7.9 - 115.9) | 34.9 (10.8 - 110) |
| | EF (%) | 66.2 (21.5 - 90) | 64.4 (45.1 - 86) |
| | TAPSE (mm) | 25 (11 - 42) | 25.5 (2.2 - 60) |
| | sPAP (mmHg) | 23 (7 - 64) | 25 (5 - 70) |
| | RVFAC (%) | 46 (14 - 70) | 45 (13 - 69) |
| | RVV (mL) | 20 (11 - 48) | 22 (6 - 97) |
| | RVD (mm) | 33 (2.4 - 55) | 34 (23 - 54) |
| | RAA ($cm^2$) | 12 (5 - 48) | 14 (5 - 51) |
| | Hr (1/s) | 75 (47 - 127) | 74 (46 - 119) |

*BMI, body mass index; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; HE, hepatic encephalopathy; Hr, heart rate; HRS, hepatorenal syndrome; PSPG, portosystemic pressure gradient (PSPG); LV-GLS, left ventricular global longitudinal strain; MELD, Model for End-Stage Liver Disease; RVFAC, right ventricular fractional area change; RAA, right atrial area; RVD, right ventricular diameter; RVV, right ventricular volume; sPAP, systolic pulmonary arterial pressure; STE, speckle tracking; TAPSE, tricuspid annular plane systolic excursion; TIPS, transjugular intrahepatic portosystemic shunt; TTE, transthoracic echocardiography. Data are given as n or as median (range).*

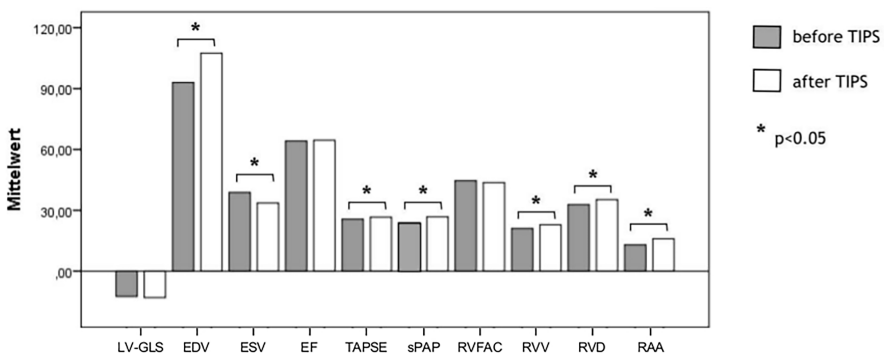


**FIGURE 2** | Assessment of cardiac function measured by echocardiographic parameters and STE (GLS) before and after TIPS. As expected, most parameters show a significant change after TIPS. EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; LV-GLS, left ventricular global longitudinal strain; p, p-value; sPAP, systolic pulmonary arterial pressure; TAPSE, tricuspid annular plane systolic excursion; TIPS, transjugular intrahepatic portosystemic shunt; RVFAC, right ventricular fractional area change; RAA, right atrial area; RVD, right ventricular diameter; RVV, right ventricular volume.

Laboratory data assessed before and after TIPS insertion are listed in **Table 2**. Interestingly, C-reactive protein, transaminase (AST, ALT) and gamma-glutamyltransferase showed an increase seven days after TIPS placement, followed by normalization at last follow-up (**Table 2**). Seven days and six weeks after TIPS placement, serum creatinine notably decreased and remained lower than at baseline (1.16 mg/dl vs. 1.04 mg/dl) at last follow-up (**Table 2**).

## Prognostic Value of Cardiac Parameters

As expected, indication, age, MELD score and ascites were shown to be predictors of survival, while in univariate

**TABLE 2 |** Laboratory values of cirrhotic patients before and after TIPS insertion.

| Laboratory values | Before TIPS | After TIPS | | |
|---|---|---|---|---|
| | | day 7 | week 6 | last follow-up |
| Sodium (mmol/L) | 138 (114 - 147) | 139 (124 - 148) | 139 (110 - 154) | 139 (121 - 154) |
| Creatinine (mg/dL) | 1.16 (0.5 - 14) | 0.95 (0.4 - 14.8) | 0.96 (0.2 - 8.1) | 1.04 (1.5 - 8.7) |
| Bilirubin (mg/dL) | 1.1 (0.1 - 11) | 1.53 (0.2 - 25) | 1.645 (0.3 - 16.9) | 1.74 (1.2 - 40) |
| CRP (mg/L) | 10 (0.2 - 99) | 14.2 (0.2 - 129) | 6.8 (0.3 - 182) | 7.14 (1.4 - 167) |
| Albumin (g/L) | 31 (14.6 - 51) | 30.65 (14.1 - 50) | 31.9 (12.7 - 47.9) | 34.5 (11.2 - 51.3) |
| INR | 1.1(0.9 - 2.3) | 1.2 (0.9 - 3.5) | 1.2 (0.9 - 5.3) | 1.2 (0.9 - 3) |
| TWBC (g/L) | 6.1 (1.3 - 51) | 6.37 (1.9 - 29.6) | 5.7 (1.5 - 31.87) | 6.17 (1.3 - 23.9) |
| Hb (g/dL) | 10.1 (5.4 - 16.4) | 9.7 (5 - 15.4) | 10.2 (4.9 - 14.8) | 10.8 (5.8 - 16.8) |
| Platelets (g/L) | 129.5 (27 - 697) | 119.5 (29 - 788) | 127.5 (19 - 667) | 120 (8 - 697) |
| GGT (U/L) | 120 (15 - 709) | 151 (19 - 866) | 133 (16 - 2160) | 123 (14 - 2062) |
| ALT (U/L) | 24 (6 - 137) | 38 (9 - 604) | 26 (6 - 825) | 26 (6 - 465) |
| AST (U/L) | 39 (11 - 361) | 48 (10 - 451) | 43 (10 - 1036) | 42 (12 - 1619) |

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; Hb, hemoglobin; GGT, gamma-glutamyltransferase; INR, international normalized ratio; TIPS, transjugular intrahepatic portosystemic shunt; TWBC, total white blood cell count. Data are given as median (range).*

analysis, only the central venous pressure could be identified as a predictor of survival (**Table 3**). However, in univariate time-to-event analysis, only RAA showed an association with survival (**Table 3**). These findings were supported by log-rank test, revealing no significant change of survival by change in EDV, ESV or EF as well as its evolution (**Figures 3A–C**).

The multivariate analysis (**Table 4**) includes all parameters that were found to be significant in the univariate analysis (**Table 3**). In this multivariate Cox regression analysis, gamma-glutamyltransferase and aspartate-aminotransferase were shown to be independent predictors of survival (**Table 4**). However, only an increase in contractility of more than 20% using STE after TIPS as parameter assessed by echocardiography could be identified as an independent predictor of survival in multivariate Cox regression analysis (**Table 4**).

Interestingly, patients with increased contractility (more negative LV-GLS) before TIPS showed better survival within a 24-month follow-up period (**Figure 3D**). In contrast, after TIPS, an increase of contractility of more than 20% was associated with worse survival (**Figure 4**) with an area under the curve (AUC) of 0.633 (p=0.05, CI 0.485-0.799).

**TABLE 3 |** Univariate time-to-event analysis shows parameters that predict survival in cirrhotic patients with TIPS insertion within a follow-up period of 24 months.

| Parameters | *P* | HR |
|---|---|---|
| Indication | 0.45 | 0.872 |
| Age | 0.028 | 1.033 |
| MELD score | 0.043 | 1.056 |
| Ascites | 0.005 | 1.414 |
| Central venous pressure | 0.032 | 0.937 |
| GGT | 0.006 | 1.003 |
| AST | 0.001 | 1.015 |
| RAA | 0.047 | 1.085 |
| LV-GLS before TIPS | 0.022 | 2.227 |
| LV-GLS More than 20% increase of contractility after TIPS | 0.048 | 2.039 |

*AST, aspartate aminotransferase; HR, hazard ratio; GGT, gamma-glutamyltransferase; LV-GLS, left ventricular global longitudinal strain; MELD, Model for End-Stage Liver Disease; P, p-value; RAA, right atrial area; TIPS, transjugular intrahepatic portosystemic shunt.*

## DISCUSSION

In this study, we performed echocardiography before and after TIPS insertion in 206 patients, which is - to the best of our knowledge - the first study to assess echocardiography before and after TIPS for such a large cohort. This study demonstrates that an increase in cardiac contractility of more than 20%, assessed by speckle tracking after TIPS, was an independent predictor of mortality.

The transjugular intrahepatic portosystemic shunt is a well-established intervention to improve survival of patients with cirrhosis and it leads to a relevant reduction of portal hypertension-associated complications, such as ascites and variceal bleeding. TIPS is known to lead to severe changes in systemic circulation. As already described by Rössle et al., total vascular conductance increases (decreasing resistance) after TIPS insertion due to unrestricted flow through the splanchnic bed. With time, the splanchnic/hepatic conductance decreases (increasing resistance) while still remaining above the pre-TIPS level. With portal decompression the circulatory dysfunction improves after approximately one year (9). However, parameters for cardiac follow-up in these patients, especially regarding cardiac dysfunction due to changed hemodynamics after TIPS, are missing and prognostic parameters need to be defined for patient follow-up to improve TIPS safety and to enable early identification of potential complications. Indeed, we do find that the recommended cut-off for CCM of <18 measured in TTE before and after TIPS does not predict outcome in this specific patients population receiving TIPS. Therefore, the increase in contractility after TIPS is counterintuitively associated with worse outcome, while the fixed cut-offs do not predict outcome (10).

Armstrong et al. examined a large cohort of 117 patients but none of the echocardiography measures pre-intervention were related to 30-day or overall transplant-free survival after TIPS insertion. Hence, there are no reliable parameters in echocardiography to predict outcome and overall survival in these patients (11, 12). In the present study, we found no correlation between TTE parameters before and after TIPS

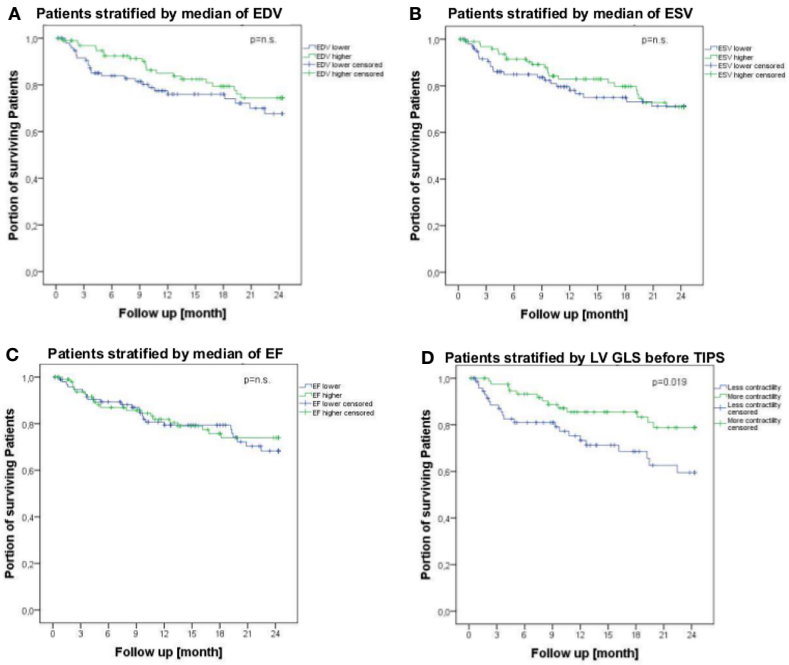


**FIGURE 3** | Survival of patients suffering from liver cirrhosis receiving TIPS stratified by conventional echocardiographic parameters [EDV **(A)**, ESV **(B)** and EF **(C)**] and speckle tracking parameter (LV-GLS) **(D)** within a follow-up period of 24 months. Lower LV-GLS indicates more contractility of left ventricle and higher LV-GLS means less contractility of left ventricle. Survival rates were analyzed by log-rank test and are shown using Kaplan-Meier curves. EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; LV-GLS, left ventricular global longitudinal strain; p, p-value; TIPS, transjugular intrahepatic portosystemic shunt.

**TABLE 4** | Multivariate Cox regression analysis (forward stepwise likelihood quotient) using significant parameters of univariate analysis (**Table 3**) to identify independent predictors of survival in cirrhotic patients with TIPS insertion within a follow-up period of 24 months.

| | *P* | HR |
|---|---|---|
| GGT | <0.001 | 1.004 |
| AST | <0.001 | 1.013 |
| LV-GLS More than 20% increase of contractility after TIPS | 0.018 | 2.639 |

*AST, aspartate aminotransferase; HR, hazard ratio; GGT, gamma-glutamyltransferase; LV-GLS, left ventricular global longitudinal strain; P, p-value; TIPS, transjugular intrahepatic portosystemic shunt.*

insertion with survival. Especially regarding parameters of the right ventricle, standard echocardiography could not identify any prognostic parameters, suggesting that the right ventricular function plays a minor role once pulmonary arterial hypertension is excluded. Also, diastolic dysfunction does not seem to be applicable for prediction of overall survival in TIPS patients as investigated by different groups (11, 13). However, a study shows that the hospitalization for cardiac decompensation after TIPS insertion can be predicted in a combining BNP or NT-proBNP levels and echocardiographic parameters including parameters of diastolic dysfunction (14). We determined the diastolic dysfunction of the patients for 118 patients. The median of the e` values was 8.5 in median. It should be noted that 99 patients had a value ≥7. The E/e` showed a value of 9.6 in median. Here, too, only 8 had a value greater than 15. Thus, by combining the two parameters, we can safely say that at 96 have no highgrade diastolic dysfunction. In the log rank analysis as well as the regression, no significant differences in survival were found when we compared these groups.

However, looking at systolic function, the scenario seems to be different. Previous studies showed TIPS insertion resulting in a significant increase of left atrial diameter, left ventricular end-diastolic diameter, and pulmonary arterial systolic pressure (15–18). However, the ejection fraction before TIPS revealed no prognostic relevance for survival in patients with TIPS. Therefore in this study, we used the technique of speckle tracking to investigate left ventricular strain to identify a new prognostic biomarker.

Speckle tracking echokardiography offers new possibilities to analyse cardiac function, whereby the left ventricular global longitudinal strain (LV-GLS) reflects left cardiac contractility in cirrhosis better than other parameters. As we have already demonstrated in previous studies, the left ventricular global longitudinal strain (LV-GLS) as a reflection of cardiac contractility is independently associated with the development of acute-on-chronic liver failure (ACLF) and overall survival of patients with cirrhosis after TIPS (8). LV-GLS is a tool which seems to reflect the severe changes after TIPS as shown in our cohort. Importantly, TIPS may unmask the underlying

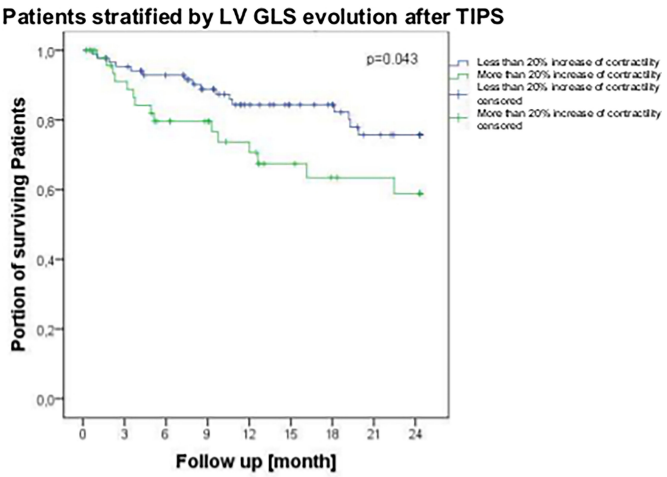


**FIGURE 4** | Survival of patients suffering from liver cirrhosis stratified by left ventricular global longitudinal strain (LV-GLS) evolution more than 20 percent after TIPS insertion within a follow-up period of 24 months. Lower GLS indicates more contractility and higher GLS means less contractility. Survival rates were analyzed by log-rank test and are shown using Kaplan-Meier curves. LV-GLS, left ventricular global longitudinal strain; p, p-value; TIPS, transjugular intrahepatic portosystemic shunt.

cardiomyopathy, while LV-GLS before TIPS may help to identify patients at risk.

Especially in Child-Pugh C patients, STE parameters were also shown to be independent predictors of transplant-free survival and time to transplantation. Therefore, STE could predict the need for liver transplantation as suggested in previous publications (19). Our present study confirms our published findings, namely that STE is a suitable noninvasive and less biased technique to predict mortality after TIPS. This underlines the robustness and reproducibility of our data. However, the question remains whether the prediction can be more accurate if the change of contractility after TIPS is assessed. The present study provides an answer to that question, identifying the change in LV-GLS as a stronger independent predictor of long-term (2-year) mortality after TIPS, together with liver function tests. The persistent increase in left ventricular contractility of more than 20% after TIPS insertion does not indicate an improvement in cardiac function but is rather a sign of aggravation of the cardiac function. This may be explained either as a consequence of the aggravating hypercirculation or the lack of adaptation of the splanchnic bed pooling after TIPS insertion.

This study has several limitations, such as its retrospective nature, limited by the technical feasibility of STE analysis. Although the patients were well characterized, a selection bias cannot be excluded because only patients with good quality TTE were included. Of course, patients who met the exclusion criteria did not receive TIPS. In addition to the excessively restricted liver function, cardiac reasons should also be mentioned here. Due to the strict protocol for TIPS-evaluation, patients with high-grade pathology in the cardiac examination by the colleagues from cardiology are excluded from a TIPS insertion. Independent validation of these findings and prospective stratification of management based on LV-GLS are tasks for future studies including markers of myocardial dysfunction (e.g., pro-brain natriuretic peptide). Finally, the study was carried out far before the new guidelines of ESC were published and could not include all currently recommended measurements (20).

In summary, this study demonstrates that a contractility increase of more than 20% after TIPS insertion assessed by STE is an independent predictor of decompensation and survival. This study advocates pre- and post-TIPS cardiac assessment by STE which may improve patients care during longer term follow-up. Although there are no recommendations for the intervalls between visits, TIPS-patients may require more stratified care for different predicted outcome. Patients with a contractility increase of more than 20% 6 weeks after TIPS insertion assessed by STE need closer follow-up, especially at mid- and longer-term follow-up. Patients with 20% increase in GLS should be seen more frequently, e.g. 3 months or even closer.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## ETHICS STATEMENT

The ethical committee of the University of Bonn approved the study in accordance with the Declaration of Helsinki (No. 121/14). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

CJ: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, statistical analysis of data, critical revision of the manuscript for important intellectual content. PN: acquisition of data, drafting of the manuscript, statistical analysis of data, critical revision of the manuscript for important intellectual content. CC: drafting of the manuscript, critical revision of the manuscript for important intellectual content. MP, JC, JL, DT, GN, CS, CM, MW, ES, CÖ, and CZ: acquisition of data, analysis and interpretation of data, drafting of the manuscript. CH and CS: analysis and interpretation of data, drafting of the manuscript, statistical analysis of data, critical revision of the manuscript for important intellectual content. JT: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, obtained funding, administrative, technical, and material support, study supervision. All authors contributed to the article and approved the submitted version.

## FUNDING

JT is supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18, CRC 1382 A09), European Union's Horizon 2020 Research and Innovation Programme (Galaxy, No. 668031, MICROB-PREDICT, No. 825694 and DECISION No. 84794), and Societal Challenges - Health, Demographic Change and Wellbeing (No. 731875), and Cellex Foundation (PREDICT).

## ACKNOWLEDGMENTS

We thank Kristin Gehrmann and Nadine Köstlmeier for excellent technical support.

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**Conflict of Interest:** JT has received speaking and/or consulting fees from Gore, Bayer, Alexion, MSD, Gilead, Intercept, Norgine, Grifols, Versantis, and Martin Pharmaceutical, without any relationship to the present work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## 3.6 Auswirkungen eines transjugulären intrahepatischen portosystemischen Shunt auf das zentrale lymphatische System bei Patienten mit Leberzirrhose

Pieper CC, Feißt A, Meyer C, Luetkens J, Praktiknjo M, Trebicka J, Attenberger U, **Jansen C. Impact of transjugular intrahepatic portosystemic shunt creation on the central lymphatic system in liver cirrhosis.** Sci Rep. 2021 Mar 29;11(1):7065. doi: 10.1038/s41598-021-86006-7. PMID: 33782430; PMCID: PMC8007746.

Zielsetzung der Arbeit – Ziel dieser Studie war es, den Durchmesser der Cisterna Chyli, in Bezug auf das Ausmaß der portalen Hypertonie, der Hämodynamik und dem Einfluss einer transjugulären intrahepatischen portosystemischen Shunt-Anlage, bei Patienten mit Zirrhose zu untersuchen.

Methoden und Ergebnisse – Es wurden 93 Patienten mit Zirrhose (57 männlich, mittleres Alter 59 Jahre) in die Studie eingeschlossen.

Die Diameter Erfassung der Cisterna Chyli erfolgte durch CT-Bildgebung vor und nach der Shunt-Anlage.

Vorab erfolgte die Einteilung der Patienten in zwei Gruppen: mit und ohne große venöse Kollateralen (d. h. > 6 mm). Die klinischen Daten und portal-hämodynamischen Parameter wurden in Bezug zum Durchmesser der Cisterna Chyli und deren Größenänderungen nach Shunt-Anlage analysiert. Bei den meisten Patienten (74,2 %) war die Cisterna Chyli in der Schnittbildgebung zu sehen. Der mittlere präinterventionelle Durchmesser der Cisterna Chyli betrug 9,4 ± 2,7 mm.

In den einzelnen Gruppen, die nach großen und kleinen Kollateralen aufgeteilt waren, zeigten sich signifikant unterschiedliche Durchmesser der Cisterna Chyli. Die Durchmesser in der Gruppe mit großen Kollateralen wurden mit 8,7 ± 2,0 mm und in der Gruppe der kleinen Kollateralen mit 10,7 ± 3,2 mm dokumentiert. Der Durchmesser der Cisterna Chyli korrelierte stark mit dem Portaldruck vor Shunt-Anlage, aber nur mäßig mit dem portosystemischen Gradienten.

Der Durchmesser der Cisterna Chyli verringerte sich signifikant von 10,2 ± 2,8 mm auf 8,3 ± 3,0 mm nach der Shunt- Anlage. Patienten ohne nachweisbarer Cisterna Chyli im CT vor Shunt-Anlage überlebten signifikant kürzer.

Schlussfolgerungen – Der Durchmesser der Cisterna Chyli ist assoziiert mit dem Portaldruck. Die Abnahmen des Durchmessers nach Shunt-Anlage, ist durch die portale Dekompression und den verringerten Rückstau in das lymphatische System zu

erklären. Ein Mangel an größeren zentralen Lymphgefäßen in der CT Bildgebung sind nach Shunt-Anlage mit einem kürzeren Überleben verbunden.

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# Impact of transjugular intrahepatic portosystemic shunt creation on the central lymphatic system in liver cirrhosis

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**The purpose of this study was to evaluate associations of cisterna chyli (CCh) diameter with portal hemodynamics and the influence of TIPS-creation in cirrhotic patients. 93 cirrhotic patients (57 male, mean age 59 years) received CT prior to TIPS-creation. 38/93 additionally underwent post-interventional CT. CCh-diameter was measured. After categorization into patients with and without large venous collaterals (i.e. > 6 mm), data were analyzed regarding associations between CCh-diameter, clinical and portal-hemodynamic parameters and diameter-changes after TIPS-creation. Patient survival post-TIPS was analyzed. Median portosystemic pressure-gradient decreased from 20 to 9 mmHg after TIPS-creation. Large venous collaterals were observed in 59 patients. In 69/93 patients (74.2%) the CCh was detectable. Mean pre-interventional diameter was 9.4 ± 2.7 mm (large collaterals: 8.7 ± 2.0 mm, no large collaterals: 10.7 ± 3.2 mm, p = 0.003). CCh-diameter correlated strongly with pre-TIPS portal-pressure (Rs = 0.685, p = 0.0001), moderately with portosystemic-gradient (Rs = 0.524, p = 0.006), liver shear-wave-elastography (Rs = 0.597, p = 0.004) and spleen size (Rs = 0.501, p = 0.01) in patients without large collaterals, but not in patients with large collaterals. Post-TIPS CCh-diameter decreased significantly from 10.2 ± 2.8 mm to 8.3 ± 3.0 mm (p < 0.001). Patients without a detectable CCh on CT survived significantly shorter. The diameter of the CCh is associated with portal-pressure and decreases after TIPS-creation in cirrhotic patients, reflecting a portal decompression mechanism via the lymphatic system. Lack of larger central lymphatics detectable on CT may be associated with shorter survival.**

The cisterna chyli is a saccular lymphatic structure located in the retrocrural space receiving lymphatic flow from the hepatic, intestinal and lumbar lymphatic truncs. Its cranial continuation is the thoracic duct—the main collecting lymphatic duct of the body—returning lymphatic fluid to the venous circulation via the left venous angle[1,2].

In the liver, fluid that is filtered out of the sinusoids into the space of Disse can flow either into the interstitial space of the portal tracts, sublobular veins or the capsule[3]. From here it enters the respective lymphatic system (portal, sublobular or superficial) with primary drainage (80%) via the portal lymphatics and the hepatic trunc into the cisterna chyli. Hepatic lymph constitutes a large amount (as much as 25–50%) of lymph flow within the cisterna chyli and the thoracic duct[3]. Hepatic and intestinal lymph production can increase even further in patients with liver disease especially with portal hypertension[4–6]. Close interrelations of excessive hepatic lymph production in cirrhotic patients, portal vein pressure and ascites formation have been suggested several decades ago[7]. More recently initial imaging studies found dilated central lymphatics in patients with liver disease possibly as a consequence of increased hepatic lymph production[8–11]. However, the role of the lymphatic system and its regulatory role in liver disease are still poorly understood.

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The purpose of this study was therefore to investigate associations of cisterna chyli diameter on cross-sectional imaging with clinical and portal hemodynamic parameters, the influence of portal decompression by transjugular intrahepatic portosystemic shunt (TIPS)-creation in cirrhotic patients with portal hypertension as well as patient outcome.

## Materials and methods

**Patient cohort and inclusion criteria.** All consecutive patients suffering from liver cirrhosis with portal hypertension who underwent TIPS creation at our institution from 2014 to 2016 were retrospectively identified and screened. Decisions for TIPS-creation were reached in interdisciplinary consensus (internal medicine, interventional radiology).

The study was approved by the local institutional review board of the University Hospital of Bonn with a waiver for informed patient consent. All examinations were performed in accordance with the relevant guidelines and regulations. Inclusion criteria of the study were successful TIPS-creation at our institution for refractory ascites or variceal bleeding, availability of clinical and hemodynamic parameters of the patients, as well as pre-interventional contrast-enhanced CT imaging. Patients receiving liver transplantation during the post-TIPS observational period were excluded from the analysis. In a subgroup of patients additional post-interventional CT was available.

**TIPS creation.** All patients underwent routine clinical and laboratory examinations prior to TIPS-creation. TIPS-creation was performed under combined ultrasound and fluoroscopic guidance with placement of a 10 mm Viatorr stent-graft (Gore Medical, Flagstaff, Arizona, USA) between the right hepatic vein and the right portal venous branch. Balloon dilatation was performed to an effective diameter of 8 to 10 mm with a non-compliant balloon (Mustang, Boston Scientific, Galway, Ireland) based on the individual needs of the patient with the goal to reduce the portosystemic gradient by half (ideally below 12 mmHg).

**Pressures measurement.** Pre- and post-TIPS intravascular pressure levels (right atrial pressure, portal venous pressure, hepatic venous pressure) were measured during TIPS-creation using a 5F pigtail catheter and a pressure transducer system (Combitrans, Braun, Melsung, Germany) with a multichannel monitor (Sirecust, Siemens, Germany). Three separate measurements were performed and results are reported as the mean of the total number of valid measurements. The difference between portal and hepatic venous pressures was defined as portosystemic pressure gradient.

**Data acquisition.** 93 patients received pre-interventional CT before TIPS-creation and were included into the study (57 men, 36 female, mean age 59.4 [range 36–86] years) with a mean time between CT imaging and TIPS-creation of 10 ± 14 days (range 0–77). In a subgroup of 38/93 patients an additional post-TIPS CT was available (mean time between TIPS-creation and follow-up CT 118 ± 164 days (range 0–660)). Patient characteristics, indications for TIPS-creation and hemodynamic parameters were retrieved from patients' medical records. Indications for TIPS-creation were bleeding in 33, therapy refractory ascites in 58 and a combination of both in 2 patients.

Contrast-enhanced CT scans were acquired in the portal venous phase with patients in a supine position using a 16-slice or 64-slice spiral CT scanner (Philips Medical Systems, Best, The Netherlands). Patients fasted for at least two to three hours before examination and received an enteral contrast agent (30 ml Gastrografin [Bayer Healthcare, Leverkusen, Germany] in 1 l of water) one hour before the CT.

Images were assessed with respect to (at least partial) discernibility of the cisterna chyli. The cisterna chyli was identified in the retrocrural space right of the aorta as an oblong structure isodens to water typically extending cranially into the lower caliber thoracic duct. The maximum axial diameter of the cisterna chyli was measured independently by two radiologists perpendicular to the long axis both on pre- and post-interventional CT (C.C.P, 9 years of experience, A.F, 7 years of experience; both blinded to patients' clinical data). To this end, 3D multiplanar reconstructions of the CT data set were created offline on a clinical PACS-workstation (IMPAX, Agfa, Germany) with axial slices perpendicular to the long axis of the cisterna chyli. Measurement of the largest diameter of the cisterna chyli (outer circumference to outer circumference) was performed by hand by both readers. In patients with post-TIPS CT scans the diameter was also measured at the site of the largest diameter of the cisterna chyli. A diameter > 6 mm was defined as dilatation of the cisterna chyli[8]. Images were additionally evaluated by both radiologists in consensus according to the presence and extent of portosystemic collateral vessels (especially gastric veins, para-umbilical veins or spleno-renal shunts). In cases with visible portosystemic collaterals, the diameter of these vessels was measured perpendicular to its long axis at the largest identifiable collateral using 3D multiplanar reconstructions. A cut-off diameter of 6 mm was employed to define large versus small porto-systemic collaterals.

**Shear-wave elastography (SWE) procedure.** SWE measurements of liver and spleen were performed in abdominal ultrasound with an abdominal 3.5-MHz curved-array transducer in three different regions of interest in the liver and the spleen as previously described using the Aixplorer US system (Supersonic Imagine SA; Aixplorer, Aix-en-Provence, France)[12–14]. The patients were placed in supine position with the right arm or left in maximal abduction and were requested to hold their breath for at least 5 s. A SWE measurement was considered accurate when it fulfilled the following criteria: (1) temporal stability of the selected liver area for at least three seconds before measurement, (2) two-dimensional quality confirmed by homogenous color in the region of interest and (3) a measurement region of at least 10 mm. Up to three separate measurements were performed and results reported as the mean of the total number of valid measurements.

**Statistical analysis.** Statistical analysis was performed using commercially available software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp., www.ibm.com) and GraphPad Prism (GraphPad Prism version 4.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com) was used to plot Fig. 2. Normal distribution of the data was assessed using Q-Q-plots and the Kolmogorov-Smirnov test. Statistical significance ($p < 0.05$) for group differences was tested with Student's t-test or ANOVA for independent samples in case of inter-subject comparisons (large collaterals vs. no large collaterals, indications for TIPS, cause of liver disease, Child–Pugh status), and with Student's t-test for paired samples in case of intra-subject comparisons (cisterna chyli diameter pre- vs. post-TIPS). Interrelations between lymphatic vessel diameters and pre-TIPS hemodynamic parameters (portal pressure, right atrial pressure, porto-systemic gradient) were assessed using Pearson's correlation coefficient. Inter-observer reliability of diameter measurements was investigated with the intra-class correlation coefficient (ICC). Kaplan–Meier curves and a log-rank test were used to analyze overall survival rates of patients in respect to gender, etiology of cirrhosis, bleeding vs. ascites, presence of large collaterals and detectability of the cisterna chyli). One-year overall survival rates were calculated.

## Results

**Patient characteristics.** Portal-pressure was successfully lowered by TIPS-creation from a mean (± SD) of 26.5 ± 5.7 mmHg (range 11–41) to 18.7 ± 4.7 mmHg (range 8–29) and portosystemic-gradient from 19.9 ± 5.6 mmHg (range 6–34) to 8.8 ± 3.8 mmHg (range 3–24). Pre-TIPS portal pressure was slightly, but significantly higher in patients receiving TIPS for variceal bleeding than in patients treated for ascites (data not shown). None of the patients in this cohort underwent liver transplantation post-TIPS. On CT large venous collaterals (> 6 mm) were identified in 59 patients. Pre-TIPS portal pressure and portosystemic gradient did not differ significantly between patients with and without large collaterals, between different causes of cirrhosis, patients with different Child–Pugh status or patients with and without a detectable cisterna chyli (Table 1). None of the patients with post-TIPS CT showed signs of TIPS dysfunction at the time of follow-up CT. Detailed patient characteristics are summarized in Table 1.

**Cisterna chyli diameter measurements.** The cisterna chyli was detectable on pre-interventional CT images in 69/93 cases (74.2%). Patient characteristics grouped by detectability of the cisterna chyli are summarized in Table 2. The mean pre-interventional diameter of the cisterna chyli was 9.4 ± 2.7 mm (range 5.3–16.2 mm). 64/69 patients (92.8%) showed a dilated cisterna chyli (> 6 mm). The cisterna chyli was significantly larger in patients without large portosystemic collaterals compared to patients with large collaterals (10.7 ± 3.2 mm vs. 8.7 ± 2.0 mm, $p = 0.003$) (Table 1). There were no significant differences in cisterna chyli diameter for the whole group as well as the subgroups with and without large collaterals between different causes of cirrhosis (alcohol, hepatitis, other), indications for TIPS-creation (bleeding or ascites) or Child–Pugh status.

The results of correlation analyses are summarized in Table 3. In patients with large collaterals there was no correlation between cisterna chyli diameter and portal-pressure, atrial pressure or portosystemic gradient. However, in patients without large collaterals the diameter of the cisterna chyli correlated strongly with pre-TIPS portal-pressure ($Rs = 0.685$, $p = 0.0001$), weakly with pre-TIPS atrial pressure ($Rs = 0.353$, $p = 0.07$) and moderately with the portosystemic gradient ($Rs = 0.524$, $p = 0.006$). Furthermore, cisterna chyli diameter correlated moderately with sheer wave elastography measurements of the liver ($Rs = 0.597$, $p = 0.004$) and spleen size ($Rs = 0.501$, $p = 0.01$).

On post-TIPS CT images the cisterna chyli was detectable in 31/38 cases (81.6%). In these patients with available post-TIPS follow-up CT, the diameter of the cisterna chyli decreased significantly after TIPS-creation in all but two cases from 10.2 ± 2.8 mm to 8.3 ± 3.0 mm ($p < 0.001$) (Figs. 1 and 2). Both patients with increasing cisterna chyli diameter showed no distinguishing clinical characteristics. One was treated for ascites, the other for recurrent bleeding. Mean diameter decrease was 1.9 ± 1.4 mm (ranging from an increase of 1.8 mm to a decrease of 5.5 mm). However, the cisterna chyli was still rated to be dilated (> 6 mm) in 26/31 cases (83.9%) after TIPS-creation.

**Interobserver reliability.** Intra-class correlation coefficients indicated almost perfect agreement between the two readers with an ICC of 0.991 [0.985; 0.994] for pre-TIPS cisterna chyli diameter and 0.997 [0.994; 0.999] for post-TIPS measurements.

**Survival.** Median overall survival after TIPS-creation was 44.8 ± 3.7 months (one-year overall survival rate 76.5%). Patients with a detectable cisterna chyli on CT survived significantly longer than those in whom the cisterna chyli was not visible on CT (49.0 ± 4.1 months vs. 17.0 ± 2.6 months; one-year overall survival rate 82.7% vs. 56.3%) (Fig. 3). There were no significant differences in survival in all other investigated grouping variables.

## Discussion

The results of the present study demonstrate that the diameter of central lymphatic vessels is closely associated with portal venous pressure in cirrhotic patients without larger venous collaterals that could provide decompression of the portal venous system. This indicates increased hepatic lymph production as a compensatory mechanism in portal hypertension. After TIPS-creation the diameter of the cisterna chyli decreases significantly, indicating decompression not only of the portal vein, but also of the lymphatic system due to decreasing hepatic lymph production.

In general, interstitial fluid and ultimately lymph is formed via filtration through the walls of blood capillaries[15,16]. Hepatic lymph primarily derives from the highly permeable sinusoids and is rich in plasma

| Parameters | All patients n = 93 | Large collaterals n = 59 | No large collaterals n = 34 | p |
|---|---|---|---|---|
| **General characteristics** | | | | |
| Gender [female/male] | 36/57 | 24/35 | 12/22 | 0.61 |
| Age [years] | 59.4 (36–85.5) | 58.7 (36–84.8) | 62.3 (38.4–85.5) | 0.12 |
| Etiology [viral/alcohol/other] | 12/56/25 | 7/36/16 | 5/20/9 | 0.92 |
| Child class [A/B/C] | 14/69/10 | 7/45/7 | 7/24/3 | 0.50 |
| MELD score | 10 (6–24) | 12 (6–24) | 9.5 (6–22) | 0.16 |
| Oesophageal varices [absent/present] | 15/78 | 8/51 | 7/27 | 0.38 |
| Ascites [absent/mild/severe] | 23/32/28 | 15/17/17 | 8/15/11 | 0.84 |
| Bleeding before TIPS [yes/no] | 35/58 | 24/35 | 11/23 | **0.01** |
| HRS [yes/no ] | 24/69 | 18/41 | 6/28 | 0.29 |
| History of SBP [yes/no] | 19/74 | 13/46 | 6/28 | 0.66 |
| HE [yes/no] | 21/72 | 19/40 | 2/32 | **0.01** |
| **Laboratory values** | | | | |
| Sodium [mmol/L] | 138 (121–155) | 139 (126–155) | 137 (121–143) | 0.14 |
| Potassium [mmol/L] | 4.12 (2.74–5.8) | 4.06 (2.74–5.8) | 4.13 (2.75–5.54) | 0.65 |
| Serum creatinine[mg/dL] | 1 (0.54–8.7) | 1 (0.59–2.5) | 1.05 (0.54–8.7) | 0.68 |
| Blood urea nitrogen [mg/dL] | 43.5 (0–168) | 48 (0–168) | 40 (9–148) | 0.18 |
| Total protein [g/L] | 60 (16–85) | 59 (16–82) | 61 (20–85) | 0.51 |
| Albumin [g/L] | 29 (10.6–45.7) | 29 (21–45.7) | 28.95 (10.6–40) | 0.70 |
| Bilirubin [mg/dL] | 1.18 (0.1–4.23) | 1.4 (0.1–4.23) | 0.97 (0.13–4) | **0.03** |
| yGT [U/L] | 121.5 (22–770) | 113 (22–770) | 128 (34–486) | 0.36 |
| ALT [U/L] | 28 (10–490) | 28 (13–174) | 27 (10–490) | 0.56 |
| AST [U/L] | 39 (11–777) | 42 (11–197) | 38 (14–777) | 0.24 |
| CRP [mg/L] | 12.5 (0.2–120) | 12.8 (0.2–114) | 12.1 (0.3–120) | 0.77 |
| INR | 1.2 (0.9–1.8) | 1.2 (1–1.8) | 1.1 (0.9–1.7) | 0.17 |
| Total white blood cell count [G/L] | 7.19 (2–15.9) | 7.24 (2–14) | 6.97 (2.45–15.9) | 0.96 |
| Haemoglobin [g/dL] | 9.6 (5.9–15.9) | 9.4 (5.9–15.9) | 10.35 (6.8–14.8) | 0.18 |
| Platelet count [G/L] | 134 (34–697) | 121 (34–332) | 158.5 (45–697) | **0.03** |
| **Hemodynamics** | | | | |
| Portal pressure [mmHg] | 26 (11–46) | 26 (11–46) | 26 (11–39) | 0.85 |
| Central venous pressure [mmHg] | 6 (0–22) | 6 (0–22) | 6 (1–15) | 0.67 |
| Portal pressure gradient [mmHg] | 20 (6–41) | 20 (8–41) | 19 (6–34) | 0.50 |
| **Cisterna chyli** | | | | |
| Detectabel on CT [y/n] | 59/24 | 43/16 | 26/8 | 0.45 |
| Diameter [mm] | 9.1 (5.3–16.2) | 8.7 (5.4–13.6) | 10.7 (5.3–16.2) | **0.01** |
| **SWE** | | | | |
| SWE meanliver [kPa] | 41.8 (12.7–132) | 40.7 (13.4–68.8) | 42.9 (12.7–132) | 0.81 |
| SWE meanspleen [kPa] | 37 ( 2–131) | 38.2 (2–131) | 35 (4–115) | 0.93 |
| Spleen size [cm] | 13.5 (8–20) | 14 (10–20) | 12.6 (8–19) | **0.03** |

**Table 1.** Patients Characteristics and comparison of patients with and without large venous collaterals. Statistically significant values ($p < 0.05$) are shown in bold.

proteins[3]. In contrast to other organs, lymph production in the liver therefore is primarily dependent of hepatic blood flow and pressure levels within the sinusoids. Average portal vein pressures of 7 mmHg in healthy individuals are usually only slightly higher than average interstitial pressure within the portal tracts (5.8 mmHg)[17], leading to a constant formation of interstitial fluid which is then primarily drained via portal lymphatics into the cisterna chyli[3].

In healthy individuals hepatic lymph constitutes 25–50% of lymph flow within the cisterna chyli and the thoracic duct, while about 40% derives from the intestine, with variation according to the nutritional status[3]. In liver disease lymph production can increase considerably due to an increasing pressure gradient between the sinusoids and the interstitial space[4,5]. In a cirrhotic rat model hepatic lymph production increased up to 30-fold[6].

This excessive lymph flow eventually exceeds the capacity of the central lymphatic system leading to leakage of hepatic lymph into the peritoneal cavity which in turn has been suggested as one of the factors responsible for ascites formation in cirrhotic patients[7].

Clinically close interrelations between hepatic blood and lymphatic vessels can be deduced from several studies. Dumont and Mulholland performed surgical drainage of thoracic duct lymph in cirrhotic patients with ascites or variceal bleeding. They observed considerably elevated pressure levels within the thoracic ducts from

| Parameters | Cisterna chyli detectable on CT n = 69 | Cisterna chyli not detectable on CT n = 24 | p |
|---|---|---|---|
| **General characteristics** | | | |
| Gender [female/male] | 20/49 | 16/8 | 0.53 |
| Age [years] | 59.4 (36–85) | 65.5 (39.1–85.5) | 0.12 |
| Etiology [viral/alcohol/other] | 10/43/16 | 2/13/9 | 0.35 |
| Child class [A/B/C] | 10/50/9 | 4/19/1 | 0.48 |
| MELD score | 10 (6–24) | 10.5 (6–20) | 0.16 |
| Oesophageal varices [absent/present] | 11/58 | 4/20 | 0.93 |
| Ascites [absent/mild/severe] | 16/25/20 | 7/9/8 | 0.56 |
| Bleeding before TIPS [yes/no] | 25/44 | 10/14 | 0.55 |
| HRS [yes/no ] | 19/50 | 5/19 | 0.79 |
| History of SBP [yes/no] | 13/56 | 6/18 | 0.54 |
| HE [yes/no] | 15/54 | 6/18 | 0.33 |
| **Laboratory values** | | | |
| Sodium [mmol/L] | 138 (121–155) | 138.5 (128–148) | 0.14 |
| Potassium [mmol/L] | 4.12 (2.75–5.54) | 4.07 (2.74–5.8) | 0.65 |
| Serum creatinine[mg/dL] | 1 (0.56–8.7) | 1 (0.54–2.2) | 0.68 |
| Blood urea nitrogen [mg/dL] | 45 (9–168) | 37 (0–143) | 0.18 |
| Total protein [g/L] | 59 (16–79) | 62 (20–85) | 0.51 |
| Albumin [g/L] | 28.6 (16.5–39) | 31.8 (10.6–45.7) | 0.7 |
| Bilirubin [mg/dL] | 1.18 (0.1–4) | 1.16 (0.21–4.23) | 0.32 |
| yGT [U/L] | 125.5 (26–770) | 115.5 (22–585) | 0.36 |
| ALT [U/L] | 28 (10–174) | 29 (14–490) | 0.56 |
| AST [U/L] | 38 (14–148) | 45.5 (11–777) | 0.24 |
| CRP [mg/L] | 11.9 (0.2–120) | 14.25 (1.7–75.1) | 0.77 |
| INR | 1.2 (0.9–1.8) | 1.15 (1–1.5) | 0.17 |
| Total white blood cell count [G/L] | 7.5 (2–15.9) | 6.38 (2.07–10.4) | 0.96 |
| Haemoglobin [g/dL] | 9.8 (5.9–15.9) | 9.4 (6.6–14.3) | 0.18 |
| Platelet count [G/L] | 121 (34–697) | 144 (41–289) | **0.003** |
| **Hemodynamics** | | | |
| Portal pressure [mmHg] | 26 (11–46) | 26.5 (11–41) | 0.84 |
| Central venous pressure [mmHg] | 6 (0–22) | 7 (1–15) | 0.66 |
| Portal pressure gradient [mmHg] | 20 (6–35) | 19.5 (8–41) | 0.50 |
| **SWE** | | | |
| SWE meanliver [kPa] | 41.1 (12.67–87.2) | 43 (13.4–132) | 0.80 |
| SWE meanspleen [kPa] | 36.3(1.36–115.4) | 37.65 (4.2–131.5) | 0.93 |
| Spleen size [cm] | 13.55 (8.2–20) | 13 (8–16.5) | **0.03** |

**Table 2.** Comparison of patients with and without a detectable cisterna chyli on CT. Statistically significant values ($p < 0.05$) are shown in bold.

| | All Patiens n = 93 | | Large collaterals n = 59 | | No large collaterals n = 34 | |
|---|---|---|---|---|---|---|
| Parameter | | p | | P | | p |
| **Hemodynamics** | | | | | | |
| Portal pressure | 0.225 | n.s | −0.102 | n.s | 0.685 | **0.0001** |
| Central venous pressure | 0.134 | n.s | −0.034 | n.s | 0.353 | **0.07** |
| Portal pressure gradient | 0.18 | n.s | −0.082 | n.s | 0.524 | **0.006** |
| **SWE** | | | | | | |
| SWE mean liver | 0.41 | **0.002** | 0.213 | n.s | 0.597 | **0.004** |
| SWE mean spleen | 0.355 | **0.013** | 0.166 | n.s | 0.415 | **0.07** |
| Spleen size | 0.306 | **0.014** | 0.261 | n.s | 0.501 | **0.01** |

**Table 3.** Correlation of cisterna chyli diameter and patients characteristics. Statistically significant values ($p < 0.05$) are shown in bold.

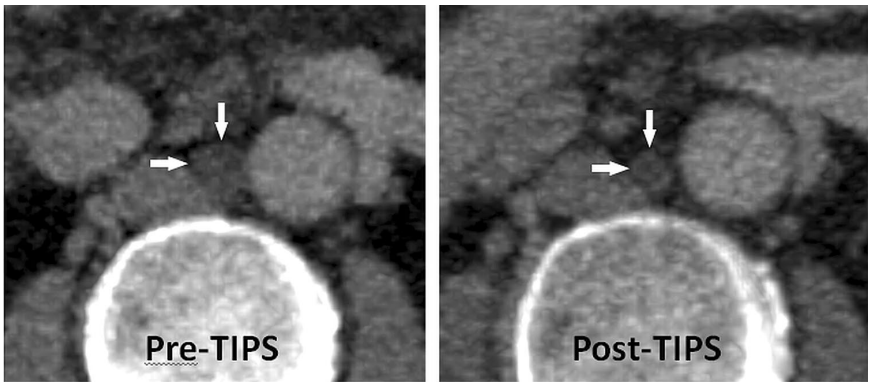



**Figure 1.** CT images of a patient before and after TIPS-creation demonstrating a considerable decrease in cisterna chyli (arrows) diameter after TIPS-creation.

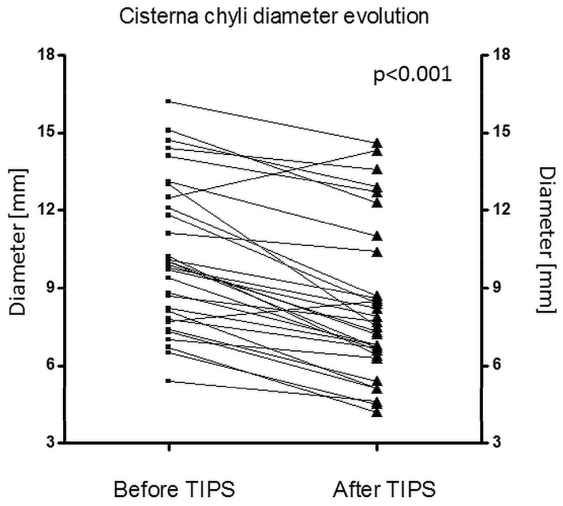


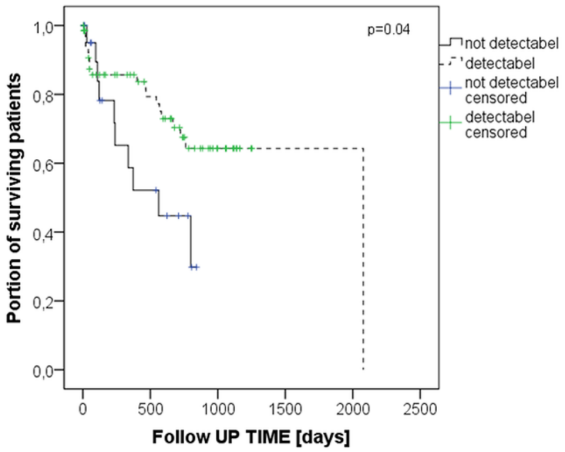

**Figure 2.** Changes in cisterna chyli diameter after TIPS creation. (GraphPad Prism version 4.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com).

15–70 cm saline (11–51.5 mmHg) (normal values 6–15 cm saline [4.4–11 mmHg])[18]. Drainage of thoracic duct lymph led to a decrease in ascites volume or termination of acute variceal bleeding. However, after discontinuation of thoracic duct drainage ascites re-accumulated and patients started to bleed again.

A close connection between hepatic blood vessels and lymphatic vessels is also reflected in that fact that changes in lymphatic flow/pressure result in morphological changes of the sinusoids and vice versa, e.g. after ligation of the thoracic duct[19]. Furthermore, the area of portal lymphatics increases in patients with idiopathic portal hypertension[20]. However, as examination of microscopic lymphatic vessels within the liver in humans in vivo is not feasible, the exact association between portal vein pressure and morphological changes of lymphatic vessels remains unknown.

Various imaging techniques can nowadays be applied in vivo to examine larger central lymphatic structures like the cisterna chyli and the thoracic duct[16,21]. Several studies primarily employing oily lymphangiography have shown that both portal lymphatics and the cisterna chyli can be dilated in patients with portal hypertension[10]. Nowadays, less invasive imaging techniques like computed tomography (CT)[22], sonography[9,11] or magnetic resonance imaging (MRI)[10,23,24] can reliably visualize central lymphatics in a large percentage of cases. This is in line with the results of our study with a detection rate of 74.2% for the cisterna chyli on standard contrast-enhanced CT.

Mean maximum transverse diameters of the cisterna chyli in healthy individuals differ slightly between studies, but seems to lie in the rage of 2–4 mm[10,22]. An MR-based study comparing cirrhotic patients with normal controls demonstrated that a cisterna chyli > 2 mm was identified significantly more often in patients suffering from liver cirrhosis compared to normal subjects (76% vs. 14%). Dilatation of the cisterna chyli (> 6 mm) was significantly more prevalent in patients with decompensated compared with compensated cirrhosis (54% vs.




| Number of patients | Day of TIPS placement | 250 days follow-up | 500 days follow-up | 1000 days follow-up | 1500 days follow-up |
|---|---|---|---|---|---|
| Detectable CCh | 69 | 46 | 37 | 11 | 1 |
| No detectable CCh | 24 | 11 | 8 | 0 | 0 |

**Figure 3.** Kaplan Meier plot demonstrating overall survival in patients with detectable (dashed line) and without detectable cisterna chyli (solid line) as well as number of patients at risk at different time points. (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp., www.ibm.com).

5%), while none of the normal subjects showed a cisterna chyli > 6 mm[10]. In our study all patients suffered from decompensated liver cirrhosis. Accordingly, in 93% of patients with a visible cisterna chyli, it was dilated > 6 mm, while the remaining 7% showed a diameter between 5.3–6 mm.

When comparing patients with and without portal hypertension Ito and colleagues found that the diameter of the cisterna chyli was significantly larger with than without portal hypertension, but without observing differences in the severity of portal hypertension. This is in line with our finding that there was no correlation between cisterna chyli diameter and portal pressure when analyzing the whole patient cohort. However, pressure levels were not invasively measured in the study by Ito and colleagues, but the presence and severity of portal hypertension was defined according to the presence and diameter of portosystemic collateral vessels[8]. This definition is problematic because although large collateral vessels may indicate the presence of severe portal hypertension, blood flow in the collaterals itself can already partially decompress the portal venous system. This is corroborated by our finding that compared to patients with large collaterals, patients without large collaterals exhibited significantly larger cisterna chyli diameters showing a strong correlation with portal pressure and a moderate correlation with the portosystemic gradient (which is the more reliable measure). Accordingly, there was a moderate positive correlation of sheer wave elastography measurements and spleen size with cisterna chyli diameter. Overall these findings suggest that there is more lymph flows within the cisterna chyli in these patients as a compensatory mechanism due to the lack of decompression via larger venous collaterals.

An established treatment option for cirrhosis-related ascites is portal decompression by TIPS-creation[25–27]. After TIPS-creation cisterna chyli diameters decreased significantly in all but two patients, indicating also a decompression of the lymphatic system due to decreasing hepatic lymph production. Both patients with slightly increasing cisterna chyli diameters showed large venous collaterals before TIPS-creation but did otherwise not differ from the remaining patients.

However, the cisterna chyli was still rated to be dilated (> 6 mm) in a large number of patients after TIPS-creation. This may be seen as a compensatory mechanism even after TIPS-creation due to still elevated hepatic lymph production. If lymphatic run-off is impaired (e.g. due to strictures of the thoracic duct or elevated central venous pressure) this may give rise to recurrent ascites. This, however, was not observed in the retrospective analysis of our patient-cohort and warrants prospective investigation.

In this respect a recent case report seems to corroborate the concept of lymphatic decompression of the portal venous system[28]. They describe decompression of the thoracic duct by placing a stent-graft across the lymphovenous junction to treat acute variceal bleeding in a patient with portal hypertension. Treatment was successful without recurrence of bleeding over a period of three months after treatment.

Interestingly patients without a detectable cisterna chyli survived significantly shorter after TIPS-creation than those with a visible cisterna chyli. At present we cannot conclusively explain this finding. A possible explanation may be that the absence of large central lymphatics may lead to an impairment of compensatory lymphatic run-off in such patients and therefore to impaired overall survival. This finding certainly has to be confirmed in larger patient cohorts as the cisterna may also not have been detectable on some CT-scans due to technical

reasons (insufficient contrast between the adjacent anatomical structures). However, the cisterna chyli is known to be anatomically absent in 20–30% of patients[29] which is in agreement of our detection rate. Further prospective studies with complete morphological and functional imaging work-up of the central lymphatic system may therefore be interesting in patients with therapy-refractory or recurrent ascites after TIPS-creation.

The results of our study are limited by its retrospective character with inherent methodological problems. First, results of measurements could not be compared to a gold standard as patients included into the study did not receive examinations specially dedicated to the depiction of the lymphatic system (such as conventional or MR lymphangiography). However, the successful use of contrast enhanced CT to visualize the cisterna chyli has already been described in literature and measurement results in our study showed almost perfect agreement between the two independent radiologist. Second, times between pre-TIPS CT and TIPS-creation, as well as between TIPS-creation and follow-up CT were not standardized. Although the mean time between pre-TIPS CT and TIPS-creation was only 10 days, it was as long as 3 months in single patients, so that the correlation of measured diameter (CT) and pressure (TIPS-creation) may be limited. Especially time intervals between intervention and follow-up CT differed so that pressure levels at the time of follow-up CT were not available as none of the patients had an indication for secondary invasive TIPS catheterization. We therefore refrained from correlation analysis of post-interventional pressure levels and cisterna chyli diameter. Furthermore, we cannot exclude a selection bias in the group of patients receiving post-TIPS follow-up CT as this cohort may represent especially the subgroup of patients with a stable clinical course after TIPS. More homogenous time intervals between CT and TIPS-creation would be needed to overcome this limitation. However, patients are not routinely submitted to CT scans before and after TIPS-creation due to radiation protection issues. Third, lymph flow within the cisterna chyli and the thoracic duct is known to increase after ingestion of food. The exact nutritional status of the patients at the time of CT could not be evaluated retrospectively, however, all CT scans showed largely empty stomachs except for enteral contrast agent. Fourth, diameter measurements may be influenced by random contraction waves of the cisterna chyli which have been observed in up to 11% of patients in a previous MR based study[8]. To exclude effects of these contraction waves repeated examinations would have been necessary, but cannot be performed in clinical routine. Width of the cisterna chyli is further affected by patient position with significantly larger cisterns in patients in a standing or sitting compared to a supine position[30]. However, all patients in our study were examined in the same supine position. Finally, the detection rate of the cisterna chyli on CT is certainly limited by available image quality. "Not detectable" therefore is not equivalent to "aplastic", but rather a combination of an actual aplasia of the cistern as well as lymph vessels that are too small to image on CT.

Due to the exploratory nature of this initial retrospective study without dedicated lymphatic imaging, we did not perform a multivariate survival analysis. Further prospective studies with lymphatic imaging are warranted to further evaluate the impact of the lymphatic system on patient survival after TIPS-creation.

## Conclusion

In conclusion the cisterna chyli can be confidently identified in about 75% of patients with end stage liver cirrhosis and portal hypertension on standard contrast enhanced CT. The diameter of the cisterna chyli was dilated in the majority of patients and was closely associated with portal pressure levels in patients without large venous collaterals that could have partly decompressed the portal venous system. Cisterna chyli diameter decreases significantly after TIPS-creation indicating decreasing hepatic lymph production and decompression of the lymphatic system after TIPS-creation. Further investigation into the association between lack of a detectable cisterna chyli and shorter overall survival after TIPS-creation is warranted.

Received: 18 November 2020; Accepted: 5 March 2021
Published online: 29 March 2021

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## Author contributions

C.C.P.: Conception and design of the study, data acquisition, analysis and interpretation, drafted the manuscript; A.F.: data acquisition and analysis, critical revision of the manuscript; C.M.: data acquisition, critical revision of the manuscript; J.L.: data interpretation, critical revision of the manuscript; M.P.: data acquisition and analysis, critical revision of the manuscript; J.T.: data interpretation, critical revision of the manuscript; U.A.: data interpretation, critical revision of the manuscript; C.J.: Conception and design of the study, data acquisition, analysis and interpretation, drafted the manuscript.

## Funding

Open Access funding enabled and organized by Projekt DEAL. The authors of this manuscript declare relationships with the following companies: CCP: Speakers Bureau: Guerbet, Philips Healthcare and Bayer Vital; Grant support: Guerbet, Medserena AG; CM: Speakers Bureau: Gore Medical; JT is supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18), European Union's Horizon 2020 Research and Innovation Programme (No. 668031) and Societal Challenges (Health, demographic change and well-being (No. 731875), and Cellex Foundation (PREDICT). UA: Speakers Bureau: Siemens Healthcare.

## Competing interests

The authors declare no competing interests.

## Additional information

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## 3.7 Die Varizenblutung hat, im Vergleich zu Nichtvarizenblutung, nur bei Männern eine erhöhte Sterblichkeit zur Folge

Erasmus HP, Bektas R, Orland K, Trifonova S, Gu WY, Uschner FE, Brol MJ, Chang J, Praktiknjo M, Strassburg CP, Trebicka J, **Jansen C. Variceal bleeding has increased mortality compared to nonvariceal bleeding only in males.** Eur J Gastroenterol Hepatol. 2021 Dec 1;33(1S Suppl 1):e94-e101. doi: 10.1097/MEG.0000000000001964. PMID: 33079784.

Zielsetzung der Arbeit – Die Grundannahme dieser Arbeit war, dass gastrointestinale Blutungen bei Patienten mit Zirrhose häufiger auftreten und eine hohe Morbidität und Mortalität bedingen. Ziel dieser Arbeit war es die Blutungen hinsichtlich Varizenblutung und Nicht-Varizenblutung zu unterschieden und ihre Relevanz für die Mortalität und Morbidität zu bestimmen.

Methoden und Ergebnisse – Insgesamt wurden 271 Patienten, mit Verdacht auf eine obere gastrointestinale Blutung, in die Studie eingeschlossen, die sich einer endoskopischen Untersuchung unterzogen haben.

Die Patienten wurden jeweils eine Woche, sechs Monate und ein Jahr nach der Aufnahme nachuntersucht. Mittels univariater und multivariater Cox Regressionsanalysen untersuchten wir die Korrelationen von prädiktiven Faktoren und klinischen Ereignissen.

Die Patienten wurden gemäß der Art der Blutung, Varizenblutung (n=115) oder Nicht-Varizenblutung (n=156) stratifiziert. Männer (n = 155) hatten eine höhere Sterblichkeit bei Varizenblutungen als bei Nicht-Varizenblutungen, während bei Frauen die Sterblichkeit bei beiden Blutungsarten ähnlich war.

Weitere, unabhängige Prädiktoren für die Mortalität bei Männern waren der MELD-Score zu Studienbeginn, Ammoniak und Alanin-Aminotransferase. Dahingegen konnte bei Frauen das Alter, die Leukozyten-Zahl, der MELD -Score; Aszites und die hepatische Enzephalopathie als Risikofaktoren für ein kürzeres Überleben idientifiziert werden.

Schlussfolgerungen – Diese Studie zeigt, dass Varizenblutungen, im Vergleich zu Nicht-Varizenblutungen, eine höhere Sterblichkeit bei Männern mit Leberzirrhose zur Folge haben. Bei Frauen konnte dieser Unterschied nicht nachgewiesen werden. Hier scheint die Art der gastrointestinalen Blutung keinen Einfluss auf die Morbidität und

Mortalität zu haben. Dies unterstreicht, dass das geschlechtsspezifische klinische Management auch in der Bewertung einer Blutung anzupassen ist.

Original article

# Variceal bleeding has increased mortality compared to nonvariceal bleeding only in males

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**Background** Gastrointestinal bleedings (GIBs) are frequent in cirrhotic patients and lead to high morbidity and mortality. Lately, there have been conflicting reports on the role of and bleeding type [variceal bleeding and nonvariceal bleeding (NVB)]. This study investigated the predictors of mortality in patients with variceal bleeding and NVB with relationship to sex differences.

**Materials and methods** A total of 271 patients with suspected upper GIB who underwent endoscopy were included. Patients were followed up at 1 week, 6 months and 1 year after admission. Univariate and multivariate logistic or Cox regression analyses investigated correlations of predictive factors and clinical outcomes. Propensity score matching was performed to control for severity of disease and compare groups for sex and bleeding type.

**Results** A total of 42 patients were excluded (cirrhosis or bleeding not confirmed). The remaining patients were classified by bleeding type into patients with variceal bleeding ($n=115$) or NVB ($n=156$). Males ($n=155$) had higher mortality in variceal bleeding than in NVB, while in females ($n=116$) mortality was similar in the two bleeding types. This was confirmed after matching in males ($n=116$) and females ($n=82$). Further independent predictors of mortality in males were model for end-stage liver disease (MELD) at baseline, blood urea nitrogen, alanine aminotransferase, while in females age, leukocytes, MELD, history of ascites and hepatic encephalopathy.

**Conclusion** This study shows that variceal bleeding has higher mortality in males compared to NVB, while in females the type of GIB does not impact the outcome. This highlights that sex-specific clinical management should be based on bleeding type after endoscopy. Eur J Gastroenterol Hepatol 33: e94–e101


## Introduction

Upper gastrointestinal bleedings (GIB) in cirrhosis can arise from either variceal bleedings or nonvariceal bleedings (NVB).

The development of esophageal varices is common in cirrhotic patients, with a prevalence of about 50%, their rupture resulting in a hemorrhagic event with high morbidity and mortality [1–9].

In recent years, mortality was reduced due to improvements in therapeutic management strategies during the acute bleeding episode and the postinterventional care period, for example, by preemptive transjugular intrahepatic portosystemic shunt (TIPS) [10,11]. There has been evidence of NVB events having better outcome than variceal bleeding, though the cause is not entirely clear. Sex seems to influence outcome independently of the type of bleeding. This is not surprising, since several processes and outcomes are dependent on sex, such as sarcopenia [12,13], creatinine and cystatin C [14], collagen III/IV deposition within liver tissue [15,16] and development of acute-on-chronic liver failure (ACLF) after surgery [17]. There also appears to be a significant difference in alcohol metabolism [18]. Though males with cirrhosis have significantly higher scores of physical body function, females have a better quality of life in areas of mental health [19].

The aim of this study was to analyze sex-specific differences and prognostic factors among patients presenting with suspected upper GIB and stratify risk between bleeding types.

## Materials and methods

This study retrospectively enrolled 313 patients of the Department of Internal Medicine, University Clinic Bonn (Germany) from June 2012 to September 2015. Patients had arrived at the hospital with suspected upper gastrointestinal-hemorrhage and suspected cirrhosis and were over the age of 18. Patients without confirmation of cirrhosis or bleeding events, or lower GIB were excluded after endoscopy. The ethics committees of the University Clinic Bonn approved the study protocol (203/13). Written informed consent for all interventions was obtained from included patients.

European Journal of Gastroenterology & Hepatology 2021, 33:e94–e101

Keywords: acute-on-chronic liver failure, acute decompensation, cirrhosis, endoscopy, gender, gender-specific medicine, liver, upper gastrointestinal bleeding, sex-specific differences, varices

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**Received** 22 July 2020 **Accepted** 24 September 2020

### *Definitions*

Upper GIB, as defined by any bleeding arising from the upper esophagus to the ligament of Treitz [20], was suspected based on patient history or clinical findings, that is, when patients presented or reported hematemesis, coffee-ground vomit or melena. Shock was defined as a positive shock-index >0.8 [21]. Upper and lower endoscopy were performed according to the German national guidelines on GIBs by the German Society of Gastroenterology (Digestive and Metabolic Diseases) [22]. Variceal bleeding was diagnosed when clinical suspicion signs were confirmed by endoscopic bleeding signs; the former were hematemesis, coffee ground vomitus, melena or hematochezia confirmed by hospital staff, the latter were active bleedings, white nipple signs, variceal erosions, red-color signs on large varices or adherent blood clots. NVB was diagnosed when the clinical signs presented without endoscopic signs of variceal bleeding and a portal-hypertensive gastropathy or ulcer bleeding were deemed the cause of bleeding.

At initial presentation, after hemostatic treatment and at follow-up intervals of 6 weeks, 6 months and 1-year data about the patients' history, physical and laboratory examination values were collected. Successful initial hemostasis was defined by stable vital signs and an absence of rebleeding signs within the first 5 days. The primary outcome was all-caused mortality during 1-year follow-up.

The esophageal varices were classified as either small or large based on their extension into the esophageal lumen; gastric varices were classified according to the criteria suggested – and Baveno consensus-endorsed in 1996 – by Sarin *et al*. who described four types: Gastroesophageal varices type 1 (GOV1) and type 2 (GOV2) are varices that lie along the lesser and greater curvature of the stomach, respectively, and extend into the esophagus. Isolated gastric varices can be of two types, type 1 (IGV1) being found in the cardia in the absence of esophageal varices and type 2 (IGV2) being those outside of the cardiofundal region or in the proximal duodenum. Bleeding was deemed active whenever blood was seen endoscopically in a jet stream or oozing from a variceal lesion.

### *Treatment*

In our center vasoconstrictors, proton pump inhibitors and prophylactic antibiotics were administered as soon as possible after admission. Transfusion of red blood cells occurred whenever the hemoglobin blood concentration dropped below 7 g/dL prior to endoscopic treatment. Based on the clinical presentation, history of the patient and partly endoscopic findings, initial hemostatic treatment comprised drugs, endoscopy or a complete lack or combination of these. Endoscopic treatment included both endoscopic band ligation and tissue glue injections. Other treatment possibilities were TIPS placements or revision of existing TIPS or the creation of surgical shunts. All procedures were performed by practitioners experienced in their field of specialty or by experienced residents under the supervision of an experienced specialist.

### *Statistical analysis*

Descriptive data are reported as median (interquartile range) for numerical and as counts (percentage of total) for categorical variables. In cases of missing values, adjusted subgroup number (*N*) is listed in square brackets behind a given value. For assessment of normal and non-normal distributed groups, the Student's *t*-test and Mann–Whitney *U* test were used, respectively; Chi-square test was used for categorical variables. Univariate and multivariate Cox regression (forward selection) analyses were performed to find correlations of predictive factors and clinical outcomes. Two-tailed *P* values of <0.05 were considered statistically significant and are individually reported to visualize the degree of significance of findings. Covariates with *P* values <0.05 in the univariate analysis were selected into multivariate model. Propensity score matching by bleeding type, age and model for end-stage liver disease (MELD) score was performed for further survival analysis showed by Kaplan–Meier curves compared between groups by log-rank tests. Univariate and multivariate analyses were performed both in all 271 patients and in sex-specific grouping to detect confounders for 1-year survival. Statistical analyses were performed with IBM SPSS Statistics 24 (SPSS Inc. Chicago, Illinois, USA).

## Results

### *Patient characteristics*

A total of 313 patients were recruited. Of these, a cumulative 42 patients were excluded either if they were not cirrhotic ($n=22$) or bleeding was not confirmed endoscopically ($n=37$). A total of 271 patients were hence included for analysis, with a slight majority of 156 (58%) NVBs to 115 variceal bleedings (42%) (Fig. 1). The median age was 59 years (51–68), and 155 (57%) patients were male. The etiology of cirrhosis was either alcohol (141, 52%), viral (53, 20%) or other causes (77, 28%) including, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). Patients with chronic hepatitis B (6/5.2% in the variceal bleeding group, 5/3.2% in the NVB group), PBC/PSC (7/6.0% in the variceal bleeding group, 12/7.7% in the NVB group), or AIH (3/2.6% in the variceal bleeding group, 1/0.6% in the NVB group), were treated according to current recommendations. No difference was observed between bleeding groups. We could not see a difference in outcome in patients treated or untreated. Median MELD-score at time of inclusion was 14 (11–19) and Child-Pugh score was 8 (7–9) with Child-Pugh class being distributed with 66 (23%), 150 (57%) and 55 (20%) of patients in classes A, B and C, respectively. At presentation, 200 (74%) of these patients had cirrhosis-related complications, such as hepatic encephalopathy, ascites, jaundice or others. After 1 year, 139 of these survived, 69 were lost to follow-up and 63 died (see Table 1). The cause of death was documented in 33 out of 63 patients. It was due to ACLF in 4 (3.4%) patients in variceal bleeding and 4 (2.6%) patients in NVB and due to sepsis in 12 (10.3%) patients in variceal bleeding and 13 (8.3%) patients in NVB. Ten patients received a liver transplant (2/1.7% in variceal bleeding, 8/5.1% in NVB). Average follow-up time including 81 patients lost during the interval was 235 days. No difference among groups regarding cause of death, liver transplants or average follow-up time was stastistically significant.

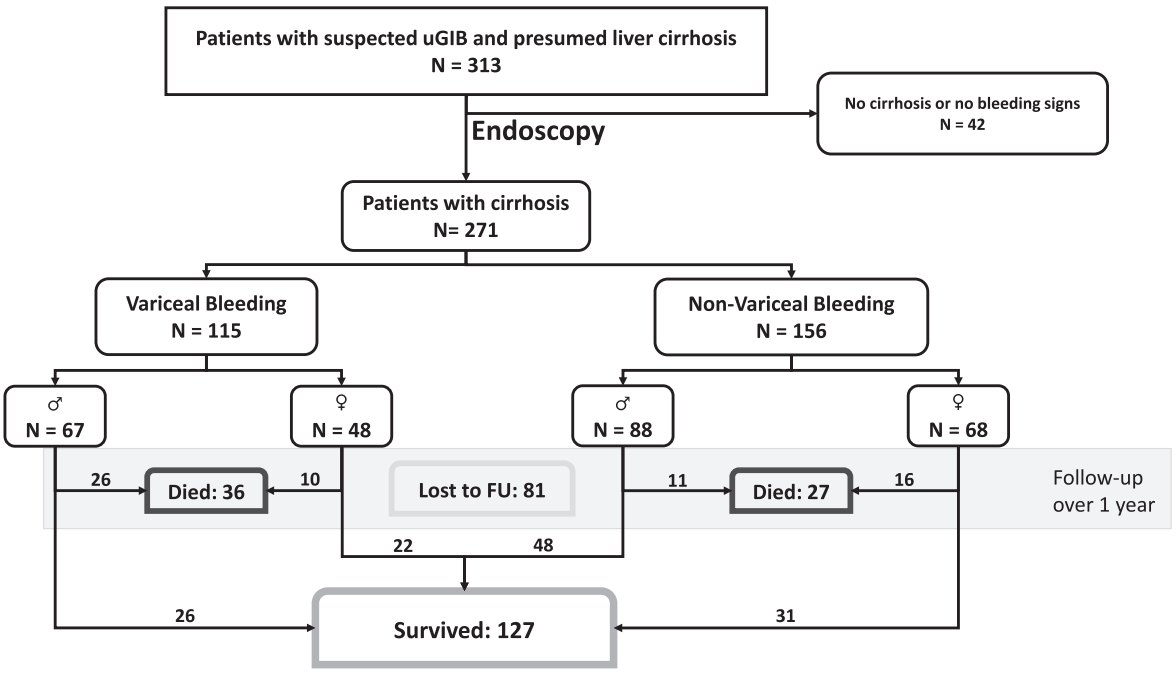


**Fig. 1.** Flowchart describing the patient cohort, enrollment and classification steps as well as the survival during follow-up.

### *Role of bleeding type*

A total of 115 (42.4%) of patients presented with variceal bleeding, 156 (57.6%) with NVB. Causes of NVB were portal hypertensive gastropathy in 116 (74.4%) cases, post-banding ulcera in 10 (6.4%) cases, of unknown source in 31 (19.9%) cases and labeled as 'other source' in 5 (3.2%) cases. Treatment of NVB was none, endoscopical only, pharmaceutical only, dual, or by TIPS revision in 63 (41.0%), 62 (39.7%), 6 (3.8%), 23 (14.7%) or 1 (0.6%), respectively. Gender (58 vs. 56% male), age (median 60 vs. 58), and etiology of cirrhosis were similar in variceal bleeding vs. NVB, as was number of past decompensations and bleedings. The severity of liver disease was also comparable with MELD of 14 vs. 15 and 8 vs. 7 Child-Pugh points, respectively. The percentage of patients presenting with either hepatic encephalopathy, ascites or jaundice was also similar. Patients in the bleeding groups differed in the hemoglobin and white blood cell count (WBC) level at admission with lower hemoglobin and higher WBC levels. Significantly more patients with variceal bleeding presented in shock and more variceal bleeding patients received endoscopy within 24 h and were treated with antibiotic prophylaxis after the bleeding event (see Table 1). Bleeding control was achieved in all 105 (90.5%) cases of variceal bleeding and 150 (96.2%) cases of NVB with no significant difference.

A Kaplan–Meier survival curve stratified by bleeding type showed a significantly worse outcome for patients with index variceal bleeding (see Fig. 2a). Cox regression in the entire cohort showed that variceal bleeding, presence of hepatocellular carcinoma (HCC) or ascites, WBC, serum AST and MELD at baseline independently increased mortality (see Table 2). Interestingly, the difference in survival was marked when stratified by gender: Males with variceal bleeding had a significantly worse outcome than in NVB, while females had comparable survival in both bleeding groups (see Fig. 2b and c).

### *Propensity score matching for males and females and role of bleeding type in mortality*

Nonetheless, as the abovementioned groups were unbalanced, propensity score matching was performed, to control for the severity of liver disease separately in males and females. The baseline characteristics of male and female patients and their bleeding types are shown in Table 3. In the matched male subgroup ($n = 116$), patients with variceal bleeding had significantly lower hemoglobin than in NVB (8.8 g/dL vs. 10.7 g/dL, $P < 0.05$) and hyponatremia was more pronounced (136 vs. 139 mmol/L, $P < 0.05$). Most importantly, 38% of patients with variceal bleeding vs. just 12% with NVB died within 1 year. In females, significant differences were found in the number of patients in shock at admission (10 vs. 0%) in variceal bleeding vs. NVB. The Kaplan–Meier analysis of the matched groups confirmed that males the results found in the entire cohort (see Fig. 3a and b). Univariate and multivariate analysis by Cox regression in the matched populations showed that NVB was independently associated with lower mortality in males only [multivariate hazard ratio: 0.05 (95% CI, 0.01–0.26), $P < 0.001$], whereas higher blood urea nitrogen (BUN), MELD and serum alanine aminotransferase (ALT) were independently associated with higher mortality in both males and females. In females only, the presence of hepatic encephalopathy, and increased age and total WBC were all independent risk factors of increased mortality (see Table 4).

**Table 1.** Baseline characteristics of overall cohort

| Parameter | Total (*n* = 271) | Variceal bleeding (*n* = 115) | NVB (*n* = 156) |
|---|---|---|---|
| Demographics | | | |
| Male sex | 155 (57%) | 67 (58%) | 88 (56%) |
| Age (years) | 59 (51–68) | 60 (50–70) | 58 (51–67) |
| Alcoholic cirrhosis | 141 (52%) | 60 (52%) | 81 (52%) |
| Viral cirrhosis | 53 (20%) | 22 (19%) | 31 (20%) |
| Other cirrhosis | 77 (28%) | 33 (29%) | 44 (28%) |
| Previous bleeding | 87 (32%) | 45 (39%) | 47 (30%) |
| Active alcoholism | 105 (39%) | 45 (39%) | 60 (38%) |
| Previous decompensation | 170 (63%) | 70 (61%) | 100 (65%) |
| Ascites | 138 (51%) | 51 (67%) | 87 (66%) |
| Hepatic encephalopathy | 71 (26%) | 35 (30%) | 36 (23%) |
| On Abx at time of bleeding* | 88 (33%) | 47 (41%) | 41 (26%) |
| PVT | 40 (15%) | 19 (17%) | 21 (14%) |
| HCC | 18 (7%) | 10 (7%) | 8 (6%) |
| Scores | | | |
| MELD at baseline | 14 (11–19) | 14 (10–20) | 15 (11–18) |
| CLIF-C AD | 51 (44–58) | 52 (46–59) | 50 (43–57) |
| Child class A/B/C | 66 (24%)/150 (55%)/55 (20%) | 26 (23%)/62 (54%)/27 (23%) | 40 (26%)/88 (56%)/28 (18%) |
| Child score | 8 (7–9) | 8 (7–9) | 7 (6–9) |
| Laboratory parameters | | | |
| Hb (g/dL)*** | 9.7 (8.2–11.7) | 9.2 (7.5–10.8) | 10.3 (8.5–12.8) |
| WBC (1000/dL)** | 7.0 (4.8–10.2) | 7.7 (5.4–10.5) | 6.6 (4.4–8.8) |
| PLT (1000/dL) | 106 (67–158) | 104 (67–143) | 110 (67–171) |
| ALT (U/L) | 30 (20–48) | 31 (19–47) | 29 (20–50) |
| AST (U/L) | 50 (33–77) | 50 (35–89) | 50 (33–74) |
| Creatinine (mg/dL) | 1.1 (0.8–1.6) | 1.1 (0.8–1.7) | 1.0 (0.8–1.5) |
| BUN (mg/dL) | 53 (30–86) | 55 (32–98) | 48 (25–78) |
| Sodium (mEq/dL) | 138 (135–141) | 138 (135–141) | 138 (135–140) |
| Bilirubin (mg/dL) | 1.8 (1.0–3.4) | 1.6 (0.9–3.5) | 1.9 (1.1–3.4) |
| INR | 1.3 (1.1–1.5) | 1.3 (1.1–1.6) | 1.3 (1.1–1.5) |
| Shock at admission* | 7 (3%) | 6 (6%) | 1 (1%) |
| Complications | | | |
| Complications at admission | 200 (74%) | 91 (79%) | 109 (70%) |
| Hepatic Encephalopathy Grade I–II/III–IV | 65 (25%) 55 (20%)/10 (5%) | 33 (28%) 28 (24%)/5 (4%) | 32 (20%) 27 (17%)/5 (3%) |
| Ascites | 164 (61%) | 78 (68%) | 86 (55%) |
| Mild–moderate/ refractory | 98 (36%)/66 (24%) | 47 (41%)/31 (27%) | 51 (33%)/35 (22%) |
| Jaundice | 51 (19%) | 22 (19%) | 29 (19%) |
| Other* | 85 (35%) | 43 (38%) | 42 (27%) |
| Treatment | | | |
| Endoscopy in <6 h**/<12 h**/<24 h** | 94/107/126 | 54/59/67 | 40/48/59 |
| Need for intubation for Endo*** | 28 (10%) | 21 (19%) | 7 (5%) |
| Use of Abx Prophylaxis*** | 178 (66%) | 91 (80%) | 87 (58%) |
| Number of RBC in 24h*** | 0 (0–2) | 2 (0–2) | 0 (0–2) |
| Outcome | | | |
| Alive at 1 year | 139 (51%) | 54 (47%) | 85 (55%) |
| Total days of hospitalization | 13 (7–23) | 15 (7–24) | 12 (6–22) |

Data presented as median (interquartile range) or *n* (%), as appropriate. The Chi-square test was used for categorical variables and the Mann–Whitney *U* test for continuous variables. The list of parameters is shown with respective overall significance values ($ <0.1, *<0.05, **<0.01, ***<0.001), compared by bleeding type with inter-group significance (Group compared to NB, same as above; Group compared to variceal bleeding: § <0.1, +<0.5, ++<0.05, +++<0.001. Statistically significant differences between patients with variceal and nonvariceal bleedings are highlighted in bold.

Abx, antibiotics; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CLIF-C AD, chronic liver failure Consortium Acute Decompensation score; Hb, hemoglobin; HCC, hepatocellular carcinoma; INR, international normalized ratio; MELD, model of end-stage liver disease; NVB, nonvariceal bleeding; PLT, platelets; PVT, portal vein thrombosis; RBC, packed red blood cells; WBC, white blood cell count.

## Discussion

Our study investigated differences in patients with liver cirrhosis presenting with upper GIB along the dimensions of both sex and bleeding type. Variceal bleeding had overall higher mortality than NVB in males, while in females variceal bleeding and NVB showed similar outcome.

Varices development and bleeding are directly caused by portal hypertension [23]. In fact, in both treatment and rebleeding prophylaxis, the major aim is to decrease portal pressure by either nonselective beta-blockers or invasively, using interventional (TIPS) or surgical portosystemic shunts [2]. On the other hand, NVB is a multifactorial and not entirely understood entity derived from several factors, other than portal hypertension, such as hypocoagulability, peptic ulcers, uremia arising from kidney failure or hepatorenal syndrome or severity of liver disease [24,25]. The severity of liver disease worsens prognosis in upper gastrointestinal hemorrhage, since it contributes to mortality likely due to a multiplicity of complications, developing in the aftermath of bleeding events [26,27]. Therefore, outcome after variceal bleeding or NVB is majorly affected by severity of liver disease. Studies so far have not been able to clarify the question whether bleeding type matters in determining the outcome, since they did not control for severity of liver disease. We could demonstrate that bleeding type matters in patient groups matched by severity of the liver disease. Yet, this difference was only significant in males, while in females bleeding type did not influence outcome. Therefore, biological sex seems to be a factor influencing outcome in patients with cirrhosis.

In fact, several studies have brought to attention that several sex-specific differences might be important in determining the outcome of cirrhotic patients [14,15,17]. The development of cirrhosis and HCC appears to be more frequent in males [28], while AIH has a higher prevalence in females [29]. However, conflicting data exist on whether males and females have a different outcome in the event of upper GIB. In our cohort of 271 patients, little more than half of the patients were male, with variceal bleeding having a worse outcome overall. When stratified by sex, no survival differences were found in the overall cohort. To analyze sex-specific survival differences and exclude as many confounding factors as possible, we matched our cohort by sex, age and severity of liver disease and analyzed the differences within either sex between bleeding groups. The abovementioned difference in survival by log-rank was confirmed by propensity score matching. Cox regression also showed bleeding type to be a significant independent factor in survival of male patients, while in females hepatic encephalopathy, ALT, WBCs and BUN were correlated with worse outcome, but the bleeding type was not.

There have been some studies that evaluated sex as a risk factor. Schemmer *et al*. analyzed it among others in a risk factor analysis of 121 consecutive patients and found that males might slightly worse ($P = 0.065$, odds ratio 4.8) [30]. However, in that study, cirrhosis was not an absolute inclusion criterion. Tandon *et al*. compared clinical outcomes as measured by the length of hospital stay (LOS) and 30-day readmission rate in cirrhotic patients with either variceal or nonvariceal bleedings [31]. In their set

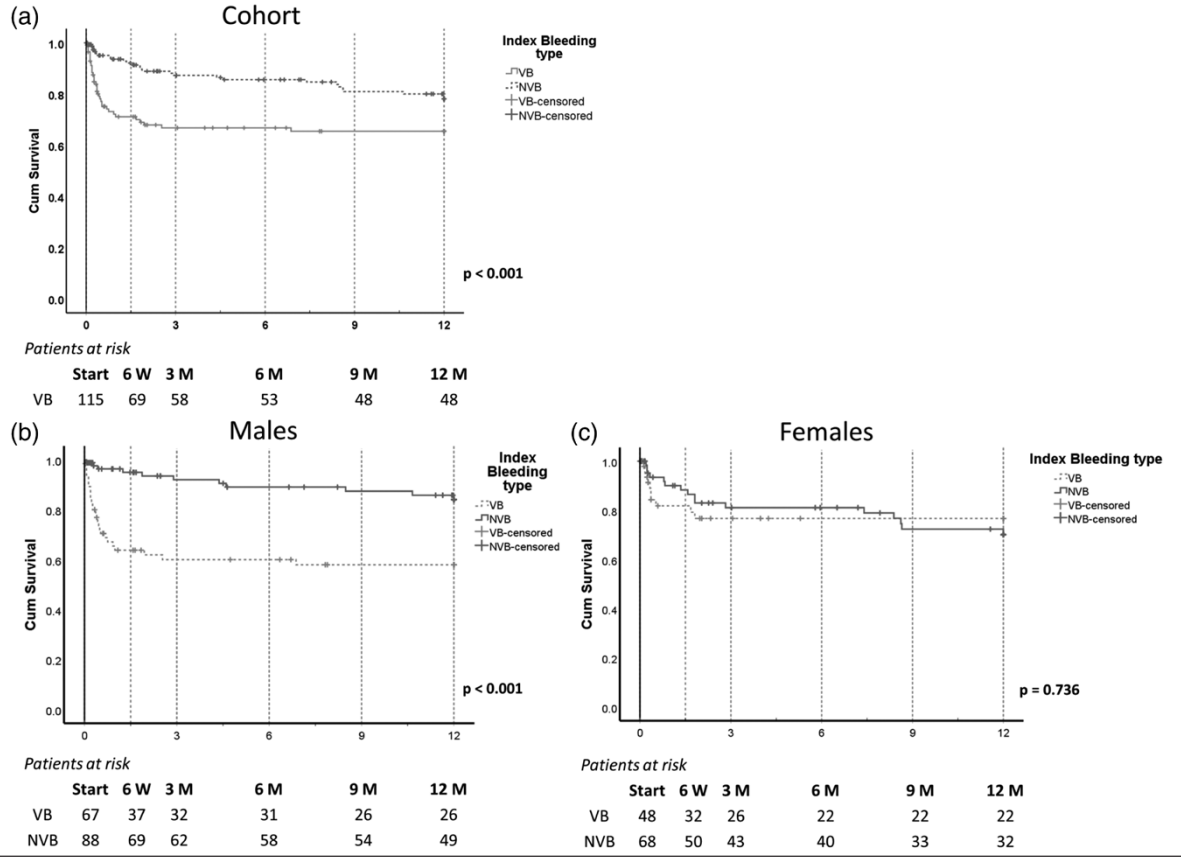


**Fig. 2.** Kaplan–Meier curves of survival at 1 year in the overall population by bleeding type. The survival curves for the overall population. Each step is a patient death, each small tick on the line is a patient lost to follow-up. (a) Whole cohort; (b) in males and (c) in females. NVB, nonvariceal bleeding.

**Table 2.** Univariate and multivariate analysis by Cox regressions in the whole population

| Parameter | Univariate hazard ratio and 95% CI | *P* | Multivariate hazard ratio and 95% CI | *P* |
|---|---|---|---|---|
| Presence of NVB | 0.47 (0.29–0.78) | 0.003 | 0.41 (0.22–0.78) | 0.006 |
| HCC | 2.98 (1.42–6.27) | 0.004 | 4.38 (1.74–11.04) | 0.002 |
| Ascites | 4.94 (2.35–10.40) | <0.001 | 4.12 (1.46–11.64) | 0.008 |
| WBCs | 1.08 (1.06–1.11) | 0.039 | 1.06 (1.02–1.09) | 0.002 |
| AST | 1.002 (1.002–1.003) | <0.001 | 1.002 (1.000–1.003) | 0.002 |
| MELD at baseline | 1.15 (1.11–1.19) | <0.001 | 1.10 (1.06–1.15) | <0.001 |
| Refractory ascites | 2.94 (1.76–4.91) | <0.001 | | |
| Jaundice | 2.68 (1.58–4.54) | <0.001 | | |
| Hepatic encephalopathy | 1.94 (1.16–3.24) | 0.012 | | |
| Hemoglobin | 0.80 (0.71–0.89) | 0.039 | | |
| BUN | 1.01 (1.01–1.01) | <0.001 | | |

Complications and lab parameters were evaluated at initial presentation. Parameters are listed with significance and hazard ratios with 95% confidence interval. AST, aspartate aminotransferase; BUN, blood urea nitrogen; CI, confidence interval; HCC, hepatocellular carcinoma; MELD, model of end-stage liver disease score; NVB, nonvariceal bleeding.

of 116 included patients, they did find a trend in mortality between variceal bleeding and NVB (15.1 vs. 9.3%). Moreover, LOS was not associated with sex and the relationship between mortality and sex was not described in the article. In our study, we observed outcomes over a longer time period, and included a larger set of patients. Moreover, we improved the analyses by adjusting for liver disease severity, age and sex to be able to compare between bleeding groups. First, our data showed that variceal bleeding was associated with a worse outcome compared to NVB. However, in the unmatched overall cohort, no difference between survival in males or females were found. Once stratified by bleeding groups, males were found to have a much worse outcome with variceal bleeding rather than with NVB, while an equally bad outcome was found for females in both bleeding groups. This association was confirmed after matching by sex and severity of disease.

To the best of our knowledge, our study is the first to compare sex-differences in survival within bleeding groups after excluding confounding. Furthermore, these findings have several clinical implications by identifying potential high-risk patients in males with variceal bleeding. In males, it is important to stratify by bleeding. Especially closer follow-up after endoscopy should be an important

**Table 3.** Cohort after propensity score matching and classified by sex

| Parameter | ♂ = 116 | | ♀ = 82 | |
|---|---|---|---|---|
| Demographics | | | | |
| Bleeding type | Variceal bleeding (*n* = 58) | NVB (*n* = 58) | Variceal bleeding (*n* = 41) | NVB (*n* = 41) |
| Age (years) | 60 (51–71) | 59 (51–67) | 61 (50–68) | 57 (51–67) |
| Alcoholic/viral/other cirrhosis | 35 (60%)/11 (20%)/12 (21%) | 32 (55%)/12 (21%)/14 (24%) | 18 (44%)/8 (20%)/15 (37%) | 17 (41%)/6 (15%)/18 (44%) |
| Previous bleeding | 22 (38%) | 23 (40%) | 17 (41%) | 8 (20%) |
| Active alcoholism | 25 (43%) | 23 (40%) | 15 (37%) | 16 (39%) |
| Previous decompensation | 37 (64%) | 40 (69%) | 24 (59%) | 32 (78%) |
| Ascites | 29 (50%) | 34 (59%) | 16** (39%) | 29 (71%) |
| Hepatic encephalopathy | 19 (33%) | 14 (24%) | 13 (32%) | 8 (20%) |
| Scores | | | | |
| MELD at baseline | 15 (11–19) | 15 (10–20) | 13 (10–17) | 14 (11–17) |
| CLIF-C AD | 55 (46–60) | 49 (43–55) | 50 (43–57) | 48 (43–59) |
| Child class A/B/C | 10 (17%)/34 (59%)/14 (24%) | 14 (24%)/34 (59%)/10 (17%) | 10 (24%)/23 (56%)/8 (20%) | 5 (12%)/29 (71%)/7 (17%) |
| Child score | 8 (7–9) | 7 (7–9) | 8 (7–9) | 8 (7–9) |
| Laboratory parameters | | | | |
| Hb (g/dL) | 8.8*** (7.3–10.8) | 10.7 (9.2–13.2) | 9.5 (8.2–11.0) | 10.1 (8.4–12.3) |
| WBC (1000/dL) | 7.1 (5.0–12.1) | 6.1 (4.4–8.8) | 8.0 (5.9–9.2) | 6.7 (3.8–8.7) |
| PLT (1000/dL) | 103 (65–127) | 107 (70–173) | 97 (70–156) | 120 (82–188) |
| ALT (U/L) | 26 (19–43) | 30 (22–55) | 31 (19–44) | 27 (18–37) |
| AST (U/L) | 49 (28–80) | 45 (30–74) | 49 (37–68) | 48 (35–72) |
| Creatinine (mg/dL) | 1.3 (1.0–1.7) | 1.0 (0.8–1.6) | 0.9 (0.7–1.3) | 0.9 (0.7–1.3) |
| Sodium (mEq/dL) | 136* (134–140) | 139 (136–141) | 139 (136–141) | 137 (134–140) |
| Bilirubin (mg/dL) | 1.6 [0.9–3.5) | 1.8 (1.0–3.1) | 1.5 (0.8–2.6) | 1.8 (1.2–3.6) |
| BUN (mg/dL) | 64 (36–104) | 57 (30–81) | 48 (31–70) | 46 (25–69) |
| INR | 1.2 (1.2–1.5) | 1.2 (1.1–1.4) | 1.3 (1.2–1.6) | 1.3 (1.1–1.5) |
| Complications | | | | |
| Shock at admission | 0 (0%) | 0 (0%) | 4* (10%) | 0 (0%) |
| No hepatic encephalopathy/ grades I–II/III–IV | 42 (72%)/15 (26%)/1 (2%) | 46 (79%)/10 (17%)/2 (3%) | 27 (66%)/11 (27%)/3 (7%) | 30 (73%)/10 (24%)/1 (2%) |
| No ascites/moderate/ refractory | 17 (29%)/24 (41%)/17 (29%) | 24 (41%)/23 (40%)/11 (19%) | 13 (32%)/17 (41%)/11 (27%) | 12 (29%)/17 (41%)/12 (29%) |
| Treatment | | | | |
| Use of Abx prophylaxis | 49** (84%) | 35 (60%) | 31** (76%) | 19 (46%) |
| Number of RBC in 24 h | 2*** (0–2) | 0 (0–0) | 0* (0–2) | 0 (0–0) |
| Outcome | | | | |
| Total days of hospitalization | 15* (10–24) | 12 (6–22) | 16 (7–27) | 15 (8–24) |
| Deceased at 1 year | 22** (38%) | 7 (12%) | 9 (22%) | 8 (20%) |

Data presented as median (interquartile range) or *n* (%), as appropriate. The Chi-square test was used for categorical variables and the Mann–Whitney *U* test for continuous variables. List of parameters after propensity score matching for bleeding type with related significance value as marked by either asterisks and dollar signs for comparison between the bleeding types in the same sex ($< 0.1, *<0.05, **<0.01, ***<0.001).
Abx, antibiotics; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CLIF-C AD, chronic liver failure Consortium Acute Decompensation score; Hb, hemoglobin; HCC, hepatocellular carcinoma; INR, international normalized ratio; MELD, model of end-stage liver disease; NVB, nonvariceal bleeding; PLT, platelets; PVT, portal vein thrombosis; RBC, packed red blood cells; WBC, white blood cell count.

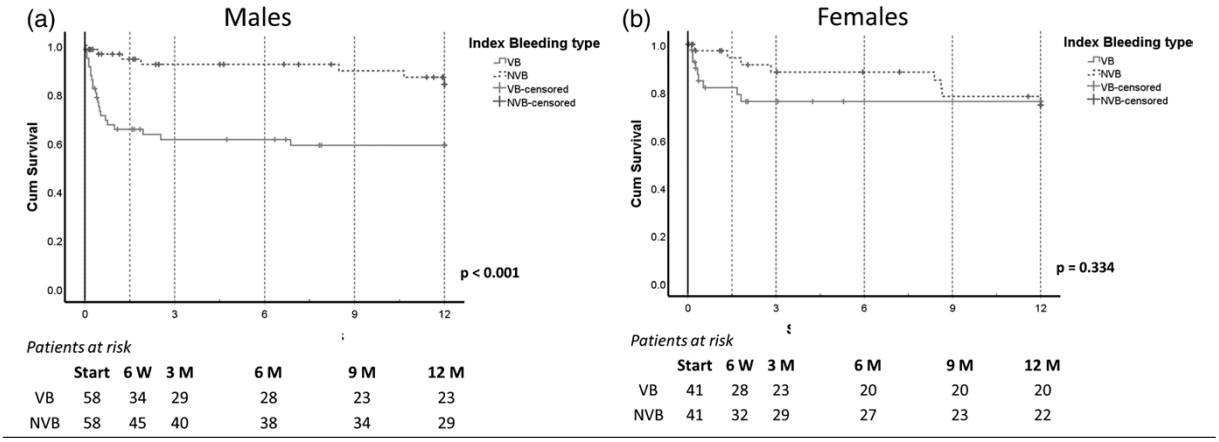


**Fig. 3.** Kaplan–Meier curves of survival at 1 year in a population matched by bleeding type. The survival curves for the matched population. Each step is a patient death, each small tick on the line is a patient lost to follow-up. (a) In males. (b) In females. NVB, nonvariceal bleeding.

focus specific to male cirrhotics with variceal bleeding as already suggested by current guidelines.

This study was limited by its retrospective observational character. Confounding factors such as sarcopenia or malnutrition were not regarded because they were not recorded in the registry. The retrospective design and the post hoc character of its analysis were further limitations of this study. Moreover, poor documentation of treatments can not be excluded, may induce further bias and may be a further limitation of this study, for example, only 80% of patients with variceal bleeding received prophylactic antibiotherapy. In summary, patient prognosis is dependent

**Table 4.** Univariate and multivariate analysis done by Cox regressions in the matched population split by sex

| Parameter | Univariate hazard ratio and 95% CI | *P* | Multivariate hazard ratio and 95% CI | *P* |
|---|---|---|---|---|
| Male | | | | |
| Presence of NVB | 0.27 (0.13–0.55) | <0.001 | 0.05 (0.01–0.26) | <0.001 |
| HCC | 3.64 (1.59–8.34) | 0.002 | | |
| Ascites | 5.29 (2.05–13.61) | 0.001 | | |
| WBCs | 1.19 (1.12–1.26) | <0.001 | | |
| MELD at baseline | 1.15 (1.09–1.21) | <0.001 | 1.20 (1.09–1.33) | <0.001 |
| Hemoglobin | 0.74 (0.63–0.86) | <0.001 | | |
| On Abx | 2.10 (1.09–4.05) | 0.027 | | |
| Shock | 19.50 (2.28–166.88) | 0.007 | | |
| BUN | 1.01 (1.01–1.01) | <0.001 | 1.01 (1.00–1.02) | 0.015 |
| Child-Pugh points | 1.34 (1.18–1.52) | <0.001 | | |
| ALT | 1.004 (1.00–1.01) | 0.047 | 1.01 (1.00–1.01) | 0.006 |
| Female | | | | |
| Previous ascites | 3.12 (1.31–7.42) | 0.010 | 4.28 (1.00–18.34) | 0.051 |
| Hepatic encephalopathy | 2.64 (1.22–5.70) | 0.014 | 4.05 (1.18–13.87) | 0.026 |
| Ascites | 4.25 (1.27–14.16) | 0.019 | | |
| Age | 1.04 (1.01–1.07) | 0.015 | 1.11 (1.03–1.20) | 0.006 |
| MELD | 1.14 (1.07–1.21) | <0.001 | 1.17 (1.06–1.29) | 0.002 |
| Child-Pugh points | 1.44 (1.18–1.76) | <0.001 | | |
| WBCs | 1.07 (1.03–1.11) | 0.001 | 1.22 (1.07–1.40) | 0.003 |
| BUN | 1.02 (1.01–1.03) | <0.001 | | |

Complications and lab parameters were evaluated at initial presentation. Parameters are listed with significance and hazard ratios with 95% confidence interval. Abx, antibiotics; ALT, alanine aminotransferase; BUN, blood urea nitrogen; HCC, hepatocellular carcinoma; MELD, model of end-stage liver disease score; NVB, nonvariceal bleeding; WBC, white blood cell count.

not only on the type of bleeding source but also on sex; different management strategies might need to be adopted based on these two factors.

## Acknowledgements

We thank Gudrun Hack and Silke Bellinghausen for their excellent technical support.

The authors were supported by grants from Deutsche Forschungsgemeinschaft (SFB TRR57 P18 and CRC1382) H2020 European Institute of Innovation and Technology (668031 and 825694) H2020 Societal Challenges (731875) and CELLEX Foundation (PREDICT). The funders had no influence on study design, data collection and analysis, decision to publish or preparation of the manuscript.

R.B., K.O., S.T. and C.J.: acquisition of data. W.Y.G., F.E.U., M.P., C.P.S., J.C. and M.J.B.: interpretation of data, critical revision of the manuscript regarding important intellectual content. J.T.: study concept and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript regarding important intellectual content, administrative, technical and material support, funding recipient and study supervision. H.P.E. and C.J.: analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript regarding important intellectual content.

### *Conflicts of interest*

There are no conflicts of interest.

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## 3.8 Die zweidimensionale Scherwellen-Elastographie sagt das Überleben bei Patienten mit fortgeschrittener chronischer Lebererkrankung voraus

Trebicka J, Gu W, de Ledinghen V, Aubé C, Krag A, Praktiknjo M, Castera L, Dumortier J, Bauer DJM, Friedrich-Rust M, Pol S, Grgurevic I, Zheng R, Francque S, Gottfriedovà H, Mustapic S, Sporea I, Berzigotti A, Uschner FE, Simbrunner B, Ronot M, Cassinotto C, Kjaergaard M, Andrade F, Schulz M, Semmler G, Drinkovic IT, Chang J, Brol MJ, Rautou PE, Vanwolleghem T, Strassburg CP, Boursier J, Ferstl PG, Rasmussen DN, Reiberger T, Vilgrain V, Guibal A, Guillaud O, Zeuzem S, Vassord C, Lu X, Vonghia L, Senkerikova R, Popescu A, Margini C, Wang W, Thiele M, **Jansen C. Two-dimensional shear wave elastography predicts survival in advanced chronic liver disease**. Gut. 2022 Feb;71(2):402-414. doi: 10.1136/gutjnl-2020-323419. Epub 2021 Jan 21. PMID: 33479052; PMCID: PMC8761995.

Zielsetzung der Arbeit – Ziel dieser Arbeit war es zu evaluieren, ob die Messung der Lebersteifigkeitsmessung, gemessen mit Shear-Wave-Elastographie geeignet ist, signifikante Fibrose und Portale Hypertension zu erkennen. Es sollte in dieser retrospektiven multizentrischen Studie untersucht werden, ob es dieses einfache Tool im klinischen Einsatz ermöglicht, Patienten mit einer erhöhten Mortalität zu detektieren.

Methoden und Ergebnisse – Klinische- und laborchemische Parameter wurden zu Studienbeginn und während des Follow-up erfasst. Die Lebersteifigkeitsmessung wurden durch Shear-Wave-Elastographie und transiente Elastographie erfasst. Der primäre Endpunkt war die Gesamtsterblichkeit. Der sekundäre Endpunkt war die Entwicklung einer ersten bzw. einer weiteren Dekompensation. Nach Screening von 2148 Patienten (16 Zentren) konnten 1827 eingeschlossen werden (55 Jahre, 62,4 % Männer). Die mittlere Lebersteifigkeit gemessen mit Shear-Wave-Elastographie betrug 11,8 kPa. Die Kombination aus MELD-Score und Lebersteifigkeit zeigte sich als unabhängiger Prädiktor für die Mortalität. Gewählt wurde ein cut-off von mehr als 20 kPa kombiniert mit einem MELD ≥10. Die 2-Jahres-Sterblichkeit und Dekompensationsraten betrugen 36,9 % bzw. 61,8 % in den einzelnen Gruppen. Der Algorithmus wurde von uns „M10LS20-Algorithmus“ benannt.

Schlussfolgerungen – Der M10LS20-Algorithmus ermöglicht es, eine Stratifizierung von Patienten mit weit fortgeschrittener Lebererkrankung vorzunehmen und Patienten mit erhöhtem Risiko einer Dekompensation und Mortalität zu erkennen.

OPEN ACCESS

Original research

# Two-dimensional shear wave elastography predicts survival in advanced chronic liver disease

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Received 20 October 2020
Revised 26 December 2020
Accepted 8 January 2021
Published Online First
21 January 2021



**To cite:** Trebicka J, Gu W, de Ledinghen V, *et al*. *Gut* 2022;**71**:402–414.

## ABSTRACT

**Objective** Liver stiffness measurement (LSM) is a tool used to screen for significant fibrosis and portal hypertension. The aim of this retrospective multicentre study was to develop an easy tool using LSM for clinical outcomes in advanced chronic liver disease (ACLD) patients.

**Design** This international multicentre cohort study included a derivation ACLD patient cohort with valid two-dimensional shear wave elastography (2D-SWE) results. Clinical and laboratory parameters at baseline and during follow-up were recorded. LSM by transient elastography (TE) was also recorded if available. The primary outcome was overall mortality. The secondary outcome was the development of first/further decompensation.

**Results** After screening 2148 patients (16 centres), 1827 patients (55 years, 62.4% men) were included in the 2D-SWE cohort, with median liver SWE (L-SWE) 11.8 kPa and a model for end stage liver disease (MELD) score of 8. Combination of MELD score and L-SWE predict independently of mortality (AUC 0.8). L-SWE cut-off at ≥20 kPa combined with MELD ≥10 could stratify the risk of mortality and first/further decompensation in ACLD patients. The 2-year mortality and decompensation rates were 36.9% and 61.8%, respectively, in the 305 (18.3%) high-risk patients (with L-SWE ≥20 kPa and MELD ≥10), while in the 944 (56.6%) low-risk patients, these were 1.1% and 3.5%, respectively. Importantly, this M10LS20 algorithm was validated by TE-based LSM and in an additional cohort of 119 patients with valid point shear SWE-LSM.

**Conclusion** The M10LS20 algorithm allows risk stratification of patients with ACLD. Patients with L-SWE ≥20 kPa and MELD ≥10 should be followed closely and receive intensified care, while patients with low risk may be managed at longer intervals.

## Significance of this study

**What is already known on this subject?**

- To date, the existing tools to stratify liver disease patients into different risks for clinical outcomes, such as the model for end-stage liver disease (MELD), may fail to predict the outcome, especially in well-compensated patients.
- The value of liver shear wave elastography (L- SWE) has only been established in the classification of liver fibrosis and clinically significant portal hypertension.
- No studies have assessed the prognostic value of SWE for different etiologies and stages of liver disease.

**What are the new findings?**

- This international multicenter cohort study provides an efficient and easy algorithm (M10LS20 algorithm) for risk stratification of advanced chronic liver disease (ACLD) with the combination of MELD with liver stiffness measured by shear wave elastography (SWE).
- The combined MELD 10 and liver-SWE (L-SWE) 20kPa cutoff (M10LS20) algorithm was confirmed in compensated and decompensated patients and was validated by TE measurements. It was externally validated in an addtional cohort of patients with point SWE.
- This M10LS20 algorithm provides early identification of at-risk patients and allows for early treatment, filling the gap in the risk stratification for complications and mortality in patients with ACLD.

## Significance of this study

**How might it impact on clinical practice in the foreseeable future?**

- This study provides a straightforward algorithm for bed-side stratification of patients with ACLD based on MELD and L-SWE.
- Patients with L-SWE ≥20kPa combined with MELD ≥10 should be followed closely and receive more intensified care, while patients with low risk may be managed at longer intervals.
- The M10LS20 algorithm supports clinicians in identifying populations at low-risk for complications as well as mortality, possibly enabling sparing of health care resources.

## BACKGROUND

Advanced chronic liver disease (ACLD) is associated with high mortality due to decompensations, which are consequences of liver failure and portal hypertension.[1] In these patients, predictors of outcomes are needed to stratify care and to allocate specific therapy and liver transplantation. The model for end-stage liver disease (MELD) is widely used to predict the prognosis of liver cirrhosis patients.[2–5] An MELD score above 15 is usually an indication of evaluation for liver transplantation. However, since the MELD score was originally designed to predict 90-day mortality after transjugular intrahepatic portosystemic shunt, it may fail to predict the outcome, especially in patients with compensated cirrhosis, who have a lower MELD score.[6]

Liver stiffness measurement (LSM) by transient elastography (TE), point shear wave elastography (p-SWE) or two-dimensional SWE (2D-SWE) is an established non-invasive technique to assess significant fibrosis in patients with chronic liver disease.[7–13] Moreover, it has proven to be a useful surrogate marker to diagnose clinically significant portal hypertension,[14] mostly shown for TE and recently for liver SWE (L-SWE).[15] Ideally, short-term and long-term risk of decompensation and mortality should be predicted to plan patient management. Also, decompensated patients with ascites may have a different natural course of disease.[16] Some patients may improve and not require intensified care. Since LSM not only reflects fibrosis or portal hypertension but also inflammation,[3 11] and given that inflammation is a marker of disease progression, LSM could be used as a predictive tool for outcomes in decompensated patients.[16–18] While LSM has been used previously to predict clinical decompensation and death in patients with compensated ACLD, data in decompensated patients are lacking. This is mostly due to TE's technical limitations, which cannot be applied to patients with ascites. L-SWE is not affected by ascites and can be applied to evaluate LSM similar to TE,[12 19 20] and it has the additional benefit of using ultrasound for HCC screening and portal vein evaluation (one-stop-shop examination).[21 22]

Therefore, the aim of this multicentre observational study was to provide a simple algorithm using L-SWE for risk stratification of all patients with ACLD, including decompensated patients with ascites.

## METHOD AND PATIENTS

### Study design and participants

We established a derivation cohort from a multicentre international study conducted in 16 centres, 14 of which are located in Europe, and two in China. Patients aged over 18 years, with suspected or confirmed chronic liver disease of viral and/or steatotic origin, requiring percutaneous liver biopsy, or who had received a liver ultrasound at baseline, were screened from July 2007 to September 2017. Inclusion criteria were presence of ACLD of any aetiology as defined in the Baveno VI concensus,[14] valid LSM measured by 2D-SWE at baseline, and at least 28 days of follow-up of outcome after the index L-SWE measurement. All of the enrolled patients received standard treatment according to the respective hospital protocols. The flow chart of patient enrolment is depicted in online supplemental figure 1. Some patients of this cohort were included in another, recently published study.[23] The number of patients from each participating centre as well as intercentre and interobserver reliability are shown in online supplemental tables 1 and 2. In centres where L-SWE is not the standard of care, patients signed written informed consent. The study has been registered in ClinicalTrials.gov as NCT03389152.

Clinical and laboratory parameters were assessed at baseline. These included demographic data, aetiologies, clinical decompensations (hepatic encephalopathy, ascites, variceal bleeding, hepatorenal syndrome, spontaneous bacterial peritonitis and/or other infections), routine blood tests, liver, kidney and coagulation function parameters and mean values of the various elastography measurements. Definitions of the non-invasive scores including fibrosis-4 index (FIB-4), aspartate aminotransferase to platelet count ratio index (APRI) and albumin-bilirubin score (ALBI) at baseline were shown in online supplemental methods.

### 2D-SWE and TE procedure and additional cohort of p-SWE

L-SWE measurements were conducted according to the European Federation of Societies for Ultrasound in Medicine and Biology.[24] One-dimension TE was measured using Fibroscan (Echosens, Paris, France). TE measurements were performed at baseline as per EASL-ALEH clinical practice guideline.[3 20] Details of the procedure for 2D-SWE and TE in derivation cohort are summarised in online supplemental methods.

An additional cohort from the Medical University of Vienna undergoing LSM by p-SWE was included (online supplemental figure 1). The same clinical data were obtained from 121 patients from September 2017 to January 2020, and 119 patients were finally included into analysis. The protocol for pSWE of the liver utilising the Elast PQ module on the Philips system was previously described[25 26] and is found in online supplemental methods.

### Outcome evaluation

After L-SWE measurement, the study patients were regularly followed for at least 28 days. The primary outcome was all-cause mortality of the patients in the cohort with 2D-SWE, and mortality or liver transplantation in the cohort with p-SWE. Development or worsening of complications and decompensations were assessed and recorded during follow-up and were set as the secondary outcome. Hepatic encephalopathy was assessed and scored according to West Haven criteria in the follow-up hospital admission.[27] Worsening of ascites was recorded by physical examination and abdominal ultrasound. Episodes of variceal bleeding, hepatorenal syndrome and bacterial infections were assessed and recorded during follow-up and compared with baseline decompensation.

### Statistical analysis

Data were presented as median with IQR or number with percentage. Mann-Whitney test or $\chi^2$ test was used for intergroup comparison where appropriate. We followed the Transparent

Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis statement for multivariate prediction model development and validation. Univariate and multivariate Cox proportional hazard regression analyses were performed to identify independent predictors of survival. We evaluated all potential risk factors in our regression analysis from a literature review, and the variables included are shown in online supplemental table 3. Patients with missing data or lost to follow-up were excluded in the corresponding analysis (online supplemental table 4). The cumulative incidence curve for secondary outcome of decompensations were plotted and compared using Gray's test with death as the competing event.

For the selection of cut-off values, we evaluated the receiver's operating characteristics (ROC) of vital status at 2 years, and the highest Youden index was determined. Sensitivity, specificity, positive predictive value and negative predictive value (NPV) were calculated together with 95% CI of each cut-off value. Model performance was assessed in the 2D-SWE and the additional p-SWE cohorts. Discrimination parameters, including the time-dependent area under the ROC (AUROC) curve, integrated AUROC and Uno's C statistic, were evaluated and compared. Calibration was assessed using 10-fold cross-validation with 200 times of bootstrapping.

A $p<0.05$ was considered statistically significant. All statistical analyses were performed with SPSS V.25.0 (SPSS), R (V.4.0, R core team, Austria), and SAS V.9.4 (SAS Institute).

## RESULTS

### General characteristics of compensated and decompensated patients

A total of 2148 patients from 16 centres were screened in the derivation cohort with 2D-SWE, 2032 of whom had valid L-SWE assessment at baseline. Eventually, 1827 patients with at least 28 days of follow-up were included in the study (online supplemental figure 1). The median follow-up was 33.1 (IQR 16.7–48.8) months. Baseline characteristics and follow-up measurements of all patients enrolled are summarised in table 1. Patient age was 55 years in median (IQR: 45.9–62.7), and the majority were male (62%). Of the enrolled patients, alcohol-related chronic liver disease accounted for 26.8% of cases, followed by non-alcoholic fatty liver disease (NAFLD) (25.2%) and hepatitis C virus-related (HCV) liver disease (17.3%, of which 16.8% sustained virological response patients). The median MELD score of all patients was 8 (IQR: 6–10), and the Child-Pugh score was 5 (IQR: 5–6). Patients included in the study had a median L-SWE value of 11.8 kPa (IQR: 7.4–24.5) (online supplemental figure 2). The haemodynamic baseline data of a subgroup of the patients is shown in online supplemental table 5.

Of the 1490 (81.6%) patients who were compensated at baseline, 174 (11.7%) decompensated during follow-up. Of the 337 (28.4%) patients who were decompensated at baseline, 106 (31.5%) developed further episodes of decompensations during follow-up as defined elsewhere.[16] The decompensated patients (at baseline) had significantly higher L-SWE (median 30.3 kPa vs 9.7 kPa, $p<0.001$) and TE (median 23.4 kPa vs 8.3 kPa, $p=0.017$) value at baseline than the compensated patients.

### L-SWE measurement is an independent risk factor of long-term mortality

Considering all patients with valid L-SWE measurements and follow-up data, the overall 28-day, 90-day, 1-year and 2-year mortality rates were 0.4%, 1.1%, 2.9% and 7.8%, respectively. As shown by the univariate and multivariate Cox regression models in online supplemental table 6, the LSM value with L-SWE was independently associated with long-term mortality in compensated patients (2-year HR: 1. 019, 95% CI 1.010 to 1.028, $p<0.001$) and all patients included (2-year HR: 1.019, 95% CI 1.008 to 1.030, $p=0.001$). If adjusted by MELD score, L-SWE remained an independent risk factor for 28-day, 90-day and 2-year mortality (table 2). L-SWE was also shown to be an independent risk factor in decompensated patients, in short-term as well as long-term follow-up (online supplemental tables 6 and 7).

### Model performance of L-SWE in combination with MELD score and best cut-off values

Online supplemental figure 3 shows the highest AUC of L-SWE combined with MELD score and compared with ALBI, FIB4 and APRI score. Figure 1 and online supplemental figure 4A show the model performance of L-SWE combined with MELD score. Among compensated patients, the MELD score combined with L-SWE (integrated AUC: 0.81) had a better time-dependent AUC than the MELD score alone (integrated AUC:0.73), especially in long-term follow-up. Interestingly, the c-index of the combined model (0.825) is significantly higher than that of the MELD and the Child-Pugh score ($p=0.0329$ and $p<0.0001$, respectively). Similar results were obtained in all of the included patients. The combined model was well calibrated, as shown in online supplemental figure 4B.

To further stratify the 2-year risk of mortality, the best cut-off value of 10 points for the MELD score and best cut-off value of 20 kPa for L-SWE was found with the highest Youden index in compensated patients and all patients (online supplemental tables 8–10). Moreover, in the group with an MELD score <10 and those with an MELD score ≥10, significant differences (log-rank $p<0.001$) were found between patients with L-SWE measurements above and below 20 kPa over 2 years in Kaplan-Meier curves (online supplemental figure 4C,D).

### Stratification M10LS20 algorithm with L-SWE and MELD score in compensated and decompensated patients

With the two cut-off values found for the MELD score and L-SWE, respectively, the algorithm (M10LS20) of a combination of L-SWE value and MELD score could easily stratify the patients into three different risks group, namely (1) patients with L-SWE measurements <20 kPa and an MELD score <10 (good prognosis group, 56.7%), (2) patients with L-SWE measurements ≥20 kPa and an MELD score ≥10 (poor prognosis group, 25.0%) and (3) the remaining patients (intermediate prognosis group, 18.3%). According to the Kaplan-Meier curve in figure 2A, the three groups have significantly different survival rates ($p<0.001$) both at short-term (90 days) and long-term follow-up (2 years) in compensated patients. The significant survival differences were also found in decompensated patients and in all patients (figure 2B and C). Interestingly, the density curve of time distribution of mortality shown in online supplemental figure 5A confirms that the algorithm identifies groups with different median survival time. The mean time to death was 11 months in the poor prognosis group, while it was 16 months in the good prognosis group.

### Predictive value of L-SWE combined with MELD score for first or further decompensation

As expected, the three groups showed significantly different median baseline L-SWE values of 8.2 kPa, 21.1 kPa and 36.0 kPa

**Table 1** Baseline characteristics of the derivation cohort

| Characteristics | All patients (n=1827) | Compensated (n=1490) | Decompensated (n=337) | P value* |
|---|---|---|---|---|
| Age | 55 (45.9–62.7) | 54.6 (44.3–62) | 57.6 (51.1–64) | <0.001 |
| Male | 1140 (62.4) | 909 (61.0) | 231 (68.5) | 0.010 |
| BMI (kg/m$^2$) | 26.5 (23.2–30.6) | 26.7 (23.2–30.9) | 25.9 (22.9–29.4) | 0.038 |
| **Scores** | | | | |
| MELD score | 8 (6–10) | 7 (6–9) | 13 (9–17) | <0.001 |
| Child Pugh score | 5 (5–6) | 5 (5–5) | 8 (6–9) | <0.001 |
| Child Pugh class (A/B/C) | 1334/206/44 (84.2/13.0/2.8) | 0/1241/77 (0.0/94.2/5.8) | 93/129/44 (35.0/48.5/16.5) | <0.001 |
| SWE at baseline (kPa) | 11.8 (7.4–24.5) | 9.7 (6.9–17.3) | 30.3 (19.3–41.1) | <0.001 |
| TE at baseline (kPa) | 8.3 (5.7–14.0) | 8.3 (5.7–13.9) | 23.4 (12.6–35.7) | 0.017 |
| **Aetiology:** Alcohol/NAFLD/HCV/HBV/other or multiple causes | 414/389/267/166/310 (26.8/25.2/17.3/10.7/20.0) | 319/359/195/142/292 (24.6/27.7/15.0/10.9/21.8) | 95/30/72/24/28 (38.2/12.0/28.9/9.6/11.2) | <0.001 |
| **Laboratory test** | | | | |
| Albumin (g/L) | 40.0 (33.8–43.0) | 41.0 (37.0–43.8) | 31.0 (26.0–36.6) | <0.001 |
| Alkaline phosphatase (U/L) | 90.0 (67.0–128.0) | 84.0 (64.8–114.0) | 132.0 (97.0–186.0) | <0.001 |
| ALT (U/L) | 44.9 (28.0–77.0) | 47 (28.3–80) | 38 (25–61) | <0.001 |
| AST (U/L) | 43.0 (30.0–69.0) | 40 (28.6–67) | 54 (38–80) | <0.001 |
| Bilirubin (mg/dL) | 0.8 (0.5–1.3) | 0.7 (0.5–1.1) | 1.6 (1–3.1) | <0.001 |
| Creatinine (mg/dL) | 0.8 (0.7–1.0) | 0.8 (0.7–1) | 0.8 (0.7–1.2) | 0.002 |
| INR | 1.1 (1.0–1.2) | 1 (1–1.1) | 1.3 (1.2–1.5) | <0.001 |
| Platelets (G/L) | 179 (122–242) | 194 (143–253) | 100 (70–149) | <0.001 |
| WCC (×10$^9$/L) | 6.2 (5.0–7.9) | 6.2 (5–7.9) | 6.3 (4.5–8.6) | 0.935 |
| CRP | 2.9 (1.1–7.0) | 2.3 (0.9–5.6) | 10 (5.2–28.4) | <0.001 |
| **Score for liver fibrosis or cirrhosis** | | | | |
| ALBI | −2.63 (−2.96–-1.74) | −2.75 (−3.01–-2.21) | −1.59 (−2.24–-1.03) | <0.001 |
| FIB-4 | 1.96 (1.11–3.97) | 1.66 (1.00–2.97) | 5.19 (3.11–8.61) | <0.001 |
| APRI | 0.58 (0.32–1.23) | 0.50 (0.30–0.96) | 1.28 (0.69–2.40) | <0.001 |
| **Clinical parameters** | | | | |
| Absence or abstinence of alcohol consumption | 1394 (76.3) | 560 (37.6) | 97 (28.8) | <0.001 |
| HCV SVR before SWE | 81 (16.8) | 74 (16.8) | 7 (16.3) | 1.000 |
| Previous variceal bleeding | 113 (6.9) | 69 (4.6) | 44 (13.1) | <0.001 |
| Previous bacterial infection | 99 (6.0) | 54 (3.6) | 45 (13.4) | <0.001 |
| Previous hepatorenal syndrome | 51 (3.1) | 20 (1.3) | 31 (9.2) | <0.001 |

*P value of the comparison between compensated and decompensated patients

†

ALBI, albumin-bilirubin score; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet count ratio index; AST, aspartate aminotransferase; BMI, body mass index; CRP, C reactive protein; FIB-4, fibrosis-4 index; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalized ratio ; MELD, model for end-stage liver disease; NAFLD, non-alcoholic fatty liver disease; SVR, sustained virologic response; SWE, shear wave elastography; TE, transient elastography; WCC, white cell count.

**Table 2** Univariate and multivariate analysis of swe with outcome of death after adjustment for platelet count, age and MELD score

| Variables | 28 days | | | | 90 days | | | | 2 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | | Univariate | | Multivariate | |
| | P value | HR (95% CI) | P value | HR (95% CI) | P value | HR (95% CI) | P value | HR (95% CI) | P value | HR (95% CI) | P value | HR (95% CI) |
| SWE at baseline | 0.001 | 1.035 (1.014 to 1.056) | 0.004 | 1.035 (1.011 to 1.060) | <0.001 | 1.027 (1.012 to 1.042) | 0.005 | 1.025 (1.007 to 1.042) | <0.001 | 1.029 (1.022 to 1.035) | <0.001 | 1.022 (1.015 to 1.029) |
| MELD score | <0.001 | 1.115 (1.077 to 1.154) | <0.001 | 1.114 (1.075 to 1.155) | <0.001 | 1.104 (1.077 to 1.132) | <0.001 | 1.105 (1.076 to 1.134) | <0.001 | 1.087 (1.072 to 1.103) | <0.001 | 1.076 (1.057 to 1.096) |
| Platelet count | 0.043 | 0.988 (0.976 to 1.000) | – | – | <0.001 | 0.982 (0.974 to 0.99) | – | – | <0.001 | 0.990 (0.987 to 0.993) | 0.002 | 0.995 (0.992 to 0.998) |
| Age | – | – | – | – | – | – | – | – | <0.001 | 1.052 (1.032 to 1.071) | <0.001 | 1.044 (1.023 to 1.065) |

HR, hazard ratio; MELD, model for end-stage liver disease; SWE, shear wave elastography.

(p<0.001) in the good prognosis, the intermediate prognosis, and the poor prognosis group (online supplemental figure 5B), respectively. Of the 1827 study patients, 182 patients developed new episodes of decompensations or worsening of ascites or HE during the 2-year follow-up period.

Among the patients without decompensation, the model of L-SWE and MELD score had the best AUROC in the prediction of decompensation development during the follow-up, compared with the MELD score and the Child-Pugh score (online supplemental figure 6A).

When considering death as a competing risk factor for developing or worsening of decompensation, L-SWE value at baseline appeared to be a statistically significant independent risk factor in the outcome of developing decompensation, after adjustment for age, MELD score, and Child-Pugh score at baseline (HR 1.020 (95% CI 1.014 to 1.026), p=0.001) (online supplemental table 11). Moreover, in the patient groups with an MELD score< or ≥ 10 points, L-SWE (cutoff ≥20 kPa) could best stratify the cumulative incidence of decompensation for up to 2 years (Gray's test p<0.001) (online supplemental figure 6B,C).

Using the M10LS20 algorithm, we could also stratify the patients into three risk groups for risk of decompensation: (1) good prognosis, (2) intermediate prognosis and (3) poor prognosis. Of the compensated patients, the group with poor prognosis had the highest incidence of development of decompensation (figure 3A). The cumulative incidence curves with Gray's test also showed significant differences in the development or worsening of ascites (p<0.001) and hepatic encephalopathy (p<0.001) in these three risk groups (online supplemental figure 7A,B). Nevertheless, in decompensated patients, significant differences were also found in the risk of worsening of decompensation in these three groups (Gray's test p=0.0025) (figure 3B). In the whole cohort, a similar effect was confirmed (figure 3C).

### Validation of the stratification M10LS20 algorithm using p-SWE

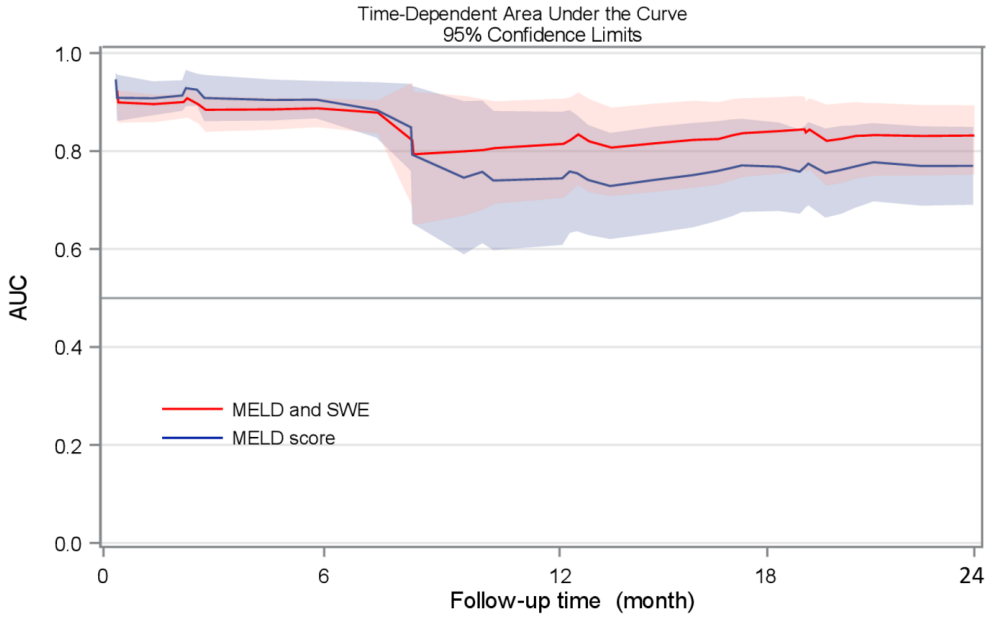
The baseline characteristics of the add groups with p-SWE are shown in online supplemental table 12). Patients in the additional cohort had higher L-SWE measured by pSWE than the 2D-SWE derivation cohort and had a higher MELD score. The pSWE combined with the MELD score model well calibrated in the additional cohort (online supplemental figure 8A) and had an integrated time-dependent AUC of 0.8407 (figure 4A and online supplemental figure 8B).

The validity of the M10LS20 algorithm using p-SWE technology was confirmed by a significant survival difference in the three prognosis groups in the additional cohort, with a log-rank p of 0.032 (figure 4B). When considering death as a competing risk for decompensation, the cumulative incidence of development or worsening of decompensation also showed significant differences in these three groups (figure 4C). Interestingly, a similar decision tree could be established in the additional cohorts, stratifying the ACLD patients into three distinct prognosis groups (online supplemental figure 9).

### Internal validation of the stratification M10LS20 algorithm in compensated and decompensated patients

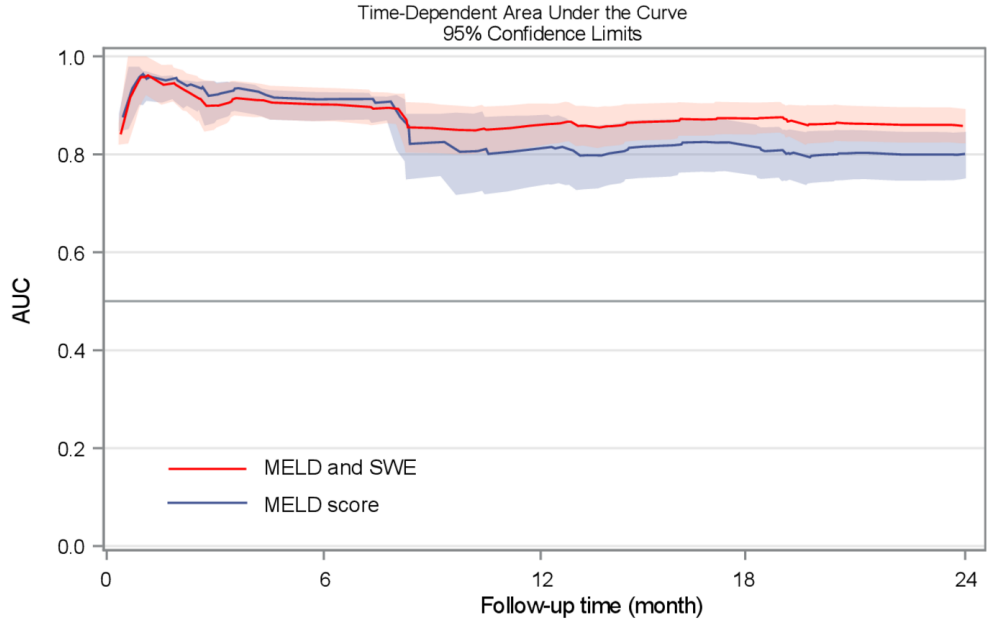
For internal validation of our stratification algorithm in both compensated and decompensated patients, the combined cohorts of 2D-SWE and p-SWE were randomly divided into

A




| Model performance measures | L-SWE and MELD | L-SWE | MELD score | Child-Pugh |
|---|---|---|---|---|
| **Compensated** | | | | |
| Time dependent AUC | 0·8151 | 0·8006 | 0·7271 | 0·5243 |
| Integrated AUC | 0·8119 | 0·7939 | 0·7841 | 0·5711 |
| C index | 0·8245 | 0·8189 | 0·7791 | 0·5810 |
| Differences estimate | 1 [reference] | -0·0002 | 0·0977 | 0·2001 |
| P value | | 0·9955 | 0·0328 | <0·0001 |

B




| Model performance measures | L-SWE and MELD | L-SWE | MELD score | Child-Pugh score |
|---|---|---|---|---|
| **All patients included** | | | | |
| Time dependent AUC | 0·8280 | 0·8165 | 0·7627 | 0·7133 |
| Integrated AUC | 0·8653 | 0·8215 | 0·8527 | 0·7449 |
| C index | 0·8466 | 0·8182 | 0·8153 | 0·7249 |
| Differences estimate | 1 [reference] | 0·0012 | 0·0915 | 0·1921 |
| P value | | 0·9626 | 0·0448 | 0·0005 |

**Figure 1** (A) Time-dependent area under the curve and 95% CI of the combined algorithm based on MELD score and SEW, and MELD score alone in compensated patients only during 2 years of follow-up. (B) Time-dependent area under the curve and 95% CI of the combined model of MELD score and swe, and MELD score alone in all patients (including decompensated patients at baseline) of the cohort with 2D-SWE during 2 years of follow-up. 2D-SWE, 2-dimensional shear wave elastography; AUC, area under the curve; MELD, the model for end-stage liver disease.

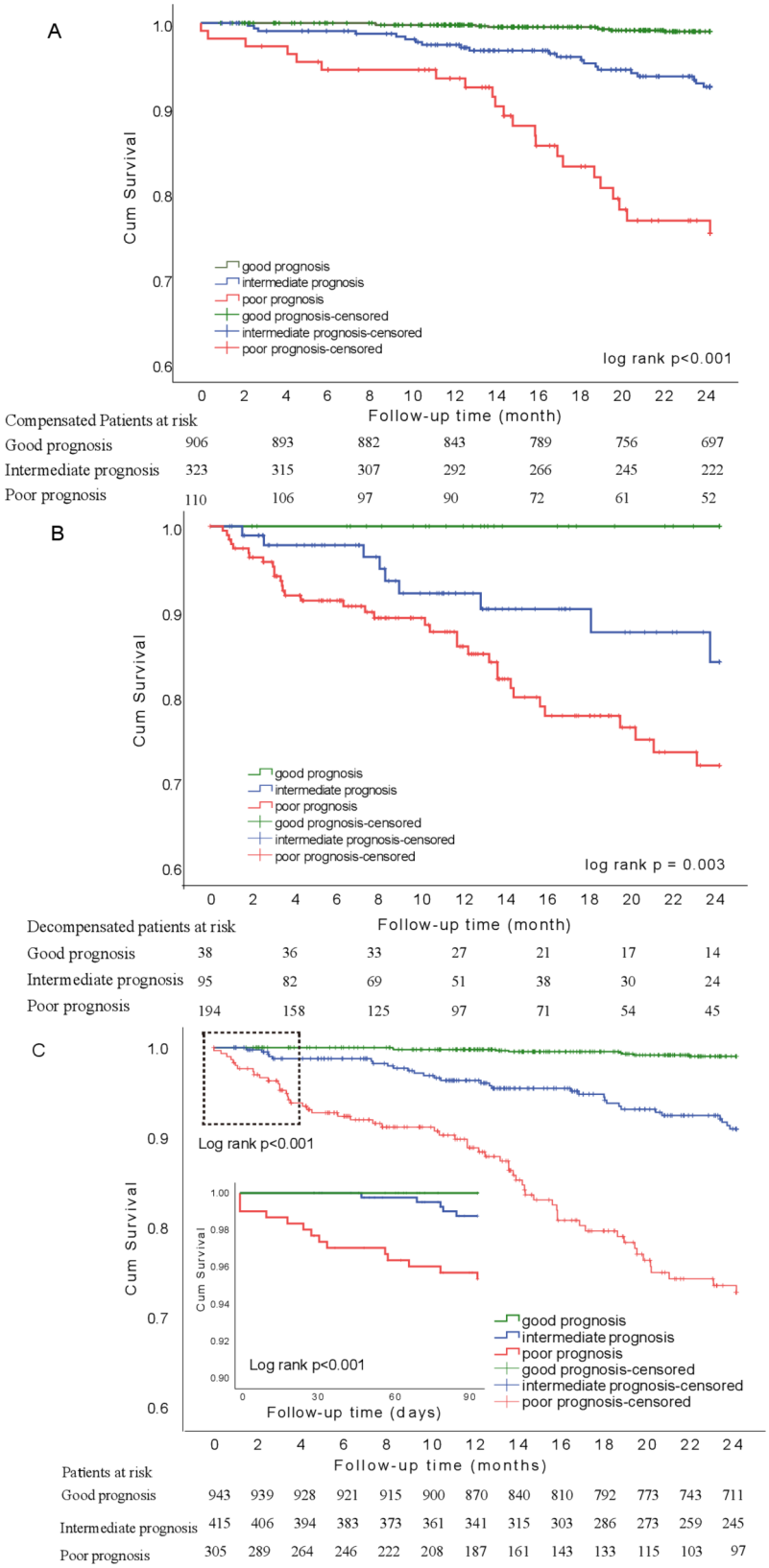



**Figure 2** Curves of patients with good, intermediate and poor prognosis. (A) Kaplan-Meier of 2-year survival curve in compensated patients. Level of significance: log-rank p<0.001. (B) Kaplan-Meier of 2 years survival curve in decompensated patients. Level of significance: log-rank p=0.003. (C) Kaplan-Meier of 2-year and 90-day survival curve of all patients of the cohort with 2D-SWE measurements. The top left rectangle in the curve of 2 years survival indicates the area of the Kaplan-Meier curve of 90-day survival depicted in the bottom left panel. Level of significance: log-rank p<0.001. 2D-SWE, 2-dimensional shear wave elastography.

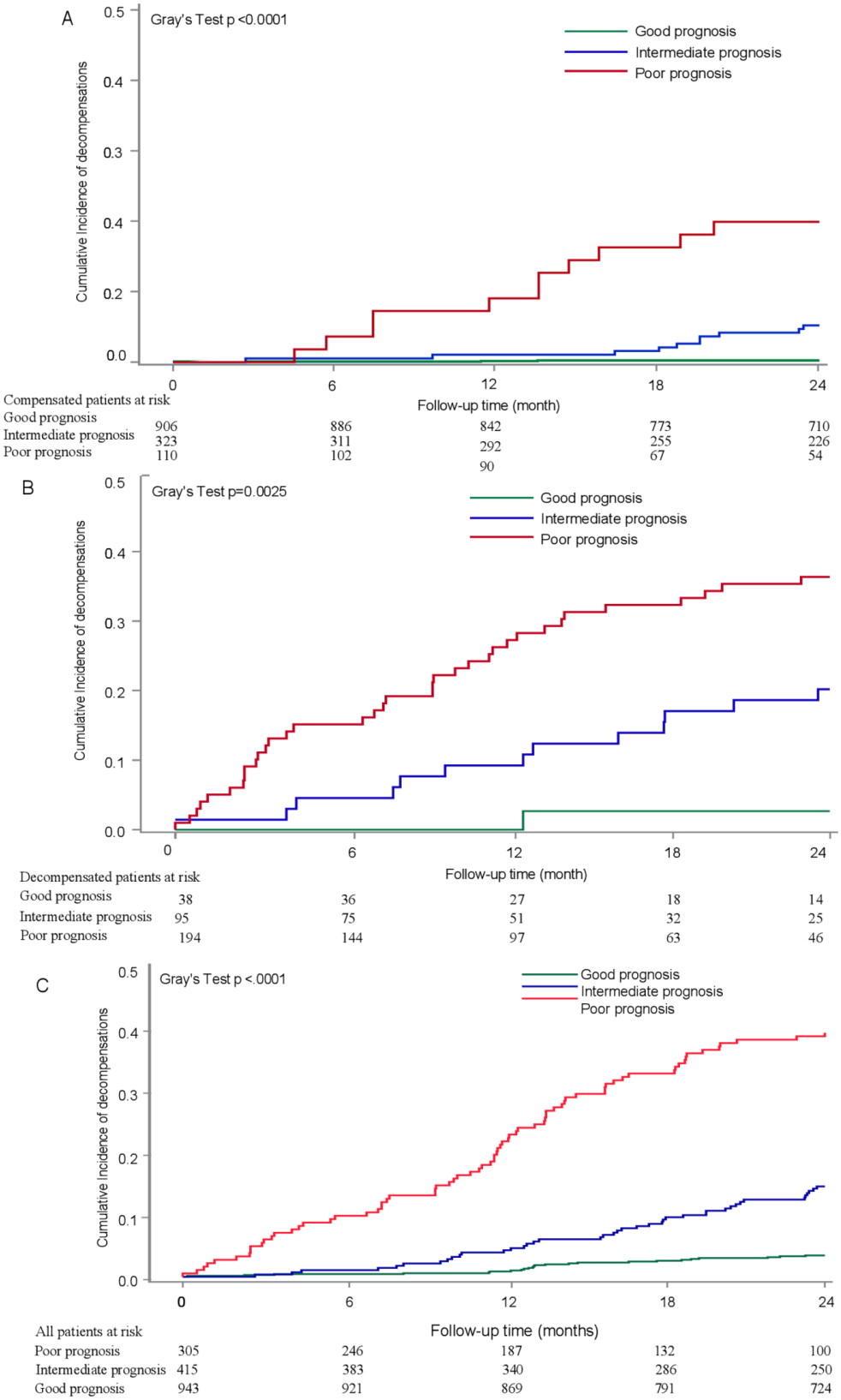



**Figure 3** (A) Cumulative incidence of development of decompensations within 2 years of patients with good, intermediate and poor prognosis in compensated patients. Level of significance: Gray's test p<0.0001. (B) Cumulative incidence of development of further episodes of decompensation within 2 years of decompensated patients with good, intermediate and poor prognosis. Level of significance: Gray's test p=0.0025. (C) Cumulative incidence of development of decompensations within 2 years of all patients included in the cohort with 2D-SWE measurements with good, intermediate and poor prognosis. Level of significance: Gray's test p<0.0001. 2D-SWE, 2-dimensional shear wave elastography.

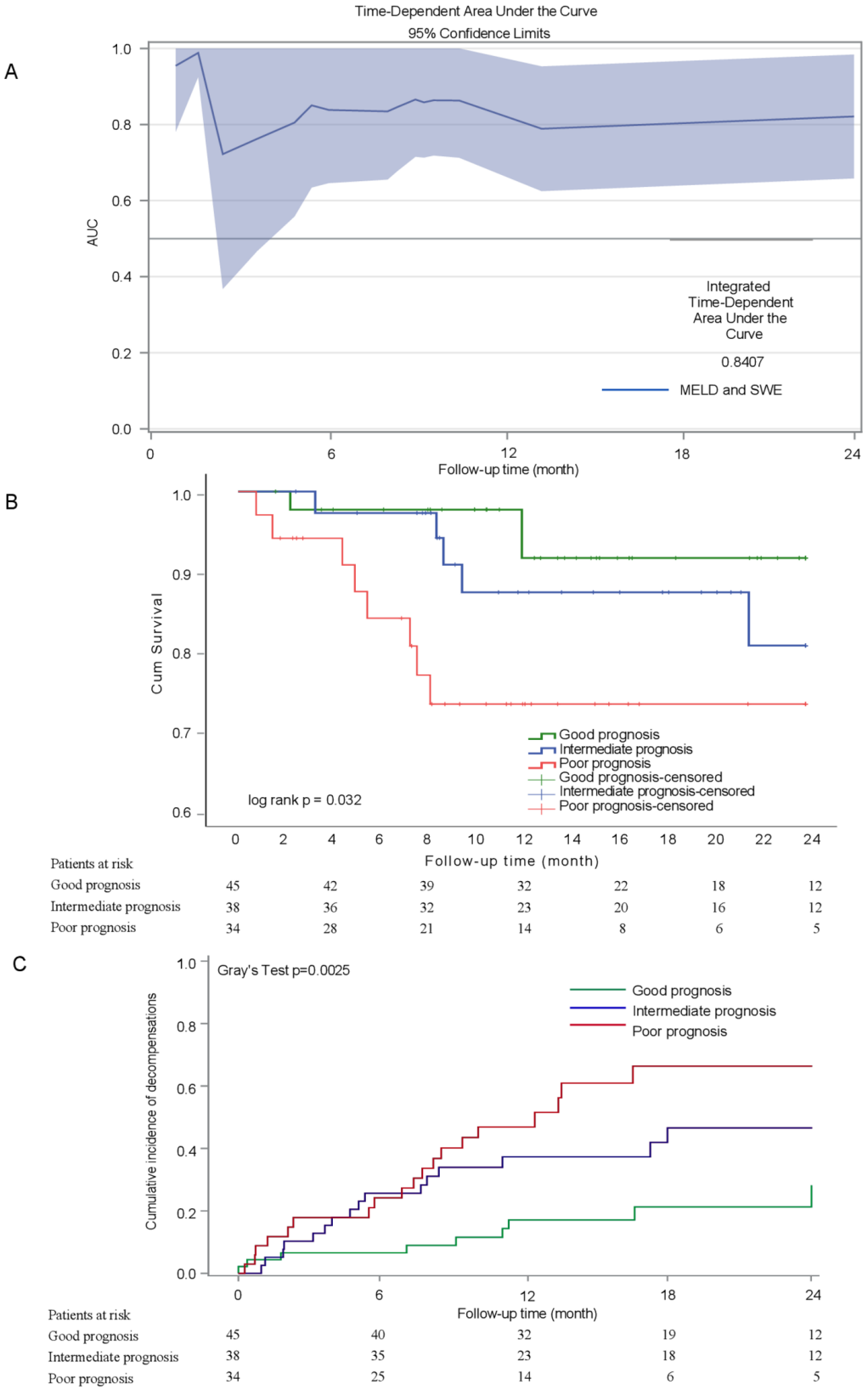



**Figure 4** (A) Time-dependent area under the curve and 95% CI of the combined model of MELD score and p-SWE in the additional cohort validated for p-SWE during 2 years of follow-up. (B) Two-year Kaplan-Meier curves of the additional cohort validated for p-SWE of patients classified with good, intermediate and poor prognosis. Level of significance: log-rank p<0.032. (C) Cumulative incidence of development of decompensations within 2 years of all patients included in the additional cohort validated for p-SWE with good, intermediate and poor prognosis. Level of significance: Gray's test p=0.0025. AUC, area under the curve; MELD, model for end-stage liver disease; p-SWE, point shear wave elastography.

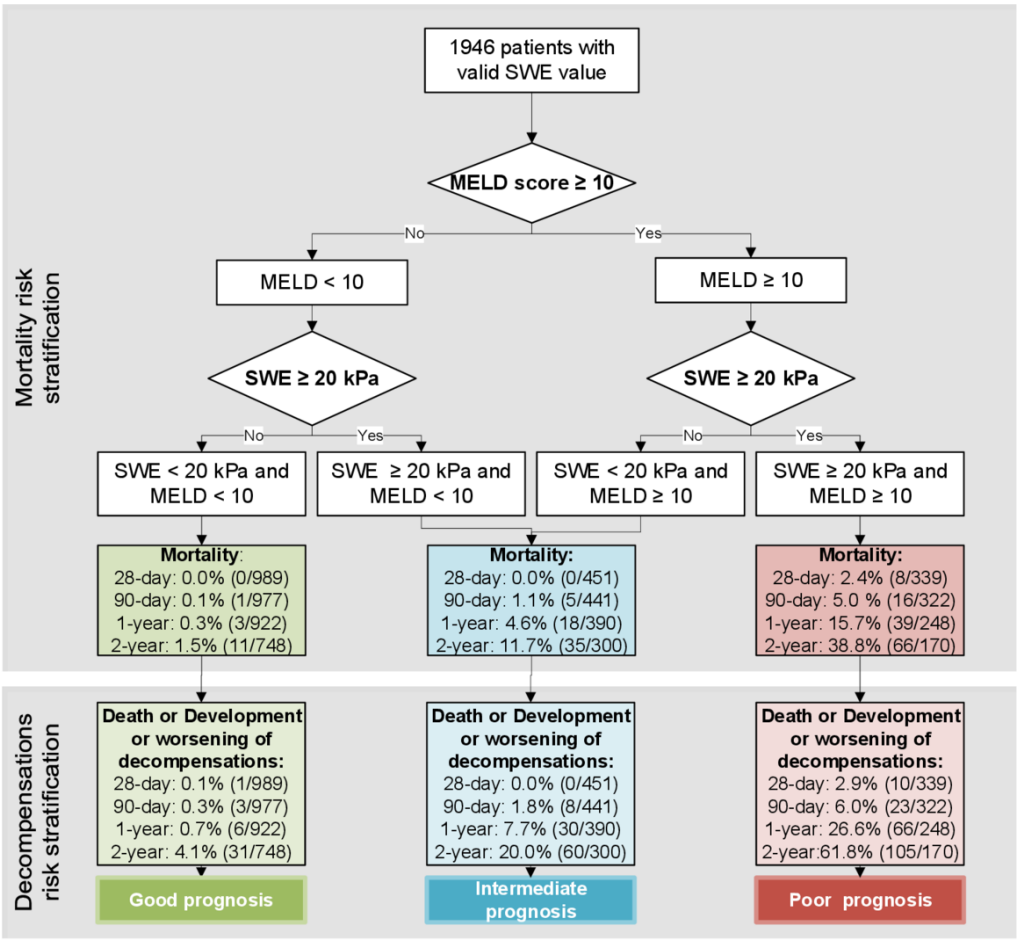



**Figure 5** Stratification model of cohort with 2D-SWE and additional cohort with p-SWE for prediction of estimated mortality (upper panel) at 28 and 90 days as well as at one and 2 years of follow-up based on MELD score <10 vs ≥10 and L-SWE <20 vs ≥20 kPa; stratification model of cohort with 2D-SWE and additional cohort with p-SWE for prediction of first/further decompensation risk (lower panel) at 28 and 90 days as well as at one and 2 years of follow-up based on MELD score <10 vs ≥10 and L-SWE <20 vs ≥20 kPa. 2D-SWE, 2-dimensional shear wave elastography' L-SWE, liver SWE; MELD, model for end stage liver disease; p-SWE, point SWE.

a 2/3 internal derivation cohort and a 1/3 internal validation cohort. This was performed for both compensated patients and decompensated patients. The baseline characteristics of both cohorts are listed in online supplemental tables 13 and 14).

Regardless whether compensated (log rank p<0.001) or decompensated (log rank p=0.042) were considered, significantly different survival functions were detected in the three different risk groups from the derivation cohorts. The strategy was subsequently validated in the 1/3 internal validation cohort, showing a significant risk of mortality within 2 years (log rank p<0.001 and p=0.038 in compensated and decompensated patients, respectively) (online supplemental figures 10 and 11).

The decision tree (figure 5) shows the strategy (M10LS20 algorithm) used for mortality risk prediction stratification in the combined cohort of cohorts with 2D-SWE and the additional cohort with pSWE. It can also be applied for decompensation, evidenced by the significantly different incidences of decompensation in these three risk groups (3.5%, 18.8% and 61.8% within 2 years, respectively).

### Validation of the stratification M10LS20 algorithm using TE and MELD score

Of the patients included in the cohort with 2D-SWE, 754 patients had LSM by TE at baseline. The median TE value was 8.3 kPa. As expected, decompensated patients had a significantly higher TE value than compensated patients (23.4 kPa vs8.3 kPa, p=0.017).

In the multivariate analysis adjusted by the MELD score, liver stiffness proved to be an independent risk factor predicting 2-year mortality (online supplemental table 15). TE could equally predict the long-term risk of death compared with SWE, as shown by similar time-dependent ROC (online supplemental figure 12A). Performance of the model of TE combined with the MELD score was also similar to 2D-SWE combined with the MELD score (online supplemental figure 12B).

The same algorithm of M10LS20 was applied in patients with TE measurement and MELD score, using the same cut-off of 20 kPa for TE. As depicted in online supplemental figure 9), the cut-off of 20 kPa could also stratify patients with the distinct outcome of death in patients with an MELD score above as well

as an MELD score below ten points. In fact, using the same algorithm and cutoffs, three distinct groups with significantly different mortality risks could be identified (online supplemental figure 13).

### Aetiological sensitivity, regional sensitivity and time-period sensitivity analysis of the stratification M10LS20 algorithm

Interestingly, the M10LS20 algorithm was confirmed in all patients with different aetiologies. As shown in online supplemental figure 14), patients with good prognosis, intermediate prognosis and poor prognosis had significantly different survival probability, regardless of aetiology as analysed separately for alcoholic-related liver disease, HCV, hepatitis B virus (HBV) and NAFLD.

When we divided the cohort with 2D-SWE into patients from Southern Europe and Northern Europe, the model still showed the highest AUROC (online supplemental figure 15A,B). Patients from Southern Europe as well as Northern Europe could be adequately stratified by the M10LS20 algorithm (online supplemental figure 15C,D).

Since our derivation cohort of 2D-SWE and the additional cohort with p-SWE were included from 2007 to 2020, we divided our cohorts into three subgroups according to different time periods of inclusion (online supplemental figure 16). The M10LS20 algorithm performed equally well regardless of the time period of inclusion, as demonstrated by the significantly different survival curves in the three stratified risk groups. Furthermore, patients with second follow-up L-SWE measurements were analysed. As shown in online supplemental figure 17), the majority of patients with good prognosis and poor prognosis remained within the same stratification group when using the M10LS20 algorithm during the follow-up. Similar results were also found in the intermediate group.

## Discussion

This international multicentre cohort study establishes and validates that the combination of MELD score with LSM measured by SWE (M10LS20 algorithm) is an accurate and easy tool for stratification of ACLD. In the study, mortality and risk of decompensation in patients with L-SWE measurements below 20 kPa and an MELD score below 10 were 1.1% and 3.5%, respectively, while patients with L-SWE measurements above 20 kPa and an MELD score above 10 had a very high risk of mortality and decompensation (36.9% and 61.8%, respectively).

Patients with ACLD represent a large and heterogeneous population with various aetiologies, such as alcoholic-related liver disease, NAFLD, chronic viral hepatitis and autoimmune liver disease. Many patients deteriorate rapidly due to severe complications and decompensating episodes and may die within a short period of time. However, even patients with ascites can recompensate and show a very low rate of further decompensation.[16] Therefore, in addition to clinical history, an accurate and practical tool to assess and predict short-term and long-term outcome in patients with high heterogeneity is urgently required. Such a tool can greatly help clinicians to stratify treatment options and reduce waste of medical resources. To identify such a tool for prediction of outcome, the mechanism leading to decompensations and death must first be identified. There is abundant evidence that advanced fibrosis is associated with progression towards decompensations in ACLD.[28–30] Moreover, portal hypertension is a driver of decompensation and death.[31 32] Finally, systemic inflammation has been identified in multiple studies as a major cause of decompensation, acute-on-chronic liver failure and death.[16 33] Of note, systemic inflammation can already be present in the early stages of ACLD.[18 34] Interestingly, hepatic fibrosis and inflammation are major components of liver stiffness.[3 11 19 35] Although LSM by TE is currently the most widely used method, it has the limitation of a high failure rate mostly due to obesity or ascites.[12 36] Our data suggest that L-SWE is useful in this complex setting. Moreover, the algorithm we established for L-SWE was also confirmed with LSM performed by TE in our study.

Additional prognostic factors in cirrhosis include the severity of liver failure and extrahepatic organ failure (eg, kidney and coagulation failure).[37 38] These markers are reflected in the MELD score.[2] To date, no easy algorithm has been available to stratify the risk of decompensation and mortality. Patients with an MELD score below 15 are the most heterogeneous in terms of clinical evolution and are especially at risk of being overlooked since 15 points is the cut-off value for putting forward the indication for liver transplantation.[39]

Our data show that LSM by 2D-SWE measurement is an independent risk predictor of death and has a high accuracy in predicting 2-year mortality. We could show that a combination of L-SWE and MELD score has the highest AUROC, superior to L-SWE alone, MELD score and Child-Pugh score. Thus, a combination of L-SWE and MELD score is an accurate tool to stratify patients with a high risk of death within 2 years. Furthermore, increased L-SWE measurements correlated with the development or further episodes of decompensation. Importantly, this large-scale multicentre study enabled the identification of an easy-to-memorise cut-off value of 10 for the MELD score and 20 kPa for L-SWE to classify heterogeneous ACLD patients into three groups with completely distinct risks of mortality. Interestingly, this cut-off value is identical to the one provided by several studies on the diagnosis of clinically significant portal hypertension.[7 11 40] The extremely high NPV (98%) indicates that this cut-off value can help clinicians exclude low-risk populations, emphasising its potential role in saving clinical resources.

A further important finding of this study is that L-SWE had predictive value for the development or worsening of decompensation, which has been missing to date. Especially in patients with ascites, which defines decompensation, this algorithm is very useful.[41] Our findings are in agreement with previous data suggesting that values of LSM indicating the presence of clinically significant portal hypertension (a major factor driving decompensation in cirrhosis[13 42] predict clinical decompensation. We confirm that this holds true across different aetiologies and stages of disease. Thus, the present study may fill the knowledge gap of the usefulness of L-SWE in the prediction of complications. From a practical point of view, routine use of L-SWE could provide early identification of patients at risk of poor outcomes, thus helping to tailor treatment options. Since non-selective beta-blockers have recently been shown to be effective in reducing clinical decompensation in patients with compensated cirrhosis and clinically significant portal hypertension irrespective of the size of varices,[43] L-SWE may be used to non-invasively select patients who could benefit from this treatment.

Of note, the M10LS20 algorithm was found to be accurate not only in compensated but also in decompensated patients. We further validated this innovative algorithm in an additional cohort with LSM measured by pSWE and confirmed the robustness and wide range of its application. The sensitivity analysis was also done in heterogeneous aetiologies and by different participating centres from various regions worldwide. Interestingly, there was no impact of aetiology on the performance and applicability of our algorithm. The M10LS20 algorithm was

found to be robust for any aetiology, including alcohol-related liver diseases, chronic hepatitis C, chronic hepatitis B and non-alcoholic fatty liver disease. Hence, it has been demonstrated that the M10LS20 algorithm is appropriate for universal use and can be easily applied in daily clinical practice. This easy algorithm may affect the time interval for outpatient follow-up treatment in patients. Thus, patients with poor prognosis and at high risk of mortality should be seen more frequently (eg, every 2–3 months), while in patients with intermediate risk the frequency of visits could be lower (eg, every 3–6 months), and patients with good prognosis are only to be seen every 6–8 months. Especially in times when person-to-person contact must be reduced, for example, during a pandemic, this algorithm can be applied successfully.[44] As a result, patients with chronic liver diseases would follow a more individualised clinical path after elastography evaluation and liver-related risk evaluation using the MELD score.

Despite the fact that our study is a large international multicentre study, it has several limitations. First, it is an observational study and, although the standard procedure of L-SWE measurement was followed in the centres, the size of the region of interest of L-SWE was freely chosen by the operator within the quality range in their clinical practice. However, excellent reliability was found in most of the operators and centres as shown by an intraclass correlation coefficient of above 90%. Second, the long time period of inclusion of this study could impact the results. However, when we stratified our patients into subgroups with different time periods of inclusion, we found that the M10LS20 algorithm could be accurately used in all subgroups. Additionally, we established a cut-off value in the study cohort with 2D-SWE, and further validated it using different techniques, including limited numbers of TE measurements and an additional cohort with p-SWE. While an external multicentre study is called for to exclude bias, our study reflects various real-life scenarios, strengthening its applicability and validity.

In conclusion, this study shows for the first time that L-SWE predicts mortality in patients with chronic liver disease regardless of aetiologies. An MELD score above 10 combined with L-SWE measurements above 20 kPa could help to stratify mortality and decompensation risk and guide patient management.

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**Correction notice** This article has been corrected since it published Online First. The provenance and peer review statement has been included.

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**Funding** JT is supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18), European Union's Horizon 2020 Research and Innovation Programme (Galaxy, No. 668031, MICROB-PREDICT, No. 825694 and DECISION No.84794), and Societal Challenges - Health, Demographic Change and Wellbeing (No. 731875), and Cellex Foundation (PREDICT). WG is supported by the China Scholarships Council (CSC: #201906230332). SF has a senior clinical research mandate from the Fund for Scientific Research (FWO) Flanders (1802154N). RZ was supported by the National Natural Science Foundation of China under grant no. 81827802.

**Competing interests** JT has received speaking and/or consulting fees from Gore, Bayer, Alexion, MSD, Gilead, Intercept, Norgine, Grifols, Versantis, and Martin Pharmaceutical. Philip Ferstl received consultancy for SNIPR Biome. Supersonic Imagine supported interaction within the groups, but without specific funding.

**Patient consent for publication** Not required.

**Ethics approval** The study was approved by the local ethics committees of the participating centres.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** All data relevant to the study are included in the article or uploaded as online supplemental information.



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## 3.9 Eine erhöhte Steifigkeit von Leber und Milz nach einem transjugulären portosystemischen Shunt ist assoziiert mit Inflammation und Mortalität.

**Jansen C***, Möller P*, Meyer C, Kolbe CC, Bogs C, Pohlmann A, Schierwagen R, Praktiknjo M, Abdullah Z, Lehmann J, Thomas D, Strassburg CP, Latz E, Mueller S, Rössle M, Trebicka J. **Increase in liver stiffness after transjugular intrahepatic portosystemic shunt is associated with inflammation and predicts mortality.** Hepatology. 2018 Apr;67(4):1472-1484. doi: 10.1002/hep.29612. Epub 2018 Feb 20. PMID: 29059466. (Impact-Faktor 14,971)

Zielsetzung der Arbeit – In dieser prospektiven Studie wurde die Lebersteifigkeit bei Patienten, die einen transjugulären portosystemischen Shunt erhalten hatten vor und nach Shunt-Anlage gemessen. Ziel war es, die Änderung der Lebersteifigkeit nach der Shunt-Anlage in Bezug auf die Mortalität und Inflammation zu untersuchen.

Methoden und Ergebnisse – Von 83 eingeschlossenen Patienten erhielten 16 eine transiente Elastographie unmittelbar vor und 30 Minuten nach Shunt-Anlage (Akutgruppe), während 67 eine Shear-Wave-Elastographie von Leber und Milz vor Shunt-Anlage und 7 Tage danach (chronische Gruppe) erhielten. Vor Shunt-Anlage erfolgte aus Kubital-, Pfortader- und Lebervenenblut die Bestimmung von Interleukinen (IL1b, IL6, IL8, IL10, IL18) und Interferon-gamma. Bei 27 Patienten (5 akut, 22 chronisch) führte die Shunt-Anlage zu einer Zunahme der Lebersteifheit von mehr 10 %. Bei 56 Patienten nahm die Lebersteifigkeit ab oder blieb unverändert. Wichtig ist, dass die durch Shear-Wave-Elastographie gemessene Milzsteifkeit bei allen Patienten (chronische Gruppe) abnahm. Keiner der klinischen oder laborchemischen Parameter unterschied sich zwischen den Gruppen der Patienten mit Zunahme der Lebersteifigkeit und solchen ohne. Bemerkenswerterweise zeigten Patienten mit erhöhter Lebersteifigkeit insgesamt höhere Werte proinflammatorischer Zytokine in der Lebervene. Diese Patienten zeigten eine höhere Inzidenz von Organversagen und ein schlechteres Überleben nach TIPS. C-reaktive Proteinwerte und Anstieg der Lebersteifheit um >10 % nach TIPS waren die einzigen unabhängigen Prädiktoren für die Mortalität bei diesen Patienten.

Schlussfolgerungen – Diese Studie zeigt, dass das Vorhandensein einer systemischen Entzündung Patienten dazu prädisponiert, nach Shunt-Anlage eine erhöhte Lebersteifigkeit zu entwickeln. Weiterhin ist es ein Prädiktor für Organversagen und Tod.

HEPATOLOGY

HEPATOLOGY, VOL. 67, NO. 4, 2018

# Increase in Liver Stiffness After Transjugular Intrahepatic Portosystemic Shunt Is Associated With Inflammation and Predicts Mortality

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**Transjugular intrahepatic portosystemic shunt (TIPS) efficiently treats complications of portal hypertension. Liver and spleen stiffness might predict clinically significant portal hypertension. This prospective study investigated liver stiffness in patients receiving TIPS regardless of indication. Of 83 included patients, 16 underwent transient elastography immediately before and 30 minutes after TIPS (acute group), while 67 received shear wave elastography of liver and spleen 1 day before and 7 days after TIPS (chronic group) and were followed further. In blood samples obtained before TIPS from cubital, portal, and hepatic veins, levels of several interleukins (IL1b, IL6, IL8, IL10, IL18) and interferon-gamma were analyzed. In 27 patients (5 acute, 22 chronic), it resulted in an increase in liver stiffness of >10%. In 56 patients, liver stiffness decreased or remained unchanged (<10%). Importantly, spleen stiffness measured by shear wave elastography decreased in all patients (chronic group). None of the clinical or laboratory parameters differed between patients with increase in liver stiffness and those without. Of note, patients with increased liver stiffness showed higher overall and/or hepatic venous levels of proinflammatory cytokines at TIPS and higher incidence of organ failure and worse survival after TIPS. C-reactive protein values and increase of >10% in liver stiffness after TIPS were the only independent predictors of mortality in these patients. *Conclusion:* This study demonstrates that the presence of systemic inflammation predisposes patients to develop increased liver stiffness after TIPS, a predictor of organ failure and death. (NCT03072615) (HEPATOLOGY 2018;67:1472-1484).**

Decompensated liver cirrhosis, often due to complications of clinically significant portal hypertension,[1] might end in acute-on-chronic liver failure (ACLF), which is associated with elevated systemic inflammation[2] and high mortality.[3] Complications of portal hypertension can be successfully treated by implantation of a transjugular intrahepatic portosystemic shunt (TIPS).[4-6] TIPS leads to immediate decompression in the portal venous system and subsequent increase of effective blood volume. It stops variceal rebleeding and improves renal function.[7] The clinical stratification of patients

*Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; CI, confidence interval; CLIF-C, Chronic Liver Failure Consortium; CRP, C-reactive protein; HR, hazard ratio; IL, interleukin; MELD, Model for End-Stage Liver Disease; SWE, shear wave elastography; TE, transient elastography; TIPS, transjugular intrahepatic portosystemic shunt; TWBC, total white blood cell count.*

*Received June 30, 2017; accepted October 18, 2017.*

*Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29612/suppinfo.*

**These authors contributed equally to this work.*

*Supported by the Deutsche Forschungsgemeinschaft (SFB TRR57), the European Union's Horizon 2020 Research and Innovation Programme (668031), and the Cellex Foundation. The funders had no influence on study design, data collection and analysis, decision to publish, or preparation of the manuscript.*



*View this article online at wileyonlinelibrary.com.*

*DOI 10.1002/hep.29612*

*Potential conflict of interest: Dr. Latz consults for IFM.*

receiving TIPS has been investigated in several studies, selecting either high-risk patients receiving TIPS for variceal bleeding[4] or patients with a lower risk receiving TIPS for refractory ascites and hepatorenal syndrome.[7] However, while the indication for TIPS might be one of the above, many patients present with other complications of portal hypertension and acute decompensation (AD) of liver cirrhosis.[8] Therefore, other predictors at TIPS insertion and at short-term clinical follow-up are needed to improve the disease management in these patients.[7]

Liver stiffness, measured by either transient elastography (TE)—recently approved by the Food and Drug Administration in the United States—or shear wave elastography (SWE), is a useful parameter in the diagnosis of clinically significant portal hypertension and varices requiring treatment.[9-15] While it has been shown that liver and spleen stiffness might detect changes in hepatic venous pressure gradient after nonselective beta-blockers, the data were not as conclusive after TIPS.[16-19] Importantly, a larger recent study could not sufficiently explain the reason behind the change of liver stiffness after TIPS.[20] This is probably due to the fact that liver stiffness is a result of various different factors, especially blood (arterial and venous) pressure, inflammation, and fibrosis.[21-23]

The aim of this prospective observational cohort study, carried out in two centers, was to evaluate the change in liver and spleen stiffness measurement after TIPS as a parameter to stratify and follow up patients.

# Patients and Methods

## PATIENTS

This prospective observational cohort study was carried out in two centers and included patients with the following criteria: (1) reliable SWE or TE measurement of the liver before TIPS insertion and (2) feasible TIPS insertion. Exclusion criteria were (1) contraindication for TIPS (including diastolic dysfunction), (2) no reliable SWE/TE measurement, and (3) patient refusal or noncompliance.

Between April 2013 and November 2015, 128 patients were screened at the Department of Internal Medicine I of the University Hospital Bonn, Germany, and at the Department of Gastroenterology, University Hospital Freiburg, Freiburg, Germany. Of these, 83 patients were enrolled, while 45 patients did not fulfill the inclusion criteria (Fig. 1). In 16 patients (acute group), liver stiffness was measured by TE immediately before and 30 minutes after TIPS insertion to detect acute changes. In 67 patients (chronic group), liver and spleen SWE was performed 1 day before and 7 days after TIPS insertion to detect chronic changes. Blood samples from portal, liver, and cubital veins were collected at TIPS insertion in 45 patients of the chronic group. The chronic group was followed in detail regarding short-term (median 7 days) and long-term follow-up. The study was registered after completion at clinicaltrials.gov (NCT03072615). All authors had access and were able to review and approve the final manuscript.

Within the context of another study, an additional 7 patients received TE and SWE of the liver before, 30 minutes after, and 7 days after TIPS.

## ETHICS CONSIDERATION

Patients gave signed written informed consent for the procedures of the study. The local ethics committees of the Universities of Bonn (no. 121/14) and Freiburg (S-384/2009) approved the study in accordance with the Declaration of Helsinki.

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## TE PROCEDURE

Liver stiffness measurements using TE were performed immediately before and 30 minutes after TIPS insertion after fasting for at least 8 hours. TE measurement was performed as described.[24-26] In this study, only procedures with at least 10 valid shots, a success rate of at least 80%, and ratio of interquartile range and median <0.3 were considered reliable and used for statistical analysis.[24-26] The XL probe was used in patients with ascites according to earlier reports.[27] Nevertheless, all patients received paracentesis before TIPS and usually a drain catheter during hospitalization. Therefore, TE or SWE measurements were not influenced by ascites.

## SWE PROCEDURE

Only accurate SWE measurements were performed after overnight fasting and considered and performed as described.[13,14] An accurate SWE measurement showed (1) stability of the selected liver area for at least 3 seconds before measurement, (2) two-dimensional quality confirmed by homogenous color in the region of interest, and (3) a measurement region of at least 10 mm. Up to three separate measurements were performed, and results are reported as the mean of the total number of valid measurements. An increase or decrease in stiffness was regarded only if SWE measurement changed >10% because this was the variance within measurements applied in the same patients in previous studies.[13,14]

## MEASUREMENTS OF PROINFLAMMATORY PROTEINS

During the TIPS procedure, to determine levels of different inflammatory proteins, first blood from cubital, then hepatic veins were collected, finally as soon as the portal vein branch was cannulated the portal venous sample was collected as well, as described.[28-32] In these patients and in 42 healthy controls, we measured interleukin 1beta (IL1$\beta$), IL6, and IL8 in the cubital vein using an enzyme-linked immunosorbent assay according to the manufacturer's guidelines (R&D Systems Inc., Minneapolis, MN).

Additionally, in 20 patients selected regarding their evolution of liver stiffness, samples taken from the cubital, liver, and portal veins of IL1$\beta$, IL6, IL8, IL10, IL18, and interferon-gamma were measured using the Luminex MAGPIX system (EMD Millipore, Darmstadt, Germany) with ProcartaPlex Mix&Match Human 15-plex (eBiosciences, Carlsbad, CA) according to the manufacturer's instructions.

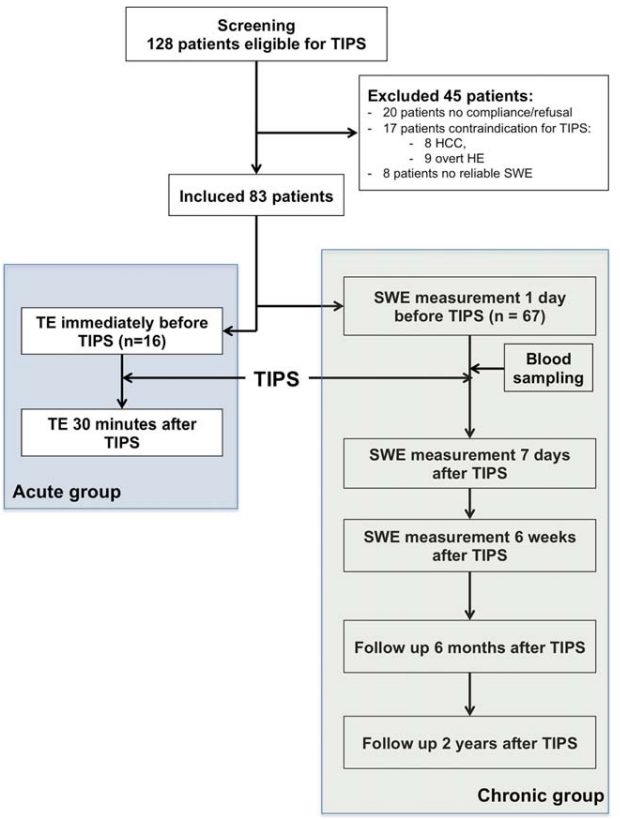


**FIG. 1.** Recruitment and study design. The flowchart illustrates screening and recruitment of the patients. In the acute group (recruitment in Freiburg), patients received liver stiffness measurements using TE immediately before TIPS and 30 minutes after TIPS. In the chronic group of patients (recruitment in Bonn), liver and spleen SWE was performed 1 day before TIPS and 7 days, 6 weeks, and 3 months after TIPS. Patients were followed for a maximum of 2 years. Abbreviations: HCC, hepatocellular carcinoma; HE, hepatic encephalopathy.

## TIPS INSERTION AND PRESSURE MEASUREMENT

TIPS (8 mm and 10 mm, Viatorr; W. L. Gore & Associates, Inc., AZ) insertion was performed as described.[6,28-33] Portal and inferior cava venous pressures were measured invasively using a multichannel monitor (Sirecust; Siemens, Germany). The difference between these pressures was defined as the pressure gradient.

**TABLE 1. General Characteristics of Patients at Baseline**

| Parameters | All | Acute | Chronic | *P* |
|---|---|---|---|---|
| Sex (male/female) | 51/32 | 12/4 | 39/28 | 0.22 |
| Age (years) | 60 (18-81) | 63 (43-77) | 59 (18-81) | 0.19 |
| Etiology (alcohol/viral hepatitis/other) | 53/11/19 | 15/1 | 38/10/19 | 0.06 |
| Indication of TIPS (bleeding/ascites/both) | 33/42/8 | 6/6/4 | 27/36/4 | 0.34 |
| Child-Pugh score | 8 (5-12) | 8 (5-9) | 8 (5-12) | 0.37 |
| MELD score | 9 (6-26) | 12 (8-22) | 9 (6-26) | 0.03 |
| Creatinine (mg/dL) | 1 (0.48-7.61) | 1.1 (0.66-4) | 1 (0.48-7.61) | 0.11 |
| Bilirubin (mg/dL) | 1 (0.16-6.3) | 1.1 (0.4-2.8) | 0.98 (0.16-6.3) | 0.64 |
| INR | 1.1 (0.9-1.63) | 1.2 (1-1.63) | 1.1 (0.9-1.5) | 0.07 |
| γGT (IU/L) | 134.5 (10-779) | 145 (33-593) | 128 (10-779) | 0.09 |
| ALT (IU/L) | 26 (4-121) | 22 (4-65) | 28 (6-121) | 0.08 |
| AST (IU/L) | 39 (13-95) | 43.5 (26-85) | 38 (13-95) | 0.08 |
| TWBC (g/L) | 5.5 (2.18-15.61) | 5 (3-11) | 5.5 (2.18-15.6) | 0.48 |
| Platelets (g/L) | 118 (39-509) | 107 (52-279) | 124 (39-509) | 0.72 |

Data are medians and (ranges). The Wilcoxon test results are shown with *P* value.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, gamma-glutamyltransferase; INR, international normalized ratio.

## STATISTICAL ANALYSIS

A nonparametric Wilcoxon test was used to compare two sets of paired data. In addition, a Friedman test was used for more than two sets of paired data. A Mann-Whitney test was used for unpaired comparisons and a Kruskal-Wallis test if more than two groups were compared. Correlations were analyzed with Spearman's correlation coefficient. Univariate time-to-event analysis was performed to identify parameters which might significantly predict survival. Cox regression analysis (forward stepwise likelihood quotient) using the significant predictors in the univariate analysis was performed to identify independent predictors of survival. Kaplan-Meier curves were used to analyze the survival rates of patients using a log-rank test. Data are presented as median and ranges unless otherwise declared. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL) and Prism 4.0 to plot a part of the graphs.

# Results

## RECRUITMENT AND GENERAL CHARACTERISTICS OF THE COHORT

In total, 128 patients were screened, of whom 45 were excluded. Seventeen patients had contraindications for TIPS: in eight cases, this was due to hepatocellular carcinoma and in nine cases, to severe overt hepatic encephalopathy. Twenty patients were excluded for noncompliance to the protocol, and in eight patients, no reliable SWE could be obtained due to obesity (Fig. 1). Thus, 83 patients were included in the study, 16 patients received in Freiburg the acute protocol using TE and 67 patients received in Bonn the chronic protocol using SWE. Of these, 16 patients were included in the acute group receiving TE measurement of the liver immediately before and 30 minutes after TIPS and 67 patients were included in the chronic group receiving SWE of the liver and spleen 1 day before and 7 days after TIPS (Fig. 1). The latter group was followed for a maximum of 24 months.

The general characteristics of the cohort and of the acute and the chronic groups are outlined in Table 1 and Supporting Tables S1 and S2. Most of the included patients were male (61.4%), with a median age of 60 years. Of these patients, 64% suffered from alcoholic liver cirrhosis, 13% from chronic viral hepatitis, and 23% from other etiologies. In the whole cohort, median Child score was 8 and median Model for End-Stage Liver Disease (MELD) score was 9 (Table 1). Median Chronic Liver Failure Consortium (CLIF-C) AD score was 45.6, while median CLIF-C ACLF score was 35 (Supporting Table S2). No major differences were found between the acute and the chronic groups, except for a higher MELD score in the acute group (Table 1).

## ACUTE EFFECT OF TIPS ON LIVER STIFFNESS

In the acute group, liver stiffness was measured using the XL probe, which is validated for patients with ascites. In this group, TIPS decreased overall liver

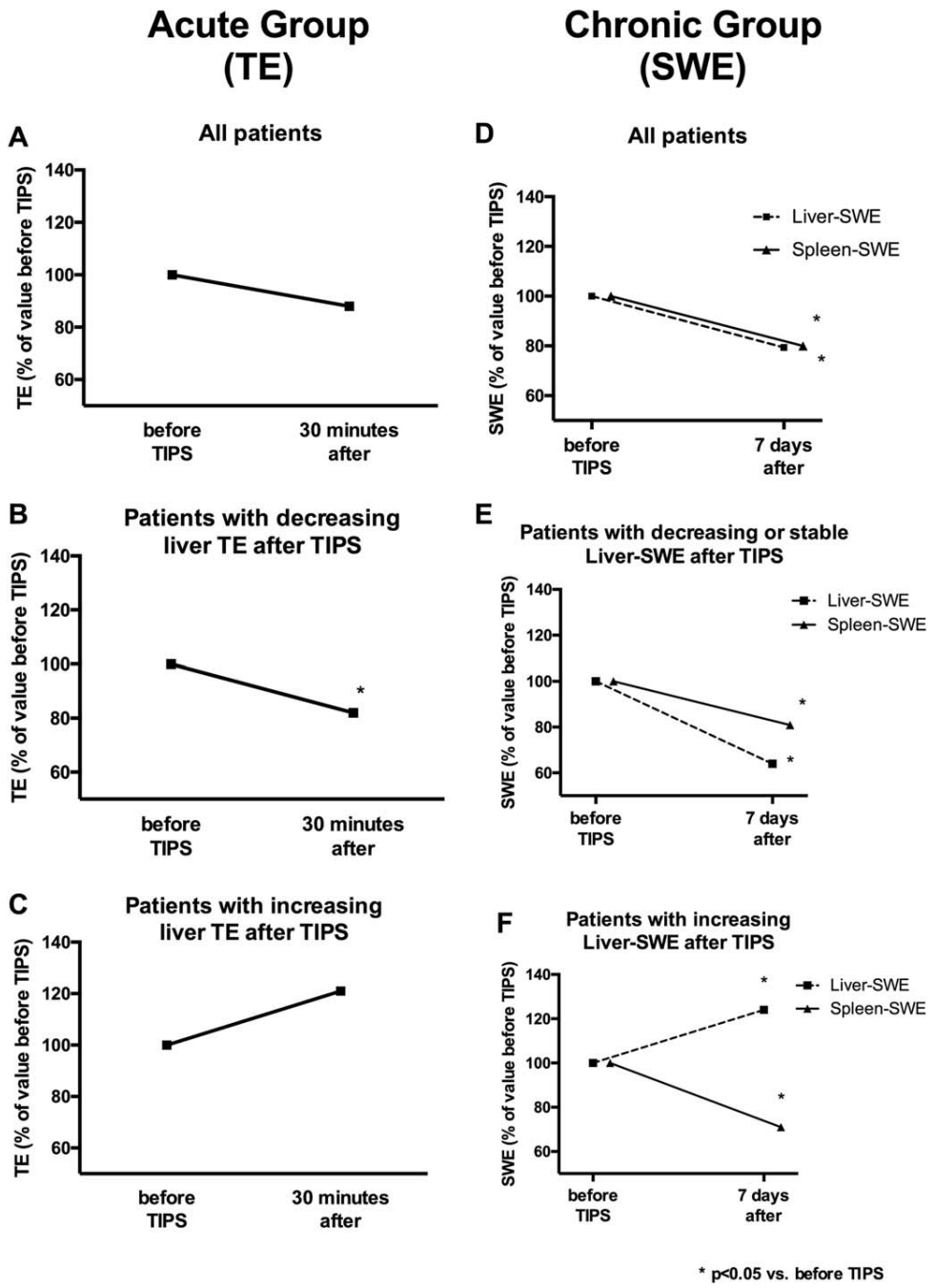


**FIG. 2.** Effect of TIPS on acute and chronic changes of liver stiffness and chronic changes in spleen stiffness. In the acute group, liver TE showed a nonsignificant trend toward decrease in liver stiffness 30 minutes after TIPS (A). In two thirds of the patients from the acute group, liver stiffness significantly decreased (B), while in one third of the patients, liver TE increased without reaching significance (C). In the chronic group, liver and spleen SWE decreased 7 days after TIPS (D). Similarly, two groups of patients could be identified: patients with a decrease in liver SWE and spleen SWE after TIPS (E) and patients with an increase in liver SWE while spleen SWE decreased (F). Data are shown as mean and were analyzed using paired nonparametric comparisons with the Wilcoxon test. Mean values are available in the Supporting Table S3, and individual data are available upon request. *$P < 0.05$ versus before TIPS.

**TABLE 2. Clinical, Biochemical, and Hemodynamic Parameters of Patients in the Chronic Group Stratified by Change of Liver SWE 7 Days After TIPS**

| | Before TIPS | | | After TIPS | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameters | Decreased or unchanged liver SWE | Increased liver SWE | *P* | Decreased or unchanged liver SWE | Increased liver SWE | *P* | *P** | *P*† |
| Child-Pugh score | 8 (5-12) | 7 (5-11) | 0.5 | 7 (5-10) | 7 (5-9) | 0.98 | 0.019 | 0.22 |
| MELD score | 9.4 (6-26) | 8.7 (6-17) | 0.22 | 11 (6-26) | 9.4 (6-21) | 0.45 | 0.13 | 0.04 |
| CLIF-C AD score | 45 (23.3-72) | 47 (31-57) | 0.47 | 47 (30-71) | 49 (37-62) | 0.12 | 0.97 | 0.025 |
| CLIF-C ACLF score | 35 (13-50) | 36 (22-44) | 0.29 | 35 (17-48) | 37 (25-46) | 0.06 | 0.84 | 0.013 |
| Creatinine (mg/dL) | 0.97 (0.48-7.61) | 0.99 (0.56-1.91) | 0.71 | 0.92 (0.46-6.36) | 1.01 (0.66-2.97) | 0.29 | <0.001 | 0.29 |
| Bilirubin (mg/dL) | 1.08 (0.16-6.31) | 0.82 (0.29-4.1) | 0.06 | 1.44 (0.23-7.88) | 1.27 (0.44-3.86) | 0.33 | 0.024 | 0.1 |
| INR | 1.1 (0.9-1.5) | 1.1 (1-1.4) | 0.73 | 1.2 (1-2.2) | 1.2 (1-1.4) | 0.63 | 0.001 | 0.21 |
| ALT (IU/L) | 24.5 (9-121) | 33 (6-90) | 0.12 | 42 (19-781) | 53 (8-1317) | 0.46 | <0.001 | 0.003 |
| AST (IU/L) | 38.5 (15-95) | 34 (13-95) | 0.42 | 51 (24-247) | 59 (12-627) | 0.9 | 0.002 | 0.016 |
| γGT (IU/L) | 131 (10-779) | 108 (53-501) | 0.8 | 172 (32-663) | 118 (61-656) | 0.16 | 0.01 | 0.65 |
| CRP (mg/L) | 10.3 (0-153) | 11.7 (0.3-106) | 0.6 | 11 (1.3-62) | 18 (1-99) | 0.13 | 0.18 | 0.022 |
| TWBC (G/L) | 5.95 (2.18-15.61) | 5.21 (2.24-8.07) | 0.1 | 5.94 (3.12-15.92) | 6.42 (3.13-12.09) | 0.9 | 0.72 | 0.04 |
| Pressure gradient (mm Hg) | 21 (10-42) | 18 (12-33) | 0.02 | 8.5 (1-36) | 7 (4-13) | 0.38 | <0.001 | <0.001 |
| Portal pressure (mm Hg) | 27 (15-46) | 26 (14-35) | 0.53 | 18 (8-43) | 19 (9-25) | 0.62 | <0.001 | <0.001 |
| Central venous pressure (mm Hg) | 6 (0-17) | 8 (2-22) | 0.09 | 10 (2-22) | 11 (1-19) | 0.19 | <0.001 | <0.001 |

Data are shown as medians and (ranges). The Wilcoxon test results are shown with *P* value.
*Intragroup comparison of patients with decreased or unchanged liver SWE after TIPS.
†Intragroup comparison of patients with increased liver SWE after TIPS.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, gamma-glutamyltransferase; INR, international normalized ratio.

stiffness measured by TE, although without statistical significance (Fig. 2A; Supporting Table S3A). Interestingly, in 11 patients a significant decrease of TE (Fig. 2B; Supporting Table S3A) and in 5 patients an increase of TE (Fig. 2C; Supporting Table S3A) was found.

In these two different groups of patients, no significant changes were observed at baseline, except for a tendency to higher bilirubin, international normalized ratio, alanine aminotransferase, aspartate aminotransferase, and total white blood cell count (TWBC) in the group with increase in liver stiffness after TIPS (Supporting Table S1).

A recent series of 7 patients received TE and SWE of the liver before, 30 minutes after, and 7 days after TIPS within a context of another study. Importantly, 30 minutes after TIPS, liver SWE and TE values remained unchanged on day 7 after TIPS (data not shown).

## EFFECT OF TIPS ON LIVER AND SPLEEN STIFFNESS AFTER 7 DAYS

In the chronic group, reliable liver SWE measurements were obtained in 67 patients and reliable spleen SWE measurements could be documented in 62 patients.

Liver and spleen stiffness measured by SWE decreased significantly after 7 days (Fig. 2D; Supporting Table S3B). Similar to the acute group, in these patients, two different groups could be distinguished. There were 45 patients with a decrease or a change in liver SWE of <10% (Fig. 2E; Supporting Table S3B) because this was the variance within measurements in the same patients in previous studies.[13,14] These patients also showed a significant decrease in spleen SWE (Fig. 2E; Supporting Table S3B), whereas 22 patients showed an increase of >10% in liver SWE 7 days after TIPS and at the same time a significant decrease in spleen SWE (Fig. 2F; Supporting Table S3B).

Comparing the baseline data of these two groups receiving SWE, no difference was found except for a tendency toward higher CLIF-C AD score, CLIF-C ACLF score, alanine aminotransferase, and C-reactive protein (CRP) but lower Child-Pugh score, MELD score, bilirubin, aspartate aminotransferase, gamma-glutamyltransferase, TWBC, and pressure gradient (Table 2). However, none of these parameters were statistically significant, except for pressure gradient

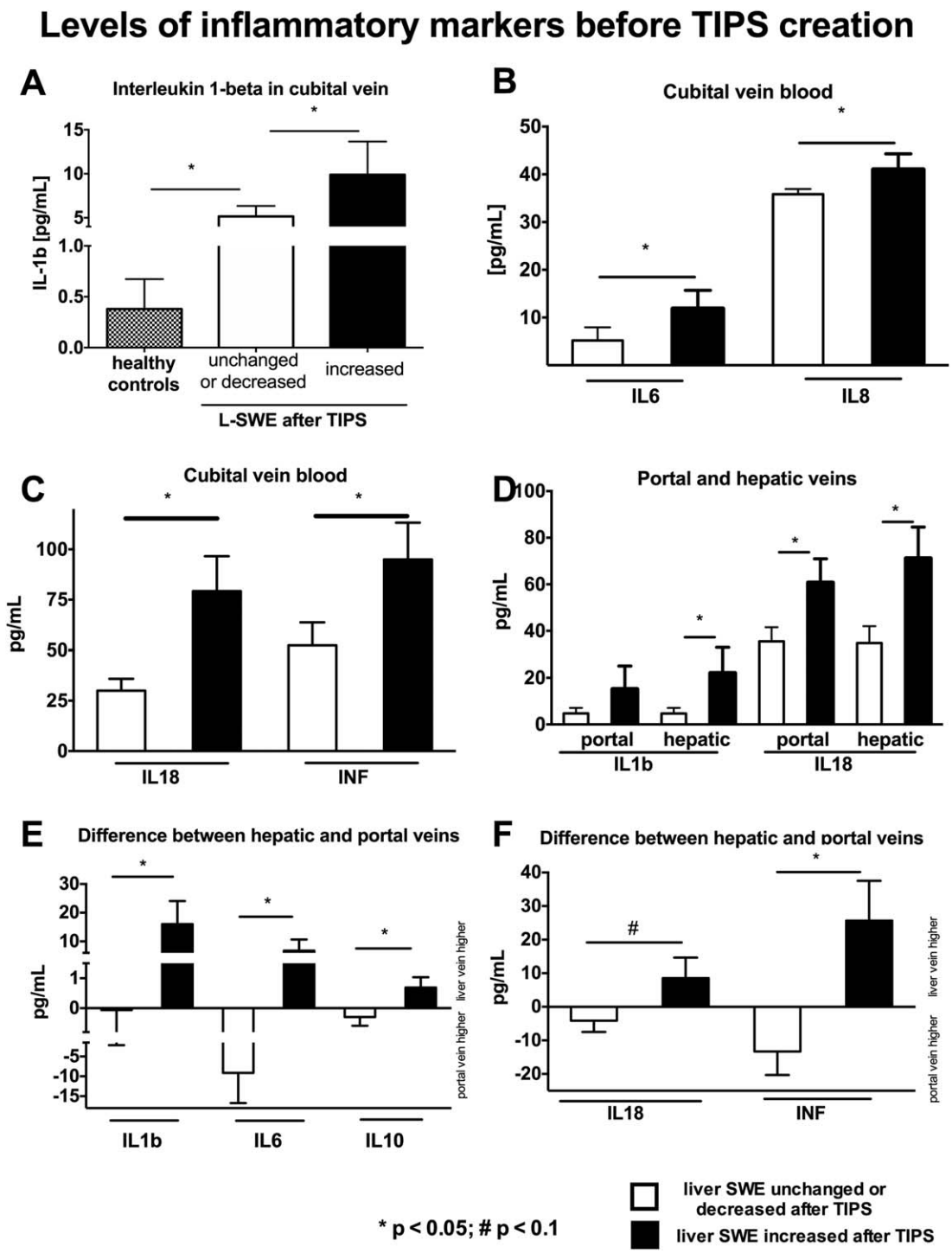


**FIG. 3.** Levels of inflammatory cytokines in samples of cubital, portal, and hepatic vein blood collected at TIPS. IL1$\beta$ levels in the cubital vein were higher in the group with increased liver SWE after TIPS (n = 15) compared to the rest of the patients (n = 39) and healthy controls (n = 42), while all patients with cirrhosis showed higher levels than healthy controls (A). Similarly, IL6 and IL8 in (B) as well as IL18 and interferon-gamma in (C) showed higher levels in the group with increased liver SWE after TIPS (n = 9) compared to the rest of the patients (n = 11). Comparing levels of IL1$\beta$ and IL18 in the portal and hepatic veins reveals similar results to findings from peripheral blood (D). Interestingly, intraindividual differences of IL1$\beta$, IL6, and IL10 between the levels found in the hepatic and portal veins were higher in the group with increased liver SWE after TIPS (n = 9) compared to the rest of the patients (n = 11) (E). Similarly, the difference between hepatic and portal venous levels of IL18 and interferon-gamma was also higher in the high-risk group (F). Data are shown as mean and standard error of mean, and comparison between groups was performed using the nonparametric Mann-Whitney U test. $^{*}P < 0.05$, $^{\#}P < 0.1$. Abbreviation: INF, interferon-gamma.

before TIPS. In summary, the baseline clinical and laboratory parameters of the entire cohort revealed no statistically significant differences (Supporting Table S2). Also, the stent diameter had no effect on development of liver SWE. Importantly, development of liver SWE was similar for the different indications for TIPS. Therefore, the cohorts of different indications were analyzed together.

## INFLAMMATORY MARKERS BEFORE TIPS AND RESPONSE OF LIVER SWE TO TIPS

Yet, it remains unclear as to why liver stiffness might increase in around one third of the patients after TIPS, despite portal pressure decreases of >50%, which is reflected by a decrease in spleen SWE (Fig. 2F; Supporting Table S3B).

To address this question, levels of proinflammatory cytokines were measured in peripheral blood samples of these patients. Interestingly, IL1$\beta$ was significantly higher in the group with increased liver SWE when compared to the other patients. As expected, patients with cirrhosis showed higher levels of IL1$\beta$ compared to controls (Fig. 3A). Similarly, peripheral blood levels of IL6, IL8, IL18, and interferon-gamma were also higher in patients with an increase in liver SWE after TIPS (Fig. 3B,C). These data suggest that systemic inflammation is already increased at TIPS insertion in patients who develop an increased liver SWE after TIPS.

However, the source of this systemic proinflammatory situation remains unclear. To address this question, cytokine levels were measured in the portal and hepatic veins. Interestingly, only IL1$\beta$ and IL18 showed higher levels in the portal and hepatic veins in the group of patients with an increase in liver SWE after TIPS (Fig. 3D). However, comparison of the difference in the levels of each cytokine between the hepatic and portal veins revealed a significant difference between the groups (Fig. 3E,F). The gradient of proinflammatory cytokines IL1$\beta$, IL6, IL10, IL18, and interferon-gamma across the liver (levels in the hepatic veins were higher than in the portal veins) was significantly higher in patients with increased liver SWE after TIPS (Fig. 3E,F). These data suggest that in these patients the liver has already reached a strong proinflammatory situation, as reflected in the higher levels of systemic inflammation measured in the peripheral blood samples (Fig. 3A-C), but without, as yet, evidence in clinical and laboratory values (Table 2).

## SHORT-TERM CLINICAL EVOLUTION AFTER TIPS AND RESPONSE OF LIVER SWE TO TIPS

Although all patients experienced a similar decrease in portal pressure gradient after TIPS (Table 2), patients with an increase of liver SWE after TIPS seem to develop increased systemic and hepatic inflammation at TIPS insertion. Therefore, the outcome after TIPS was observed in more detail (Table 2).

Patients with decreased or unchanged liver SWE after TIPS showed a significant improvement in Child-Pugh score and creatinine, while liver function tests deteriorated significantly and MELD, CLIF-C AD, and CLIF-C ACLF scores, as well as TWBC and CRP, remained unchanged (Table 2). By contrast, patients with an increase in liver SWE after TIPS showed significantly higher MELD, CLIF-C AD, and CLIF-C ACLF scores, as well as higher levels of alanine aminotransferase, aspartate aminotransferase, CRP, and TWBC (Table 2). The latter patients showed no signs of congestions (heart failure) or hepatic ischemia. Moreover, while ascites disappeared in a considerable proportion of the patients with decreased or unchanged liver SWE after TIPS, the response to TIPS was significantly less in patients with an increased liver SWE after TIPS (Fig. 4A). These data suggest that increased liver SWE after TIPS is associated with an inflammatory response mainly of hepatic origin, preventing clinical improvement in these patients after TIPS.

## DEVELOPMENT OF ORGAN FAILURE AND LONG-TERM SURVIVAL AFTER TIPS

Because systemic inflammation is a hallmark of the development of organ failure and ultimately ACLF in liver cirrhosis, the incidence of organ failure was assessed 6 months after TIPS (Fig. 4B). Indeed, there was a significantly higher incidence of organ failure in patients with an increase in liver SWE after TIPS (Fig. 4B). Moreover, 2-year survival was significantly worse in patients with an increase in liver SWE after TIPS (Fig. 4C). None of the patients has been transplanted.

When analyzing the parameters associated with survival in these patients, decreased or unchanged liver SWE 7 days after TIPS correlated significantly (hazard ratio [HR], 0.064; 95% confidence interval [CI],

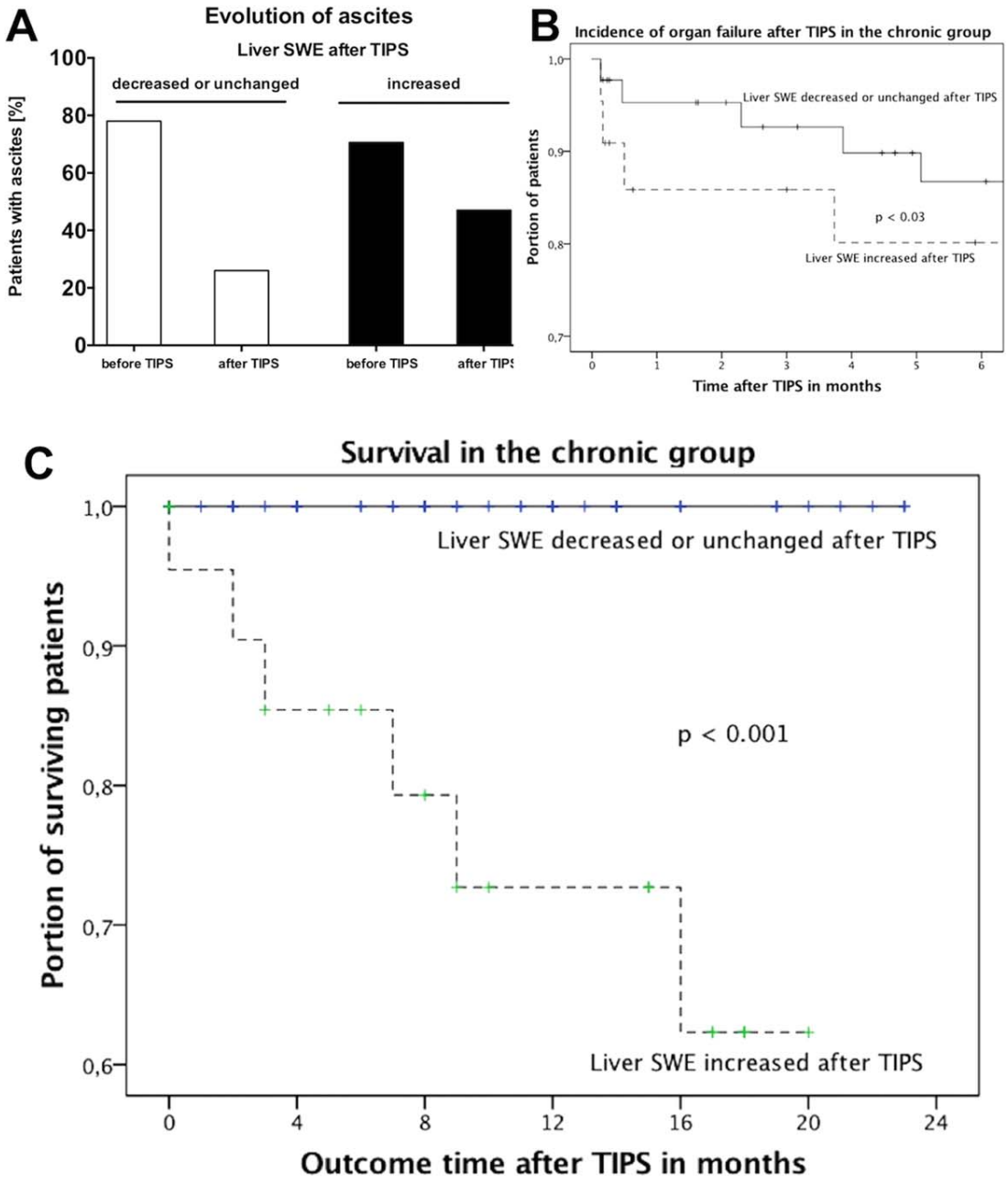


**FIG. 4.** Outcome of the patients in the follow-up. Among patients with increased liver SWE after TIPS, response of ascites to TIPS occurred in only 20% of patients compared to 60% of patients in the group with decreased or unchanged liver SWE after TIPS (A). Incidence of organ failure after 6 months was significantly higher in the high-risk group, as shown by Kaplan-Meier plot and analyzed by log-rank test (B). Importantly, the overall survival rate was unimpaired in patients with decreased or unchanged liver SWE after TIPS, while mortality was increased in patients with increased liver SWE after TIPS, as shown by Kaplan-Meier plot and analyzed by log-rank test (C).

0.007-0.547; $P = 0.012$) with survival (Table 3). Further parameters, such as age, CLIF-C AD score, creatinine 7 days after TIPS, as well as CRP 7 days after TIPS were identified as predictors of survival in univariate time-to-event analysis (Table 3), while small liver size, low platelet count, and low hemoglobin levels were not significantly associated with survival in this cohort. Importantly, the MELD score showed an

**TABLE 3. Univariate and Multivariate Time-to-Event Analysis and Cox Regression Analysis (Forward Stepwise Likelihood Quotient) in the Chronic Group to Predict Survival With Death as Endpoint**

| Parameters | *P* | HR | 95% CI for HR |
|---|---|---|---|
| *Univariate time-to-event analysis* | | | |
| Age at time of insertion | 0.033 | 1.099 | 1.008-1.199 |
| CLIF C AD score before TIPS | 0.042 | 0.103 | 0.012-0.925 |
| CRP after TIPS | 0.008 | 1.051 | 1.013-1.09 |
| Creatinine after TIPS | 0.044 | 0.019 | 0.001-0.906 |
| Liver SWE decreased/unchanged | 0.012 | 0.064 | 0.007-0.547 |
| *Forward stepwise likelihood quotient* | | | |
| CRP after TIPS | 0.03 | 1.044 | 1.004-1.084 |
| Liver SWE decreased/unchanged | 0.022 | 0.079 | 0.009-0.690 |

HR of 1.001 with a 95% CI of 0.83-1.19 and a *P* value of 0.58. Therefore, the MELD score was also not included in the multivariate analysis.

Multivariable Cox regression analysis of these significant variables identified CRP (HR, 1.044; 95% CI, 1.004-1.084; $P = 0.03$) and decreased or unchanged liver SWE (HR, 0.079; 95% CI, 0.009-0.690; $P = 0.022$), both 7 days after TIPS, as independent predictors of survival (Table 3).

# Discussion

This prospective observational cohort study demonstrates that liver stiffness after TIPS is a stratification tool for high-risk patients. Moreover, this study possibly explains the increase of liver stiffness after TIPS in some patients, while it also confirms the important role of hepatically derived systemic inflammation in the outcome of patients with decompensated cirrhosis.

Liver stiffness has been introduced in the field of digestive diseases to identify significant liver fibrosis.[26] Liver stiffness assessed by TE[9-11,24,26,34,35] or SWE[12,36] might identify clinically significant portal hypertension. However, liver TE and SWE very much depend on other factors, such as alcohol and meal ingestion, right heart function, volume changes, arterial pressure, cholestasis, and inflammation.[22,23,37-42] While TIPS decreased portal pressure by >50%, liver stiffness was not proportionally decreased, as assessed by two different techniques of measuring liver stiffness, at different time points after TIPS and in different centers. In a similar portion of patients, liver stiffness increased after TIPS, regardless of TIPS indication or diameter.

Moreover, the present study defines the prognostic impact of an increase in liver stiffness after TIPS. These patients could only be identified by the increase of liver stiffness after TIPS and not by any other clinical or routine laboratory parameters. The increase occurred very early after TIPS insertion, as shown in the acute cohort, and continued up to the next follow-up visit. This finding outperforms the MELD score for overall survival, which was designed for outcome assessment 3 months after TIPS.[43]

The pathophysiological processes responsible for the deterioration of clinical outcome and increase in liver stiffness in this subgroup of patients receiving TIPS are insufficiently explored. Frequently, the opening of the route for portal venous blood into the systemic circulation by TIPS has been blamed for increased systemic inflammation in TIPS patients. The present study clearly demonstrates that systemic inflammation had already increased in these patients before TIPS, as shown by higher levels of IL1$\beta$, IL6, IL8, IL18, as well as interferon-gamma in peripheral blood. Moreover, levels of IL1$\beta$ and IL18 were higher in the hepatic and portal veins of patients receiving TIPS. Interestingly, in patients who developed increased liver stiffness after TIPS and who had poor outcome, much higher inflammatory markers were found in the hepatic veins than in the portal veins. This suggests that the source of systemic inflammation in these patients is the diseased liver, while in patients who recovered after TIPS and who had a good prognosis, levels of inflammatory cytokines in the hepatic veins were lower than in the portal veins. Apart from the acute testing of liver stiffness in these patients, the difference between portal and hepatic levels of cytokines appears also to be fitting to distinguish between patients with better or worse prognosis. Especially the increase of IL6, which is a marker of systemic inflammation, seems to derive from the injury the liver had sustained already before TIPS insertion. IL6 has been shown to predict variceal bleeding as well as mortality in patients with ACLF.[2,44] In this study, we suggest that it might derive from the immune cells of the injured liver. Moreover, at the next follow-up, systemic inflammation aggravated and was clinically evident, as shown by increases in CRP and

TWBC. This was also mirrored by AD and ACLF scores, which predict the incidence of ACLF and survival.[45,46] Recently, the close relationship between systemic inflammation, organ failure, ACLF, and mortality has been shown in the CANONIC cohort.[2] Indeed, the increase of liver stiffness after TIPS identified patients developing organ failure and independently predicted mortality in patients receiving TIPS, which is also supported by previous observations of our group ascribing an important prognostic value to inflammatory cytokines.[28-30,32]

However, it is difficult to predict whether patients with increased liver stiffness after TIPS would not have developed organ failure or ACLF and would not have shown a higher mortality without TIPS, a question to be addressed in future studies. Turning the argument around, TIPS might prevent ACLF in patients with decreased stiffness because portal hypertension is a predisposing factor for ACLF.[47] TIPS might prevent ACLF in several situations[7]; e.g., early TIPS prevents the inflammatory response induced by further bleeding and improves survival.[4] Apart from these open questions, the present study demonstrates and offers an interesting and easy approach for the clinical routine to identify high-risk patients after TIPS.

Our data suggest that liver stiffness increases immediately after TIPS. Interestingly, these patients do not show higher stiffness before TIPS. Although liver stiffness might change after alcohol withdrawal[37] and correlates with liver damage,[48] the rather prompt change of liver stiffness only minutes after the TIPS procedure suggests pressure changes as the underlying cause. In general, liver stiffness measurement is an integrative measure of fibrosis, inflammation, and sinusoidal pressure.[21-23] In this system, TIPS might decrease the portal pressure and portal perfusion of the sinusoids but increase arterial perfusion of the sinusoids,[49] while in our patients, fibrosis and arterial pressures remained unchanged. The only difference between the groups of patients in whom liver stiffness increased after TIPS was the presence of hepatic and systemic inflammation. More importantly, it has recently been shown that systemic inflammation increases intrahepatic sinusoidal resistance.[50] Thus, to speculate, one is tempted to assume that challenge of the sinusoids with twice the hepatic arterial perfusion[49] might be the reason for the acute increase in stiffness after TIPS.

Another important finding of this study is that liver SWE is unsuitable to reflect changes in the portal pressure gradient in decompensated patients. Previous data have shown that liver TE or SWE possibly reflects the effect of nonselective beta-blockers[16,17] and that SWE also excludes or includes with high accuracy the presence of clinically significant portal hypertension,[12,17,36] a prerequisite for decompensation, complications, and, ultimately, ACLF.[1,7,47] Recent studies have suggested that spleen SWE is very important for the diagnosis of portal hypertension and changes in portal pressure.[13,14,19,20] Indeed, our study might provide an explanation for these findings.

Despite the fact that our study is a prospective observational study carried out in two centers, with two different well-characterized patient cohorts at two different time points, several limitations must be mentioned. First, this is an observational study, and due to its exploratory nature, it lacks a clear hypothesis and sample size calculation. Although we used the XL probe for TE, which has been validated for ascites, this may be cause for some criticism regarding reliability. Although in the patients of this study, we could not provide data of TE and SWE from the same patients at the same time points, within the context of another study, we recently performed a small series of 7 patients with SWE and TE of the liver at the same time, before, 30 minutes after, and 7 days after TIPS. Another limitation is that in the smaller TE group, no follow-up data were collected because this was an acute study. However, recently, together with others, we found very similar results in the TE and the SWE response to different challenges to the portal venous system.[38] Therefore, we assume that this is also the case in the present study. A further limitation is the rather small size of the cohort. However, this is the largest cohort in patients with TIPS. Despite the rather small group of patients, our study demonstrated high examiner independency because at two different sites, using two different techniques at different time points to measure liver stiffness, the results were very similar. Finally, because post-TIPS SWE change is a marker of poor prognosis after TIPS, it is not helpful to select patients before TIPS. However, it might be helpful for risk stratification in patients receiving TIPS because they will receive TIPS for a clear indication unrelated to SWE. Moreover, the measurement of systemic inflammation, as demonstrated in our cohort, might be useful to stratify patients before TIPS insertion.

In conclusion, this study demonstrates that it is mainly liver-derived systemic inflammation that predisposes patients receiving TIPS to develop organ failure and ACLF. These patients show an increased liver stiffness after TIPS immediately and at follow-up.

Therefore, inflammation at TIPS insertion and increase of liver stiffness after TIPS insertion predict organ failure and death in these patients, who require closer management.

*Acknowledgment:* We thank Gudrun Hack, Silke Bellinghausen, Nadine Köstlmeier, Julia Groffy, Teresija Boscnic, and Kristin Gehrmann for excellent technical assistance and Sabine Dentler for critical reading.

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Author names in bold designate shared co-first authorship.

# Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29612/suppinfo.

## 3.10 Die Rolle von miRNA-34a als prognostischer Biomarker für Patienten mit Portal Hypertension

**Jansen C,** Eischeid H, Goertzen J, Schierwagen R, Anadol E, Strassburg CP, Sauerbruch T, Odenthal M, Trebicka J. **The role of miRNA-34a as a prognostic biomarker for cirrhotic patients with portal hypertension receiving TIPS**. PLoS One. 2014 Jul 28;9(7):e103779. doi: 10.1371/journal.pone.0103779. PMID: 25068403; PMCID: PMC4113430.

Zielsetzung der Arbeit – Es ist bekannt, dass die zirkulierende miRNA-34a im Blut von Patienten mit verschiedenen Lebererkrankungen im Vergleich zu Gesunden erhöht ist. Der Ursprung von miRNA-34a und seine mögliche Beziehung zur Hämodynamik und zur Mortalität bei Patienten mit Leberzirrhose sollte untersucht werden.

Methoden und Ergebnisse – Wir schlossen 60 Patienten mit einer Leberzirrhose ein, die einen transjugulären intrahepatischen portosystemischen Shunt zur Prävention einer erneuten Varizenblutung oder aufgrund von Aszites erhalten hatten. Die miRNA-34a-Spiegel wurden in Pfortader- und Lebervenenblut mittels qPCR gemessen. Hämodynamische und klinische Parameter wurden vor und während der Nachsorge beurteilt. Die Spiegel von miRNA-34a waren in der Lebervene höher als in der Pfortader. Zirkulierende miRNA-34a in der Lebervene korrelierte mit ALT, CHE und der Natriumausscheidung nach transjugulärer intrahepatischer portosystemischer Shunt-Anlage. Die miRNA-34a zeigte keine Korrelation mit dem Portaldruck, aber inverse mit dem Stauungsindex der Pfortader. Interessanterweise zeigten die Spiegel der miRNA-34a im Portal und Lebervene eine umgekehrte Korrelation mit dem arteriellen Druck. Darüber hinaus hatten aber höhere Spiegel von miRNA-34a in der Lebervene einen prädiktiven Wert für das Überleben. Es war aber zu dokumentieren, dass der MELD-Score, Kreatinin im Kurzzeit-Follow-up von 14 Tage nach transjugulärer intrahepatischer portosystemischer Shunt-Anlage und der Portaldruck nach Shunt-Anlage bessere Prädiktoren waren.

Schlussfolgerungen – Diese Studie zeigt zum erstmals, dass miRNA-34a zu einem großen Teil aus der Leber stammt und einen prognostischen Marker darstellt. Klassische prognostische Parameter können aber das Überleben in diesem Kollektiv besser voraussagen.

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PLOS ONE

# The Role of miRNA-34a as a Prognostic Biomarker for Cirrhotic Patients with Portal Hypertension Receiving TIPS

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## Abstract

***Background:*** Circulating miRNA-34a is increased in blood of patients with different liver diseases when compared to healthy controls. However, the origin of miRNA-34a and its possible relationship with hemodynamics and outcome in cirrhotic patients with portal hypertension is unknown. We analyzed the levels of miRNA-34a in cirrhotic patients with severe portal hypertension.

***Methods:*** We included 60 cirrhotic patients receiving TIPS for prevention of rebleeding and/or therapy-refractory ascites. miRNA-34a levels were measured using qPCR and normalized by SV-40 in the portal and hepatic venous blood of these patients taken at TIPS procedure. Hemodynamic and clinical parameters were assessed before TIPS and during follow-up.

***Results:*** Levels of miRNA-34a were higher in the hepatic vein than in the portal vein. Circulating miRNA-34a in the hepatic vein correlated with ALT, CHE and sodium excretion after TIPS. miRNA-34a showed no correlation with portal pressure, but its levels in the portal vein correlated inversely with the congestion index. Interestingly, the levels of miRNA-34a in the portal and hepatic vein showed inverse correlation with arterial pressure. Furthermore, levels of miRNA-34a in the hepatic vein had a predictive value for survival, but MELD, creatinine at short-time follow-up 14 days after TIPS-insertion and portal pressure after TIPS performed better.

***Conclusion:*** This study demonstrates for the first time, that miRNA-34a may originate to a large extent from the liver. Even though higher levels of miRNA-34a are possibly associated with better survival at long-term follow-up in cirrhotic patients with severe portal hypertension receiving TIPS, classical prognostic parameters predict the survival better.

**Citation:** Jansen C, Eischeid H, Goertzen J, Schierwagen R, Anadol E, et al. (2014) The Role of miRNA-34a as a Prognostic Biomarker for Cirrhotic Patients with Portal Hypertension Receiving TIPS. PLoS ONE 9(7): e103779. doi:10.1371/journal.pone.0103779

**Editor:** Alfons Navarro, University of Barcelona, Spain

**Received** February 19, 2014; **Accepted** July 2, 2014; **Published** July 28, 2014



**Funding:** The study was supported by grants from Deutsche Forschungsgemeinschaft (SFB TRR57 P18 to J.T./T.S.) and from J. & W. Hector-Foundation (to J.T.). This study was further supported by the Research and Education program of the Medical Faculty of the University of Cologne and funded by the German Ministry of Education and Research (BMBF), Grant No. 01KI0601 (to M.O.) and the German Liver Foundation (to M.O). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interest exist.

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## Introduction

Chronic liver injury of different etiologies leads to hepatic fibrosis, which might progress to end-stage liver disease. Portal hypertension often develops in these patients and is responsible for severe complications, such as variceal bleeding, refractory ascites and hepato-renal syndrome [1,2]. Hyperdynamic circulation presenting with increased portosystemic shunting and arterial hypotension is a hallmark for the development of complications. Certain complications of portal hypertension can be treated using transjugular intrahepatic portosystemic shunt (TIPS), which leads to an immediate decompression of the portal venous system [2]. TIPS insertion might improve survival in well-selected patients with bleeding, refractory ascites and hepato-renal syndrome [3–6]. However, TIPS increases porto-systemic shunting and therefore leads to hepatic hypoperfusion, which can cause or aggravate hepatic encephalopathy. Therefore, careful selection of patients is mandatory, and new biomarkers might be useful in this regard.

Circulating micro ribonucleic acid (miRNA) have been increasingly evaluated as potential biomarkers in different disorders, including liver diseases [7–10] miRNA are small noncoding RNAs consisting of 19 to 25 nucleotides. miRNA modify the posttranscriptional processes of mRNA and regulate important cellular processes, such as differentiation, proliferation, metabolism and apoptosis [11]. It has been shown that circulating levels of miRNA-34a are associated with liver diseases. Since miRNA-34a has been associated also with other disorders besides liver diseases [12,13], the major source of miRNA-34a remains unclear. Furthermore, there are hints that miRNA-34a might mirror the severity of liver disease. In human, as well as in mouse model of NASH, positive correlations of miR-34a levels with disease severity were already described [7–10]. Another study proposed a link between miR-34a and TGF-β in HBV positive

patients, which is a major cytokine in development of liver fibrosis and progression of liver disease [14]. However, its role as a biomarker for severity of liver cirrhosis with portal hypertension remain unknown to date.

In the present study, circulating levels of miRNA-34a were measured in the portal and hepatic vein of cirrhotic patients with severe portal hypertension receiving TIPS. Our aim was to investigate the portentiol role of miRNA-34a as a predictor of complications and survival for cirrhotic patients receiving TIPS.

## Patients and Methods

### Patients and data collection

We retrospectively included 60 patients with liver cirrhosis underwent TIPS implantation. General clinical characteristics are shown in Table 1. Clinical, hemodynamic and biochemical parameters were assessed during the study. The patients signed a written inform consent for the procedures in the study. The local ethics committee of the University of Bonn approved the study (029/13).

### Study design

The patients received TIPS for therapy-refractory ascites (n = 27), recurrent bleeding (n = 26), or both indications (n = 7). TIPS (8–10 mm Wallstent, Boston Scientific, MA, USA) insertion was performed as previously described [3,15,16]. Portal and hepatic venous pressures were measured invasively using a pressure transducer system (Combitrans, Braun Melsung, Germany) and a multichannel monitor (Sirecust, Siemens, Germany). The difference between these pressures was defined as the portal hepatic venous pressure gradient (PHPG). Arterial pressure and heart rate were monitored non-invasively. Biochemical parameters, as well as portal and systemic hemodynamics, were measured and recorded at TIPS placement and during follow-up (Table 2 and 3). Biochemical parameters were analyzed using standard methods. In follow-up, portal vein flow and velocity, cross-sectional area of the portal vein were measured by ultrasound after a median of 14 days. We calculated the congestion index, which represents the ratio between the cross-sectional area and the blood flow velocity of the portal vein. The congestion index, first described by Moriyasu [17], mirrors best the congestion due to portal hypertension.

### miRNA-34a isolation and quantification by real-time PCR

During the TIPS procedure, blood from the portal and hepatic veins to determine levels of miRNA-34a was collected from all patients as soon as the right branch of the portal vein was cannulated as previously described [3,15,16,18]. Blood samples were centrifuged at 3000 rpm for 15 minutes at 4°C and stored at −80°C. RNA was isolated from serum samples using the Qiazol reagent following the instructions of the supplier (Qiagen, Hilden, Germany) as previously described. SV40-miRNA (Qiagen) was added to serum samples (2 pmol/200 µl) prior to the RNA isolation procedure for later normalization of circulating miRNA-34a levels, while RNA quantity was determined by $A_{260}$-measurement using the ND-1000 NanoDrop spectrophotometer (NanoDrop, Wilmington, DE, USA) and quality was assessed by microcapillary electrophoresis (2100 BioAnalyser, Agilent Technologies, Waldbronn, Germany).

miRNA was analyzed by a two-step real-time PCR using the miScript-Reverse Transcription Kit and the miRNA-SYBR Green PCR Kit (Qiagen, Hilden, Germany). miRNA-34a and SV-40 primers used for cDNA synthesis and real-time PCR were selected and purchased from the GeneGlobe Search Center (Qiagen, Hilden, Germany). All steps were performed in triplicate and in agreement with the supplier's guidelines. For normalization of extracellular miRNA-34a levels, spike-in SV40-miRNA (Qiagen, Hilden, Germany) was used.

### Statistical analysis

In our study, we used the non-parametric Wilcoxon test to compare paired data and the Mann-Whitney test for unpaired comparisons. Correlations were analyzed with the Spearman correlation coefficient. Univariate time-to-event analysis was performed to identify parameters, which significantly predict survival. Cox-regression analysis (forward step-wise likelihood-quotient) using the significant predictors in the univariate analysis

**Table 1.** Clinical parameters of the patients (n = 60) at TIPS placement.

| Parameters | Values | miRNA-34a>median | miRNA-34a<median |
|---|---|---|---|
| gender (female/male) | 24/36 | 13/17 | 11/19 |
| age median (range) | 58 (40–77) | 57. 5 (41–77) | 57.5 (40–72) |
| etiology (alcohol/hepatitis/other) | 45/7/8 | 21/4/5 | 24/3/3 |
| Child category (A/B/C) | 11/36/13 | 7/15/8 | 4/21/5 |
| Child score median (range) | 8 (5–12) | 8 (5–11) | 8 (5–12) |
| MELD score median (range) | 10 (6–30) | 9.5 (7–30) | 10 (6–25) |
| indication (recurrent bleeding/refractory ascites/both) | 26/27/7 | 14/13/3 | 12/14/4 |
| esophageal varices (absent and Grade I/Grade II and III) | 19/41 | 8/22 | 10/20 |
| ascites (absent/Grade I/Grade II) | 11/16/33 | 5/11/14 | 6/5/19 |
| hepato-renal syndrome (absent/present) | 46/14 | 25/5 | 21/9 |
| hepatic encephalopathy (absent/present/undocumented) | 21/10/29 | 12/7/11 | 9/3/18 |
| antibiotic therapy (yes/no) | 18/42 | 8/22 | 10/20 |
| betablockers therapy (yes/no) | 12/48 | 5/25 | 7/23 |

There was no significant difference in any variable between the groups.
MELD, model for end-stage liver disease.
doi:10.1371/journal.pone.0103779.t001

**Table 2.** Biochemical and hemodynamic parameters before TIPS insertion and at a short-term follow-up after a median of fourteen days (n = 60) compared using with Wilcoxon test.

| | median | median | |
|---|---|---|---|
| **Parameters** | **before TIPS** | **after TIPS** | **p-Value** |
| bilirubin (mg/dL) | 1.2 | 1.5 | 0.009 |
| AST (U/L) | 18 | 22 | 0.049 |
| ALT (U/L) | 17 | 20 | 0.009 |
| γGT (U/L) | 53 | 105 | <0.001 |
| INR | 1.1 | 1.2 | 0.003 |
| sodium (mmol/L) | 135 | 137 | 0.002 |
| serum creatinine (mg/dL) | 1.00 | 0.93 | 0.048 |
| BUN (mg/dL) | 43 | 31 | 0.001 |
| portal vein flow velocity (cm/s) | 15 | 33 | <0.001 |
| portal vein flow (mL/min) | 1,31 | 2,51 | <0.001 |
| PHPG (mmHg) | 21 | 9 | <0.001 |
| Congestion index | 0.101 | 0.046 | <0.001 |

BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, gamma-glutamyl transpeptidase; INR, International Normalized Ratio; PHPG, portal hepatic pressure gradient.
doi:10.1371/journal.pone.0103779.t002

was performed to identify independent predictors of survival. Kaplan-Meier curves were used to analyze the survival rates of patients using the Log-rank test. Statistical analysis was performed by means of SPSS 22 for Windows SPSS Inc. Chicago,IL, USA).

## Results

### Clinical, biochemical and haemodynamic characteristics

The clinical characteristics of the included patients are listed in Table 1. The median age of the included patients (36 male, 24 female) was 58 years with a range of 40 to 77 years. The etiology of cirrhosis was alcohol in 45 patients, chronic hepatitis in seven patients, while eight patients had other diseases. Eleven patients presented with Child A, 36 with Child B and 13 with Child C liver cirrhosis. The MELD score showed a median of 10, with a range from 6–30 points. While patients had experienced hepato-renal syndrome, ten patients had experienced at least one episode of hepatic encephalopathy. The indication for TIPS was recurrent bleeding in 26 cases and refractory ascites in 27 cases. In seven patients, both indications for TIPS were present. Before TIPS, 47 patients were treated with non-selective beta-blocker, while 41 patients had received prior antibiotic therapy for infections (data not shown).

The short-term effects of TIPS on the biochemical parameters are shown in Table 2. TIPS led to significant increase of bilirubin ($p = 0.009$), AST ($p = 0.049$), ALT ($p = 0.009$), γGT ($p<0.001$) and INR ($p = 0.003$). Significant higher levels of sodium ($p = 0.002$) were found in patients after TIPS, when compared with levels before TIPS (Table 2), whereas levels of creatinine ($p = 0.048$) and BUN ($p = 0.001$) decreased after TIPS (Table 2).

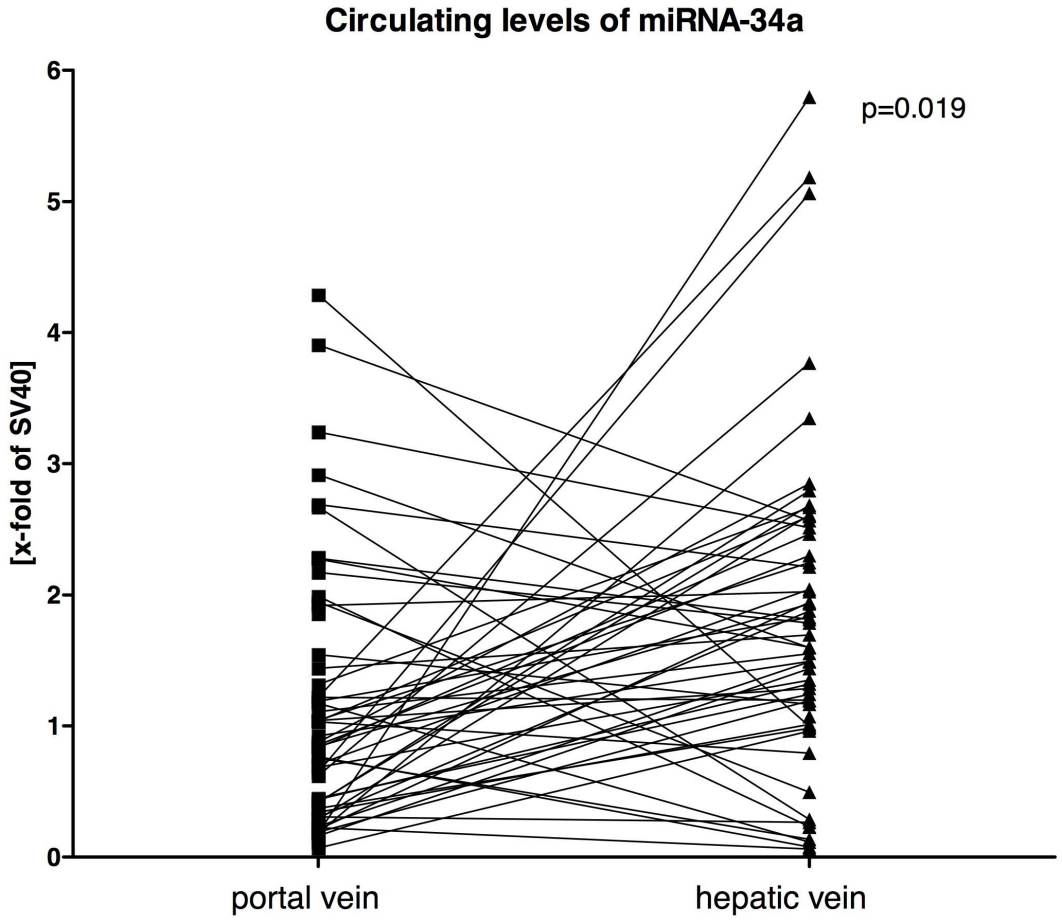
Furthermore, after TIPS portal vein flow velocity ($p>0.001$) and portal vein flow ($p<0.001$) increased, while levels of portal vein pressure gradient ($p<0.001$), as well as the congestion index ($p<0.001$) decreased as marker of successfully TIPS intervention treatment of portal hypertension (Table 3).

Interestingly, the percentage of portal pressure gradient reduction after TIPS showed a median value of 56.7% (21–91.7). Patients with a greater reduction in portal pressure gradient than the median showed a better survival after 5 years ($p = 0.035$).

**Table 3.** Univariate time-to-event analysis of patient characteristics (including variables of table 1, 2 and 3). In the table are shown only significant variables.

| | | 95% confidence interval | | |
|---|---|---|---|---|
| **Parameters** | **hazard ratio** | **low** | **upper** | **p-value** |
| miRNA-34a in hepatic vein | 0.889 | 0.775 | 1.019 | 0.091 |
| MELD | 1.129 | 1.053 | 1.21 | 0.001 |
| creatinine in short-term follow-up | 1.469 | 1.159 | 1.863 | 0.001 |
| sodium before TIPS | 0.857 | 0.794 | 0.925 | <0.001 |
| portal pressure before TIPS | 1.056 | 1.005 | 1.109 | 0.03 |
| portal pressure after TIPS | 1.215 | 1.094 | 1.35 | <0.001 |

MELD, model for end-stage liver disease.
doi:10.1371/journal.pone.0103779.t003


Circulating levels of miRNA-34a
p=0.019
[x-fold of SV40]
0
1
2
3
4
5
6
portal vein
hepatic vein

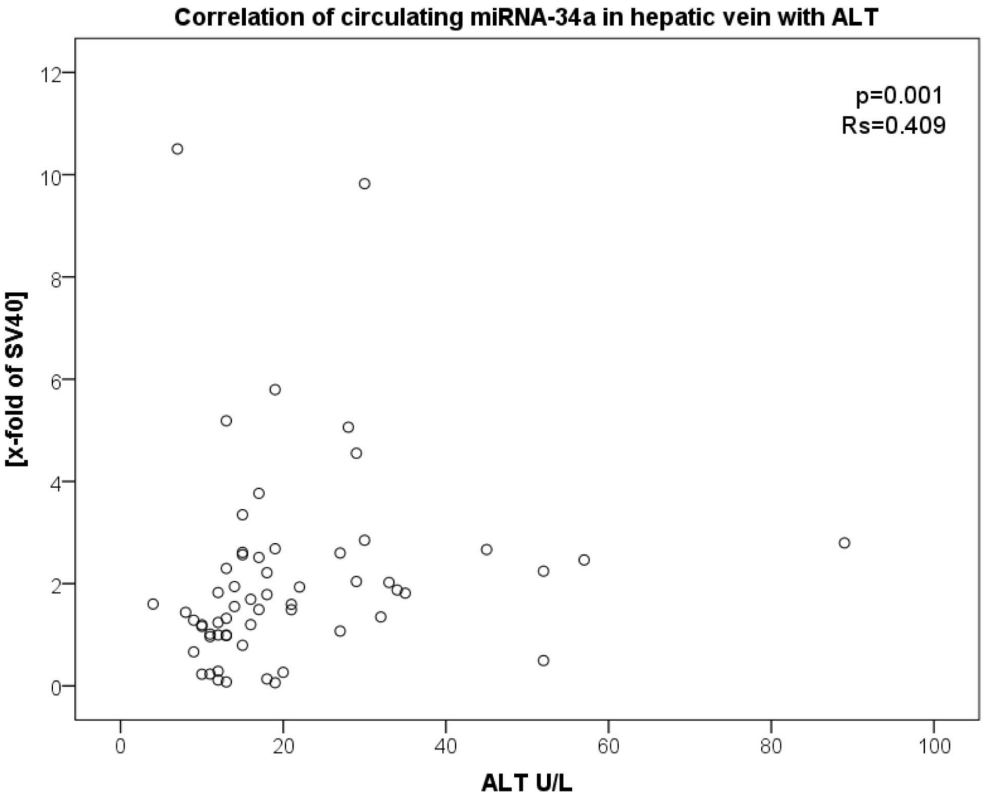

Correlation of circulating miRNA-34a in hepatic vein with ALT
p=0.001
Rs=0.409
[x-fold of SV40]
0
2
4
6
8
10
12
0
20
40
60
80
100
ALT U/L

**Figure 1. Serum levels of miRNA-34a in portal and hepatic vein before TIPS placement and its correlation with ALT-levels.** (A) The levels of miRNA-34a measured in portal vein and hepatic vein before TIPS showed significant increase of miRNA-34a levels across the liver ($p = 0.019$). Data were shown paired and analyzed by Wilcoxon test. Of note, data of six patients lay outside of the shown range, and were not shown to increase readibility. (B) Levels of circulating miRNA-34a in the hepatic vein correlated significantly with ALT before TIPS ($r_s = 0.409$; $p = 0.001$). Data are presented using Spearman coefficient $r_s$ and p-values. The levels of miRNA-34a were normalized to SV40 and are displayed as the x-fold of SV40.
doi:10.1371/journal.pone.0103779.g001

### Circulating levels of miRNA-34a derive from the liver and correlate with hepatic and renal parameters

Interestingly, the levels of miRNA-34a were significantly higher in the hepatic vein compared to the portal vein ($p = 0.019$) (Figure 1A).

We observed a relationship between miRNA-34a and hepatic and renal parameters. Levels of circulating miRNA-34a in the hepatic vein correlated significantly with ALT before TIPS ($r_s = 0.409$; $p = 0.001$; Figure 1B), CHE before TIPS ($r_s = 0.372$; $p = 0.025$) and sodium excretion in 24 h urine in the short-term follow-up after TIPS ($r_s = 0.449$; $p = 0.005$).

Furthermore, the levels of miRNA-34a in the portal and hepatic vein showed a significant inverse correlation with systolic (portal vein $r_s = -0.316$; $p = 0.018$ hepatic vein $r_s = -0.271$; $p = 0.04$) and diastolic (portal vein $r_s = -0.275$; $p = 0.04$; hepatic vein $r_s = -0.262$; $p = 0.018$) pressure measured non-invasively. There was no correlation of miRNA-34a with HPVG (data not shown). Additionally, the levels of miRNA-34a in the portal vein correlated inversely with the congestion index ($r_s = -0.297$; $p = 0.0031$) before TIPS. However, miRNA-34a measured in the portal vein showed inverse correlations with the cross sectional area in the portal vein before TIPS ($r_s = -0.316$; $p = 0.02$).

### The association of miRNA-34a measured in portal vein and hepatic vein with survival rates in patients receiving TIPS

There was no significant correlation between survival and circulating levels of miRNA-34a in the portal or the hepatic vein. However, the patients with higher levels of miRNA-34a in the hepatic vein showed a tendency towards better survival in the long-term follow-up after TIPS (Table 4) (HR = 0.889; 95%CI: 0.775–1.019; $p = 0.091$; Table 4). Further parameters, such as MELD (HR = 1.129; 95%CI: 1.053–1.21; $p = 0.001$), creatinine in short-term follow-up (HR = 1.269; 95%CI: 1.159–1.863; $p = 0.001$), serum sodium before TIPS (HR = 0.857; 95%CI: 0.794–0.925; $p<0.001$), portal pressure before TIPS (HR = 1.056; 95%CI: 1.005–1.109; $p = 0.03$), and portal pressure after TIPS (HR = 1.215; 95%CI: 1.094–1.35; $p<0.001$) were identified as predictors of survival using the univariate time-to-event analysis (Table 4, Figure 2). When stratifying this collective of patients using the median value of circulating miRNA-34a levels in the hepatic vein, patients with higher levels of miRNA-34a tended towards better survival (Figure 2 A).

Multivariable Cox regression analysis of the significant variables identified MELD (HR = 5.1; 95%CI: 1.7–15; $p = 0.003$), creatinine in short-term follow-up (HR = 103; 95%CI: 2.6–3,971; $p = 0.013$) and portal pressure after TIPS (HR = 1.4; 95%CI: 1.04–1.75; $p = 0.023$) as independent predictor of survival (Table 4).

## Discussion

This study demonstrates for the first time that miRNA-34a originates to a large extent from the liver and that levels of circulating miRNA-34a in hepatic vein correlated with parameters of hepatic dysfunction. Interestingly, miRNA-34a levels in the portal vein correlated inversely with portal venous congestion. Also, higher levels of circulating miRNA-34a in the hepatic vein might have a predictive value for survival in patients receiving TIPS.

Liver cirrhosis with portal hypertension is a major cause of morbidity and mortality world-wide [19–31]. TIPS is a widely accepted and used therapy for portal hypertension. TIPS placement decompresses the portal venous system. It reduces portal venous perfusion, with higher risk of hepatic encephalopathy and liver failure [28,30–33]. Therefore, careful selection of patients receiving TIPS is required and new markers for the predictive of complications after TIPS are needed.

Circulating miRNAs have been discussed as biomarkers for different disorders. This study demonstrates for the first time that miRNA-34a originates to a large extent from the liver. In the setting of TIPS-insertion, blood from the portal vein and from the hepatic vein was withdrawn simultaneously and the gradient showed a significant increase of miRNA-34a across the liver. This finding is supported by the positive correlation of the levels in the hepatic vein with aminotransferases and cholinesterase.

Interestingly, miRNA-34a in the portal venous compartment showed an inverse correlation with the cross-sectional area of portal vein measured by ultrasound and the portal congestion index. The congestion index, first described by Moriyasu, is calculated from parameters measured by ultrasound. This calculated index mirrors best the congestion due to portal hypertension [17]. The inverse correlation of miRNA-34a level in the portal vein with the portal venous congestion may be explained by lower hepatic perfusion with increasing congestion of the portal vein. Even though the systemic circulation was not assessed invasively at TIPS-procedure, one might be speculated that the decreased hepatic perfusion compared to systemic circulation could be the reason for the decreased release of miRNA-34a into the circulation and therefore lower levels in the arterial blood and consequently in the portal venous blood. The relationship of miRNA-34a and organ perfusion is further supported by the finding that systemic arterial pressures correlated inversely with levels of miRNA-34a in both vascular compartments. Hence, an increased splanchnic perfusion pressure in the situation of portal hypertension means higher portal venous congestion with consequently lower miRNA-34a levels, as found for the congestion index.

Although, levels of miRNA-34a in the portal vein possibly explain the portal vein congestion in the situation of severe portal hypertension, these levels showed no predictive value after portal venous decompression using TIPS. In contrast, the levels of circulating miRNA-34a measured in the hepatic vein tended to predict survival in these patients in univariate analysis. This finding might be explained by the correlation of these levels in the hepatic vein with ALT and CHE, which possibly mirror conserved liver function. Indeed, when stratifying the patients using the median of the miRNA-34a levels in the hepatic vein, patients with higher circulating levels of miRNA-34a had a better survival. After TIPS, patients with better conserved liver function showed a better survival, since MELD was an independent predictor of mortality in multivariate analysis in our collective besides renal function and portal pressure after TIPS. Interestingly, circulating miRNA-34a correlated with sodium excretion after TIPS as a marker of

**Table 4.** Cox regression analysis (forward step-wise likelihood-quotient) using the significant variable from univariate analysis (table 4) to predict survival.

| | | 95% confidence interval | | |
|---|---|---|---|---|
| **Parameters** | **Hazard ratio** | **lower** | **upper** | **p-value** |
| MELD | 5.0 | 1.7 | 14.9 | 0.003 |
| creatinine in short-term follow-up | 103 | 2.6 | 3,970 | 0.013 |
| portal pressure after TIPS | 1.35 | 1.04 | 1.75 | 0.023 |

MELD, model for end-stage liver disease.
doi:10.1371/journal.pone.0103779.t004

restored renal function. Thus, both the preserved hepatic function before TIPS and the restored renal function after TIPS may play a role in survival and are associated with the hepatic venous levels of miRNA-34a. Therefore, levels of miRNA-34a in the hepatic vein might represent a valuable biomarker for the outcome of patients receiving TIPS, but not in the same degree as MELD; creatinine in short-term follow-up and portal pressure after TIPS. In this collective of patients MELD, creatinine and portal pressure are more appropriate to predict outcome than the levels of miRNA-34a.

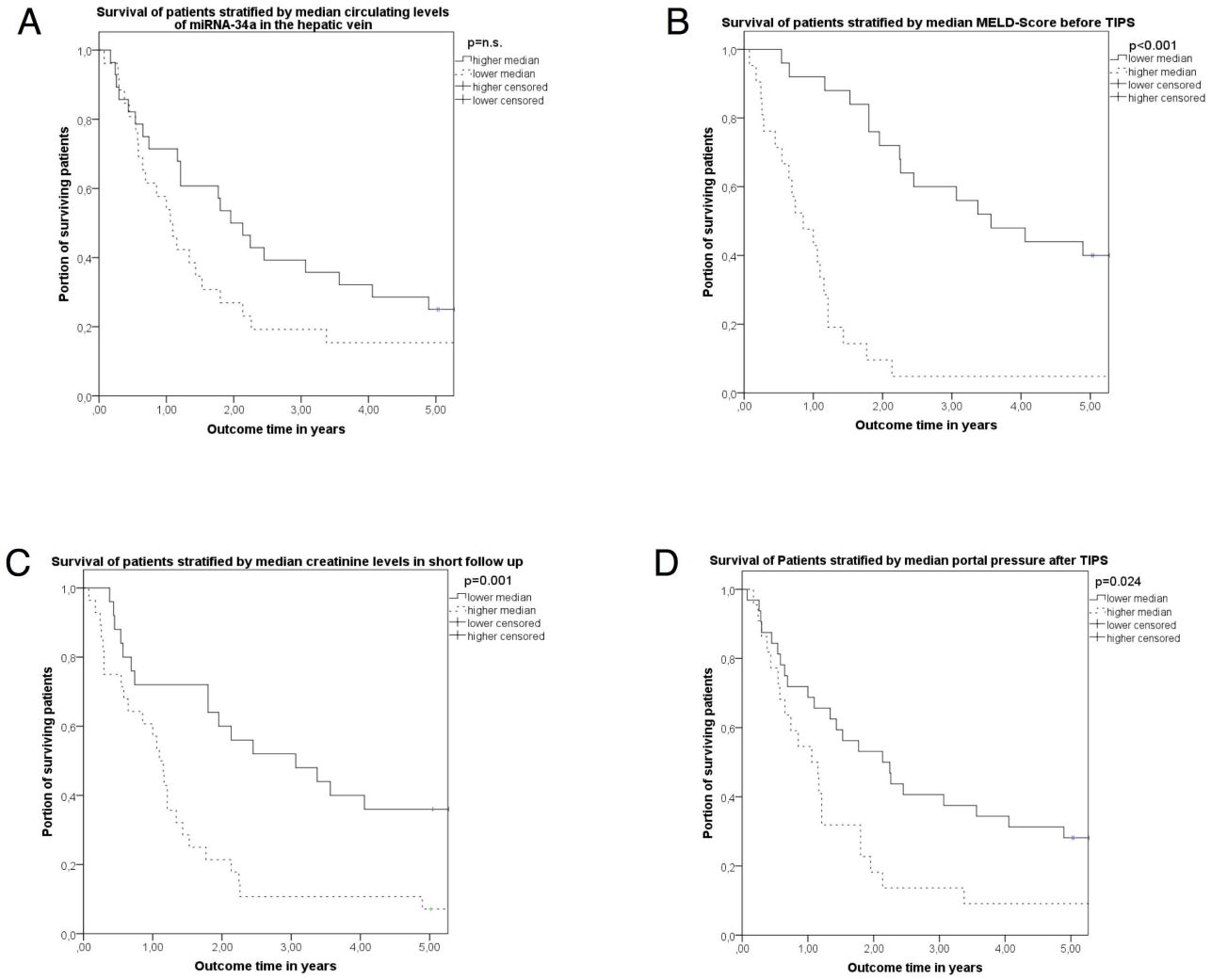



**Figure 2. Survival 5 years after TIPS stratified using the median of circulating miRNA-34a (A) in the hepatic vein as well as MELD (B), creatinine in short-term follow-up (C) and portal pressure after TIPS (D).** Patients were stratified for circulating miRNA-34a (A) measured in the hepatic vein, MELD-Score before TIPS (B), creatinine in short-term follow-up (C) and portal pressure after TIPS insertion (D) to higher and lower levels of the median of these parameters. Survival rates are shown using Kaplan-Meier plots and analyzed by log-rank test.
doi:10.1371/journal.pone.0103779.g002

Although our patient collective was very well characterized before and after TIPS with a long-term follow up, our study has several limitations. First, the extraction of miRNA was performed phenol-based and therefore, we did not distinguish between vesicle bound and free miRNA. The analysis of the patient data was retrospective and could not elucidate the cause of death in these patients. Despite these limitations, the studied cohort was consistent with other studies, since MELD, creatinine in follow up and portal pressure after TIPS were independent predictors of mortality.

As already shown by Malinchoc et al, MELD-Score is the best predictor of survival in patients receiving TIPS [34]. However, we could not confirm the role of bilirubin or age as prognostic marker in these patients as described previously [4,35]. However, the level of portal pressure gradient seems to be a good marker of survival as it has been described for HVPG [36].

In conclusion, this study demonstrates for the first time that miRNA-34a might originate to a large extent from the diseased liver and hepatic venous level of miRNA-34a might be a predictor of outcome in cirrhotic patients receiving TIPS. Future studies are needed to confirm these findings.

## Acknowledgments

We thank Dagmar Bammer, Gudrun Hack and Silke Bellinghausen for their excellent technical assistance.

## Author Contributions

Conceived and designed the experiments: JT. Performed the experiments: CJ HE JG RS MO JT. Analyzed the data: CJ JT HE MO. Contributed reagents/materials/analysis tools: JG EA CPS TS MO JT. Wrote the paper: CJ JG EA CPS TS MO JT.

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# 4 Diskussion

Die Arbeiten, die dieser Schrift zu Grunde liegen, zeigen nicht-invasive diagnostische Methoden, die es ermöglichen, das Vorhandensein und das Ausmaß der zirrhotische Kardiomyopathie im klinischen Alltag zu detektieren. Es konnte gezeigt werden, dass die Ergebnisse dieser Diagnostik einen zusätzlichen prognostischen Wert für die Risikoabschätzung bei Patienten mit fortgeschrittener Leberzirrhose darstellen. Bei der Holter Elektrokardiogramm Diagnostik und der transthorakalen Echokardiographie, handelt es sich um ubiquitär vorhandene Untersuchungsmethoden. Die Auswertung der Rohdaten mittels Speckle-Tracking-Analyse und Herzrhythmus-Analyse ist in einem Zentrum auch für zuweisende Kliniken möglich. Es ist jedoch anzumerken, dass in den letzten Jahren auch die Speckle-Tracking-Analyse mehr und mehr in neue Ultraschallgeräte integriert ist. Der Gebrauch in der klinischen Routine ist jedoch noch eingeschränkt. Die Shear-Wave-Elastographie hingegen ist aufgrund der Repräsentation den aktuellen Leitlinien bereits in jeder Klinik möglich. Die Bestimmung von Biomarkern außerhalb von BNP ist zu Zeit leider nur in Studien etabliert. Ziel der Diagnostik ist es, Hochrisiko-Patienten mit Leberzirrhose zu identifizieren und die Intervalle der klinischen Visite an das persönlichen Risikoprofil des Patienten, auch unter Berücksichtigung des Geschlechts anzupassen.

Die Herzrhythmus Variabilität ist eine nicht-invasive Methode zur Analyse der autonomen Funktion des Sinusknotens bei Patienten mit und ohne kardialen Erkrankungen (Berntson et al. 1997; Stein und Pu 2012; Omerbegovic 2009; Vukasovic et al. 1999; Kolasińska-Kloch et al. 2002). Es konnten gezeigt werden, dass durch die Herzrhythmus Variabilität Patienten identifiziert werden können, die ein erhöhtes Risiko für einen plötzlichen Herztod aufweisen (Kurths et al. 1995). Andere Studien konnten bereits zeigen, dass die systemische Entzündung eine entscheidende Rolle in der Entwicklung des ACLF spielen. Auch die Beeinträchtigung der Herzfrequenzdynamik scheint damit verbunden zu sein (Bhogal et al. 2018; Haddadian et al. 2013). Eine Assoziation zu erhöhten Zytokinspiegeln konnte bereits dokumentiert werden (Mani et al. 2009). Ein Versuch die reduzierte Variabilität des Herzrhythmus bei Patienten mit Leberzirrhose zu erklären, ist die erhöhte bakterielle Translokation als treibende Kraft der systemischen Entzündung im Verlauf der Erkrankung (Mani et al. 2009). Dies wird durch die Beobachtung untermauert, dass hohe IL-6 Spiegel mit reduzierter Variabilität assoziiert zu sein scheinen (Mani et al. 2009). Diese

Beobachtung zeigte sich auch bei anderen systemisch entzündlichen Erkrankungen (Aronson et al. 2001; González-Clemente et al. 2007; Tateishi et al. 2007). Ein anderer Ansatz, um die reduzierten Variabilität zu erklären, ist das Vorhandensein einer autonomen Neuropathie (Ates et al. 2006; Coelho et al. 2001; Hendrickse et al. 1992; Lazzeri et al. 1997).

Tiermodelle bilden in diesem Fall nicht alle Aspekte unserer Patient ab, aber es wurde veröffentlicht, dass eine Verringerung der cholinergen Innervation im Zusammenhang mit schweren systemischen Entzündung auftritt (Gholami et al. 2012; Hajiasgharzadeh et al. 2011). Auch bei zirrhotischen Ratten nach Endotoxin Injektionen wurde über eine reduzierte Variabilität berichtet (Taghipour et al. 2016). Die Messung der Herzrhythmus Turbulenz stellt die kardiale Reflexantwort auf den kurzzeitigen Verlust des Herzzeitvolumens nach einer ventrikulären Extrasystole dar. Dieser kurzzeitige hämodynamische Einbruch führt über eine Aktivierung des sympathischen Nervensystems zu einem initialen Anstieg der Herzfrequenz. Die erhöhte ventrikuläre Füllung nach der kompensatorischen Pause führt wiederum zu einer erneuten Anpassung der Frequenz. Störungen in diesem Regulationsmechanismus können in der verminderten oder reduzierten Effektivität von myokardialer Beta-Adrenozeptoren begründet sein (Lee et al. 1990; Vasina et al. 2012). Die reduzierte Dichte der Beta-Adrenozeptoren wurde bereits beschrieben bei Patienten mit Leberzirrhose (Gerbes et al. 1986, 1986). Die autonome Dysfunktion bei Patienten mit Leberzirrhose trägt zur Entwicklung der zentralen Hypovolämie bei. Die zentrale Hypovolämie ist begründet durch den Verlust des Gefäßwiderstandes mit Erhöhung des ineffektiven Blutvolumens. Diese tritt vor allen bei hepatisch dekompensierten Patienten mit Aszites auf (Møller und BERNARDI 2013). Dies bedingt im Rahmen der dekompensierten Leberzirrhose die Hyperzirkulation und die hyperdyname Kreislaufsituation (Villanueva et al. 2016; Møller et al. 2000; McAvoy et al. 2016). Dies wiederum führt zu veränderten Druckverhältnissen im splanchnischen Einstromgebiet. Diese Änderung führt zu einem entsprechenden Anstieg der Steifigkeit der Leber in der Shear-Wave-Elastographie Messung. Wir konnten zeigen, dass neben der Fibrose auch hier das Maß der Inflammation als auch die Druckverhältnisse vor und nach der Leber Einfluss nehmen. Eine kardiale Vorlasterhöhung nach Shunt-Anlage mit ggf. Rückstau in die Leber nach transjugulärer intrahepatischer portosystemischer Shunt-Anlage einen negativen diagnostischen Faktor dar. Bei Patienten im Status der Dekompensation findet man weiterhin erniedrigte Serumalbumin-Spiegel. Dies führt

zu einem verminderten kolloidosmotischen Druck, welcher die zentrale Hypovolämie verstärkt (Garcia-Martinez et al. 2013). Wir konnten zeigen, dass durch die pathologisch veränderte Herzrhythmus Variabilität und Turbulenz eine adäquate Anpassung an diese Kreislauflage im Rahmen der Dekompensation nicht mehr möglich zu sein scheint. Bereits im Vorfeld wurde publiziert, dass die zentrale Hypovolämie und ein niedriger Hämoglobinspiegel unabhängige Prädiktoren für eine hepatische Dekompensation und die Entwicklung eines ACLF darstellen (Piano et al. 2017). Die pathologische veränderte Herzrhythmus Variabilität und Turbulenz scheint Ihren Teil dazu beizutragen.

Die Hypovolämie kann physiologisch durch eine Steigerung der kardialen Kontraktion kurzzeitig kompensiert werden. Dies zeigt sich zum Beispiel im Rahmen eines Schocks, sei es nun bedingt durch Blutung oder generelle Infektionen. Wir konnten zeigen, dass die Mortalität, zumindest in Nicht-Varizenblutungen, geschlechterspezifisch zu sein scheint. Betrachtet man z.B. schwere Trauma-Blutungen in Verbindung mit hämorrhagischem Schock, so ist untersucht, dass der Erhalt der Herzfunktion und der vaskulären Reaktionsfähigkeit entscheidend ist für die Aufrechterhaltung der hämodynamischen Stabilität. In dieser Hinsicht haben Studien gezeigt, dass die Verabreichung von Östrogensulfat nach schwerem Blutverlust die Ergebnisse in hämorrhagischen Schockmodellen verbesserte (Hubbard et al. 2015; Miller et al. 2014). Darüber hinaus zeigte die exogene Estradiolverabreichung nach schwerem Blutverlust eine positive Wirkungen im Sinne einer verbesserten Myokardfunktion sowie der vaskulären Reaktionsfähigkeit (Li et al. 2014; Soliman 2015). Ob damit das weibliche Geschlecht in dem Kollektiv der Patienten mit Leberzirrhose besser in der Lage ist den Kreislauf aufrecht zu erhalten und inwieweit diese Mechanismen der zirrhotischen Kardiomyopathie entgegenwirken können, ist noch nicht untersucht.

Die Therapie sieht einen bilanzierte Volumengabe vor (Meyhoff et al. 2022; Rivers et al. 2001), wohingegen der kardiale Schock eine restriktive Volumengabe erfordert (Combes et al. 2015). Die Volumengabe in der beginnenden hepatischen Dekompensation scheint jedoch nur bedingt möglich zu sein (Belcher et al. 2022).

Die kardiale Kontraktion ist auf Grund der zirrhotischen Kardiomyopathie ebenfalls pathologisch verändert. Sie folgt dabei nicht einem linearen Verlauf. Unsere Daten zeigen, dass die Kontraktilität in Ruhe im Stadium Child B abfallen und dann wieder

im Stadium C ansteigen. Die longitudinale Kontraktilität befindet sich aber im Mittel immer noch unter den Werten eines gesunden Probanden. Die vermehrte Kontraktilität scheint somit ein Marker der Hypovolämie zu sein. Speckle-Tracking-Echokardiographie ist in der Lage, eine frühe und noch subklinische myokardiale Dysfunktion in diversen kardialen Erkrankungen aufzzueigen (Dedeoglu et al. 2016; Yin et al. 2013; Maruo et al. 2015; Winter et al. 2007). Die Literatur in diesem Gebiet ist nicht eindeutig auszuwerten. Chen et al. (2016) und Sampaio et al. (2014) beschrieben einen reduzierten linksventrikulären Strain bei zirrhotischen Patienten im Vergleich zu gesunden Patienten (Chen et al. 2016; Nazar et al. 2013). Wir konnten jedoch zeigen, dass besonders Patienten mit einer Leberzirrhose im Stadium Child C sich dem gesunden longitudinalen linksventrikulären Strain annähern. Die Patienten mit mehr Kontraktilität in unsere Studie zeigten ein kurzes transplantationsfreies Zeitintervall. Dies wiederum unterstützt unsere Erkenntnisse, dass die Hypovolämie auf Grund der Steigerung des nicht-effektiven Blutvolumens nicht über die Herzfrequenz kompensierbar ist (Møller und Henriksen 2002). Wir gehen davon aus, dass der verlängerte Strain bei Patienten im Stadium der Child C Leberzirrhose nicht als Ausdruck kardialer Reserve zu werten ist, sondern sich Patienten mit einer erhöhten myokardialen Kontraktilität bereits im “Hyperzirkulationssyndrom” befinden und somit ein erhöhtes Risiko zur Dekompensation aufweisen. Das Herz ist nicht mehr in der Lage suffizient auf Stresssituationen wie z.B. Infektionen, Blutungen oder auch TIPS-Anlagen reagieren zu können.

Daten zu herkömmlichen Echokardiographie und Transplantation sind schon gut untersucht (Park et al. 1985; Kia et al. 2013; Sonny et al. 2018, 2018; Chang et al. 2001). Arbeiten zur prognostischen Aussagekraft der Speckle Tracking Echokardiographie bei Patienten mit einer Leberzirrhose und Transplantation sind nur sehr rar. Wir konnten zeigen, dass die linksventrikuläre Kontraktilität einen unabhängigen Prädiktor für Mortalität und das Leberversagen, insbesondere bei Patienten mit Leberzirrhose im Stadium C nach Child-Pugh-Score darstellt. Eine wichtige Erkenntnis ist aber, dass eine erhöhte Kontraktilität keinen Überlebensunterschied nach einer Lebertransplantation vorhersagen kann. Dies wiederum unterstützt die Annahme über das Krankheitsbild der zirrhotischen Kardiomyopathie, dass es sich um ein Leber-assoziiertes und reversibles Phänomen handelt.

Um den latenten Charakter des Krankheitsbildes der zirrhotischen Kardiomyopathie besser zu verstehen, untersuchten wir die Aussagekraft der Speckle-Tracking-Echokardiographie in Bezug auf die zirrhotischen Kardiomyopathie bei Patienten, die einen transjugulären intrahepatischen portosystemischen Shunt erhielten. Die Entwicklung eines ACLF assoziiert mit einer gesteigerten Mortalität, ist die schwerwiegendste Komplikation nach dieser Intervention. Diese gefürchtete Komplikation steht im Mittelpunkt vieler Untersuchungen. Diverse Parameter wurden ermittelt, um Hochrisikopatienten zu identifizieren (Hernández-Gea et al. 2019; Coronado et al. 2020; Lee et al. 2021; Praktiknjo et al. 2019; Tuifua et al. 2022; Chang et al. 2022; Chang et al. 2021). Wir konnten zeigen, dass der kardiale Biomarker miRNA-34a zu einem großen Teil aus der Leber stammt und einen prognostischen Marker darstellt in diesem Kollektiv. Teil des ACLF ist die Entwicklung einer zirkulatorischen Dysfunktion (Moreau et al. 2013), die zum Versagen von Organsystemen führt (Moreau et al. 2013). Der hohe Shuntfluss führt zur Verschiebung von portalvenösem Blut in den systemischen Kreislauf. Dadurch kommt es zu einer akuten Erhöhung der Vorlast, die nur von Patienten mit ausreichender myokardialer Reserve toleriert werden kann. Ein wichtiger Unterschied zu unserem Kollektiv von Patienten, die sich auf der Warteliste zur Transplantation befanden, ist das bessere Child Stadium dieser Patienten. Die Evaluation bezüglich der Shunt-Anlage beinhaltet einen Ausschluss von Patienten mit schweren Herzerkrankungen, wie hochgradigen Trikuspidalinsuffizienzen oder schwerer pulmonaler Hypertonie. Unsere Daten konnten zeigen, dass es bei Patienten mit mehr myokardialer Kontraktilität seltener zur Entwicklung eines ACLF oder zum Eintritt des Todes nach Intervention kommt, im Besonderen bei jenen Patienten, bei denen Aszites als Indikation für diese Intervention bestand. Ziel ist es durch die Senkung des porto-systemischen Druckgradienten, eine Normalisierung der Hormonsysteme zu erreichen, die die Hyperzirkulation und zentrale Hypovolämie unterhält. Daher untersuchten wir die kardiale Funktion mittels Speckle-Tracking-Echokardiographie auch nach transjugulärer intrahepatischer portosystemischer Shunt Anlage.

Es wurde bereits im Vorfeld publiziert, dass nach der Intervention die linksatrialen- und linksventrikulären diastolischen Durchmesser vergrößert sind. Nicht selten ist ein Anstieg des systolischen pulmonal arteriellen Drucks nachzuweisen (Wannhoff et al. 2016; Merli et al. 2002; Huonker et al. 1999).

Unsere Daten zeigen, dass die Patienten, die Ihre Kontraktilität nach transjugulärer intrahepatischer portosystemischer Shunt-Anlage um weitere 20 Prozent steigern mussten, also kein Ansprechen des Hyperzirkulationssyndrom zeigten, ein schlechteres Überleben aufwiesen. Weiterhin konnten wir die wichtige Rolle der Kardiomyopathie und ihren prognostischen Wert in der Diagnostik und Risikostratifizierung in diesem Kollektiv zeigen. Wir konnten ebenfalls zeigen, dass im Rahmen der Zirrhose die Kontraktilität zunächst abfällt und dann im Verlauf der Erkrankung ansteigt. Die Differenzierung zwischen strukturellem Unvermögen und kompensatorischer Aktivität scheint Stadium abhängig zu sein. Die aktuelle Studienlage zeigt, dass die zirrhotische Kardiomyopathie ein wichtiger Bestandteil des ACLF darstellt. Die kardiale Reserve einer hyperdynamischem Kreislaufsituation entgegen wirken zu können, scheint ein wichtiger Faktor für das Überleben dieser Patienten darzustellen. Weitere Studien zur Untersuchung dieser komplexen Zusammenhänge zwischen Kontraktilität, Rhythmusanpassung, invasiven Messungen der Hämodynamik und Ausmaß der Hypovolämie in Bezug auf das Entstehen des ACLF sind zwingend notwendig, um das Krankheitsbild besser zu verstehen. Wichtig ist festzuhalten, dass sich unsere nicht-invasiven Methoden im Vergleich zu etablierten Risiko Scores wie MELD und CHILD als gleichwertig erwiesen oder sie in ihrer prognostischen Aussagekraft übertrafen.

# 5 Zusammenfassung

Zusammenfassend habe ich in dieser Arbeit neue, nicht-invasive diagnostische Methoden evaluiert, die in Zukunft dabei helfen sollen, Hochrisikopatienten frühzeitig zu identifizieren. In Anbetracht der fehlenden kurativen Maßnahmen bei Patienten mit weit fortgeschrittenen Lebererkrankungen, abgesehen von der Lebertransplantation, ist die Vermeidung der Dekompensation und des akut- auf-chronischen Leberversagens (ACLF) entscheidend für die Langzeitprognose der Patienten.

Ich konnten zeigen, dass Herz Rhythmusanalysen bei Patienten mit weit fortgeschrittener Leberzirrhose pathologische Veränderungen bezüglich der Rhythmus Variabilität und Turbulenz aufweisen. Die Parameter der Rhythmus Turbulenz korreliert mit dem Schweregrad der Lebererkrankung. Weiterhin korreliert die Rhythmus Variabilität mit dem Auftreten von Dekompensation und Entwicklung eines ACLF. Die Rhythmus Variabilität stellt einen unabhängigen Prognosefaktor für die Sterblichkeit dar.

Unter Verwendung der echokardiographischen Untersuchungen konnten wir zeigen, dass eine erhöhte myokardiale Kontraktilität gemessen mittels Speckle-Tracking-Echokardiographie auf eine drohende Dekompensation hinweist und dies nach einer Lebertransplantation reversibel zu sein scheint. Um den latenten Faktor dieser Erkrankung besser zu verstehen, untersuchten wir die zirrhotische Kardiomyopathie in Bezug auf auslösende Ereignisse für eine Dekompensation und Ausbildung eines ACLF. Hier konnten wir zeigen, dass ein reduzierter Strain vor transjugulärer intrahepatischer portosystemischer Shunt-Anlage und ein fehlendes Ansprechen der Hyperzirkulation nach dieser Intervention, welche sich in einer Verkürzung des Myokards um mehr als 20 Prozent ausdrückt, prädisponierende Faktoren für eine Entwicklung von ACLF und Mortalität sind. Routine Diagnostiken in der Behandlung der Leberzirrhose wie die Shear-Wave-Elastographie sind durch kardiale Ereignisse beeinflussbar. In Kombination mit neuen Biomarker werden wir in Zukunft drohende Dekompensationen früher erkennen können.

# 6 Überlappung mit anderen Habilitationsschriften

Inhaltliche Überlappungen mit anderen Habilitationsschriften sind ausgeschlossen, da jede der hier kumulierten Originalarbeiten ausschließlich in dieser und keiner anderen Habilitationsschrift eingereicht wurden.

# 7 Danksagung

Meine wissenschaftliche Tätigkeit begann nicht wie üblich während meines Studiums, wie ich es bei meinen Kollegen häufig lese oder höre. Vielmehr kam ich an die Universitätsklinik Bonn, um zumindest einen Doktortitel für meinen weiteren Werdegang vorweisen zu können. Dieser war zu diesem Zeitpunkt noch nicht klar gezeichnet.
Interessierte ich mich im Studium noch für die Psychiatrie, so kristallisierte sich gegen Ende die Hausarztmedizin heraus, die im PJ dann doch von der Kardiologie weit zurückgelassen wurde. Aus diesem Grund bewarb ich mich zum damaligen Zeitpunkt in der medizinischen Klinik II bei Herrn Prof. Nickenig. Meine ersten Gehversuche in der Welt der Forschung erlebte ich dann bei Herrn Prof. Hammerstigel, der mich für die Echokardiographie begeistern konnte. Aber die wunderbare Welt der Rhythmologie, an der mich Herr Prof. Schwab teilhaben ließ begeistere mich auch.
In der Notaufnahme traf ich dann auf einen Kollegen, der gerade dabei war, sich zu habilitieren mit Namen Trebicka. Jonel zeigte mir der Vielfältigkeit der inneren Medizin. Gastroenterologe und Hepatologe waren ab sofort erstrebenswerte Ziele.
Daraufhin erfolgte die Bewerbung bei Herrn Prof. Strassburg, dem ich für die Chance des Wechsels herzlich danke und für die Möglichkeit in der Arbeitsgruppe von Herrn Prof. Trebicka meine Habilitation anzustreben.
Herr Prof. Trebicka nahm mich nicht nur in seine Arbeitsgruppe auf, sondern förderte und forderte mich auch im klinischen Alltag als Oberarzt der Station Hufeland. Für dieses Vertrauen und das Mentoring werde ich ihm immer von ganzem Herzen dankbar sein. Sein unermüdliches Engagement und seine Art, das Menschliche nie außer Acht zu lassen, haben mich für immer geprägt. Auch nach seinem Wechsel nach Frankfurt und schließlich nach Münster wird diese Verbindung weiter bestehen.
Ich danke Herrn Prof. Jacob Nattermann, Herrn PD Dr. Jan-Christian Wasmuth, Herrn PD Dr. Philipp Lutz und Herrn Prof. Jürgen Rockstroh für Ihre tatkräftige Unterstützung um mich in die Rolle als Oberarzt einzufinden. Weiterhin danke ich Herrn Prof. Spengler, der auch nach dem er nicht mehr in unserem Haus tätig ist, für sein offenes Ohr zu jeder Zeit, um interessante Fälle zu besprechen. Ich danke besonders Frau Dr. Nina Böhling, Herrn Dr. Johannes Chang, Herrn Jan Görtzen-Patin, Frau Dr. Jennifer Lehmann-Bell und Frau Dr. Alessandra Pohlmann für das wunderbare Klima in unsere Arbeitsgruppe. Ich danke Herrn PD Dr. Michael Praktiknjo, der uns leider auch nach

Münster verließ für die wunderbare Zusammenarbeit. Ich bedanke mich bei allen Doktoranden, die ich mit Herrn Prof. Trebicka und Prof. Rockstroh betreuen durfte. Die hervorragende Unterstützung von Frau Nadine Köstlmeier und Karin Märtens möchte ich ganz besonders erwähnen. Ich bedanke mich bei allen Kollegen, die mir immer zur Seite standen, Herrn Dr. Carsten Meyer, Herrn PD Dr. Julian Luetkens, Herrn PD Dr. Claus Pieper, Herrn Priv.-Doz. Dr. Daniel Kütting, Herrn Dr. Patrick Kupczyk, Frau Dr. Tatjana Dell, Frau Dr. Julia Wagenpfeil und Herrn PD Dr. Philipp Lingohr.
Martina Wiersberg danke ich für die kritische Durchsicht dieser Schrift.

Ich danke von ganzem Herzen meinen Eltern für ihre selbstlose Unterstützung, die es mir und meinen Geschwistern ermöglicht haben, unser Weg zu finden.

Besonders danke ich meiner “kleinen Familie“, die an so manchen Abenden, Familienfeiern und Wochenenden auf mich verzichtet haben. Meine Tochter Frieda saß schon während ich meine Doktorarbeit schrieb auf meinen Schoß, wenn Sie nachts nicht schlafen konnte. Auch Jakob kennt schon seit dem dritten Lebensjahr die Worte, dass muss heute noch fertig werden. Ihr beide habt einen großen Teil zu dieser Arbeit beigetrage.

Besonders danke ich dir Christine, dass du mich zu jeder Zeit unterstützt hast auf diesem Weg. Ohne dich wäre dies alles nicht möglich gewesen.

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